The influence of cerebellar transcranial direct current stimulation (tDCS) on motor skill learning in swallowing

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Abstract

The cerebellum is highly involved in motor skill learning and has a primary role in the coordination of movements and error correction. Transcranial direct current stimulation (tDCS) applied over the cerebellum increases cerebellar excitability and enhances motor skill learning of healthy individuals in the corticospinal system. Whether these effects also occur for corticobulbar-related motor functions like swallowing is unknown. Cerebellar tDCS, as an adjuvant technique to motor skill training in swallowing, may offer new directions for enhancing functional and physiological outcomes for neurological swallowing rehabilitation.

Based on outcomes of cerebellar tDCS combined with skill training in limb function, it was hypothesised that anodal tDCS would enhance, and cathodal tDCS would inhibit motor performance and motor skill learning in this study of swallowing. In Behavioural study I, a double-blind randomised controlled trial (RCT) was performed to evaluate the effects of cerebellar tDCS on motor performance and learning in swallowing. Thirty-nine healthy adults were assigned to one of three conditions (anodal tDCS, cathodal tDCS, and sham). Two swallowing skill training sessions, with preceding cerebellar tDCS, were completed on consecutive days. The sessions consisted of a 2 mA current applied over midline cerebellum for 20 min, followed by skill training using sEMG biofeedback to target volitional control of timing and magnitude of submental muscle activation during swallowing. Similar to the corticospinal literature, cathodal tDCS inhibited motor skill learning of temporal accuracy gains compared to the sham condition ($p < .05$). However, anodal tDCS also inhibited the temporal aspects of motor skill learning in swallowing compared to sham ($p < .05$), which is in contrast to the hypotheses and the corticospinal literature. This suggests differences in the effects of cerebellar tDCS on corticobulbar and corticospinal motor functions. Furthermore, polarity dependent mechanisms of cerebellar tDCS need to be addressed in future research, since cathodal tDCS was not the behavioural inverse of anodal tDCS, as is seen in limb literature.

Behavioural study II represented a preliminary exploration of cerebellar tDCS on motor performance and learning in patients with neurological impairment. In this proof-of-concept study, six patients with oropharyngeal dysphagia following stroke were

randomly assigned to one of the three conditions (anodal tDCS, cathodal tDCS or sham). The same experimental procedure as in Behavioural study I was performed, however, a behavioural swallowing exam prior to commencement of the study and in the follow-up assessments was added. The assessment of swallowing skill learning, without visual feedback, was too challenging to complete in four out of the six patients. Only the patients in the cathodal group were able to complete the assessment, which hinders comparisons of learning between the three conditions in this study. Future studies will require development of an alternate measure of swallowing skill learning. All patients were able to complete the skill training protocol and the assessment of motor performance (with visual feedback). The outcomes in the assessment of performance did not change considerably from baseline in any patient over time regardless of stimulation condition. This is in contrast to the results of healthy participants in Behavioural study I where only one session of swallowing skill training was sufficient to significantly improve swallowing performance that remained over time. This indicates that two days of treatment may not be sufficient to increase swallowing performance in neurologically impaired patients. These patients may need multiple skill training sessions to increase volitional control over swallowing behaviours.

The majority of studies looking at the effects of cerebellar tDCS have been performed in the corticospinal motor system and have utilised a mono-hemispheric electrode placement. This placement has been validated by showing significant changes in cerebellar excitability, i.e. a significant increase in cerebellar-brain inhibition (CBI), immediately following cerebellar tDCS. However, unlike limb motor control, swallowing involves midline structures that are bilaterally innervated by the corticobulbar motor system. Therefore, this proof-of-concept study aimed to assess, if *midline* placement of the tDCS electrode over the cerebellum could achieve the same for a bilaterally innervated midline function. Changes in cerebellar excitability were assessed in fifteen healthy individuals using paired-pulse TMS over the cerebellum and motor cortex by measuring MEPs from the submental muscle group and the first dorsal interosseus muscle (FDI) of the dominant hand. Although MEPs from the submental muscles at rest were reported in previous research, no reliable MEP responses from this muscle group could be collected at a reasonable stimulator output (below 80% maximal stimulator output) in the current study. There were no statistically significant

differences between the active tDCS and sham group when evaluating tDCS effects of the cerebellum on the FDI motor circuits over time. This may suggest that a midline electrode placement for cerebellar tDCS of 2 mA applied over 20 minutes is not sufficient to induce neurophysiological changes within the corticospinal system. However, the lack of difference may likely be due to the large inter-individual response variability. This hypothesis is supported by findings in Behavioural study I, where behavioural inhibition was demonstrated using the same electrode placement. The results of this study provide guidance for adapted or newly developed assessment protocols that evaluate the effects of cerebellar tDCS at midline for swallowing, e.g. using pharyngeal MEP measures.

This research programme was the first to investigate the use of cerebellar tDCS for motor skill learning in swallowing in healthy individuals and patients with dysphagia following stroke. Cerebellar tDCS in combination with motor skill training, using the protocol as proposed in this research programme, inhibited motor skill learning in healthy individuals and may well be contraindicated for patients in swallowing rehabilitation using skill training. Further research is required to confirm this. However, swallowing skill training without tDCS was demonstrated to improve motor performance and motor skill learning in swallowing. This provides a strong indication for future research into the potential implementation of skill training in swallowing rehabilitation.

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Preface

This PhD thesis conforms to the referencing style recommended by the American Psychological Association Publication Manual (6th ed.) and spelling recommended by the Oxford Dictionary (http://oxforddictionaries.com). The candidate was enrolled in the Department of Communication Disorders at the University of Canterbury between May 2014 to April 2018, while the research for this PhD thesis was carried out. The research took place at the New Zealand Brain Research Institute until December 2014, after which the research was based at the UC Rose Centre for Stroke Recovery and Research. Prof Maggie- Lee Huckabee and Dr Phoebe Macrae supervised this research. Prof Richard Jones was part of the supervisory committee until December 2016 and Dr Sebastian Doeltgen was involved in the supervision of the methodological study. The research was conducted with support from the New Zealand Brain Research International Doctoral Scholarship and a travel scholarship from the Christchurch Rotary Club.

Conference Presentations

- Stroke Rehab: From No-Tech to Go-Tech Conference (Christchurch, New Zealand, 2015)
- Biomouth Symposium (Christchurch, New Zealand, 2016)
- UC Postgraduate Showcase (Christchurch, New Zealand, 2016)
- 25th Annual Meeting of the Dysphagia Research Society (Portland, USA, 2017) - Reciving the Springer Publishing Junior Investigator Scholarship
- Stroke Rehab: From No-Tech to Go-Tech Conference (Christchurch, New Zealand, 2018)

List of Abbreviations

AICA	anterior inferior cerebellar artery
AMPA receptors	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors
AMT	active motor threshold
CBI	cerebellar brain inhibition
CBF	cerebellar brain facilitation
CN	cranial nerve
CNS	central nervous system
CS	conditioning stimulus
DOSS	dysphagia outcome severity scale
EAT-10	eating assessment tool
EEG	electroencephalography
fcMRI	functional connective magnet resonance imaging
FDI	first dorsal interosseus
fMRI	functional magnet resonance imaging
fMRI-BOLD	functional magnet resonance imaging blood-oxygen-level dependent
GABA	gamma-aminobutyric acidergic
ICF	intracortical facilitation
ISI	interstimulus interval
KP	knowledge of performance
KR	knowledge of results
LTD	long-term depression
LTP	long-term potentiation
M1	primary motor cortex
MDTP	mcneill dysphagia therapy program
MEG	magnetoencephalography
MEP	motor evoked potential
MSO	maximal stimulator output
NIBS	non-invasive brain stimulation
NMDA	n-methyl-d-aspartate
PET	positron emission tomography
PICA	posterior inferior cerebellar artery

PMEP	pharyngeal motor evoked potential
PNS	peripheral nervous system
rCBF	regional cerebral blood flow
RMT	resting motor threshold
rTMS	repeated transcranial magnetic stimulation
S1	somatosensory cortex
SAT	speed/accuracy trade-off
SCA	superior cerebellar artery
SCA3	spinocerebellar ataxia type three
SCA6	spinocerebellar ataxia type six
sEMG	surface electromyography
SICF	short-interval intracortical facilitation
SICI	short-interval intracortical inhibition
SMA	supplementary motor area
SWAL-QOL	swallowing-related quality of life questionnaire
TBI	traumatic brain injury
TBS	theta burst stimulation
tDCS	transcranial direct current stimulation
TMS	transcranial magnetic stimulation
TOMASS	test of mastication and swallowing study
TS	test stimulus
TWST	timed water swallowing test
VEES	videoendoscopic evaluation of swallowing
VFS	videofluoroscopy
VFSS	videofluoroscopy swallowing study

PART I: LITERATURE REVIEW

1. Introduction

Dysphagia (swallowing disorders) is a common phenomenon after stroke or related to other neurological disorders. It can cause severe consequences for health, such as aspiration pneumonia, dehydration, malnutrition and quality of life. Enabling patients with dysphagia a way to swallow food or liquid in a safe manner is the main focus of swallowing rehabilitation. Common strategies of swallowing management are compensatory and/or behavioural. Compensation describes short-term solutions that change the environmental conditions such as posture or bolus flow, not resulting in longer-term benefits for the patient. Behavioural rehabilitative strategies primarily focus on muscles strengthening, assuming muscle weakness as the underlying cause of the swallowing impairment. However, neurological damage can lead to a variety of causes for motor dysfunction, such as spasticity (O'Dwyer, Ada, & Neilson, 1996) or apraxia (Ziegler, 2008). Muscle strengthening may therefore only be indicated when a specific diagnosis of muscle weakness exists, since strengthening approaches for swallowing can result in adverse effects, including mistiming of tongue base movements (Garcia, Hakel, & Lazarus, 2004) or deeper penetration of retentions in a patient with severe pharyngeal dysfunction (Bülow, Olsson, & Ekberg, 2001).

Skill training is an alternative to peripheral muscle strengthening exercises. It can be used to restore impaired motor behaviours following stroke by modulating cortical behaviour, achieving permanent improvements of motor skills (Kitago & Krakauer, 2013). Skill training for swallowing, using an isolated tongue protrusion task, evoked changes in corticomotor plasticity (Svensson, Romaniello, Arendt-Nielsen, & Sessle, 2003; Svensson, Romaniello, Wang, Arendt-Nielsen, & Sessle, 2006), providing neurophysiological evidence for its use in swallowing rehabilitation. Although most research indicating the potential benefit of skill training comes from other areas such as physical rehabilitation of limb motor function (Krakauer, 2006), recent research suggested behavioural benefits of swallowing skill training in neurologically impaired patients (Athukorala, Jones, Sella, & Huckabee, 2014; Huckabee, Lamvik, & Jones, 2014).

The majority of skill training protocols for swallowing use surface electromyography (sEMG) to provide visual feedback about temporal and magnitude aspects of submental muscle contraction during swallowing. These skill training protocols, using sEMG

biofeedback during swallowing, have successfully been used with healthy and swallowing impaired volunteers (Athukorala et al., 2014; Sella, 2012; Stepp, Britton, Chang, & Merati, 2011). Hence, swallowing skill training using submental sEMG may successfully be used to regain control over the timing and strength of muscles contraction during swallowing for patients with dysphagia following stroke.

Transcranial direct current stimulation (tDCS) is an non-invasive brain stimulation technique which has been used to facilitate neural changes resulting from skill training during limb motor recovery in stroke patients (Lefebvre et al., 2012; Madhavan & Shah, 2012). When tDCS is applied over neural structures, such as the motor cortex (Paulus und Nitsche, 2001) or the cerebellum (Galea et al., 2009), it changes the neuronal excitability of these structures. Furthermore, it has been proposed to strengthen interneuronal connections, when used in combination with motor skill practice (Stagg & Nitsche, 2011). For example, when tDCS is applied over the motor cortex in addition to behavioural motor skill training for impaired limb function, it has been found to enhance rehabilitation outcomes over skill training independently (Buch et al., 2016). The application of tDCS over the motor cortex has been explored in dysphagia rehabilitation after stroke (Pisegna, Kaneoka, Pearson, Kumar, & Langmore, 2015). Results from this research suggest additional benefits of tDCS over behavioural dysphagia rehabilitation without tDCS (Pisegna et al., 2015). Unfortunately, these research findings were weakened by significant methodological limitations (e.g. no blinding to the stimulation condition of patient or researcher) and the interpretation of results is hampered since heterogeneous stimulation protocols (e.g. stimulating the left or right motor cortical hemisphere) have been used. Most importantly, tDCS was combined with unspecific behavioural treatment approaches that were not tailored to the pathophysiology. Improved study designs, e.g. using double-blinded study protocols, and greater specificity in the application of tDCS for its potential usage in swallowing rehabilitation warrants investigation.

This research programme addressed the current limitations identified in the literature and proposed a possible new direction for the application of tDCS in swallowing rehabilitation. Swallowing skill training, based on the principles of motor learning, has been identified to be a specific treatment approach, targeting motor recovery of swallowing by increasing volitional control of swallowing motor behaviours. Facilitation of neural changes underlying motor skill learning in limb function has

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previously been demonstrated when tDCS was applied over the cerebellum (Cantarero et al., 2015; Buch et al., 2016). This approach is based on the important role of the cerebellum in motor learning, being responsible for error correction and coordination of movements (Ito, 2000; Sokolov, Miall, & Ivry, 2017).

This research programme evaluated if the reported facilitating effects of cerebellar tDCS on motor skill learning in limb function also translate to facilitation of motor skill learning in swallowing. The effects of cerebellar tDCS were tested on healthy volunteers and in a smaller sample of patients with dysphagia following stroke, using study protocols with randomisation and double-blinding. Enhancing motor skill learning in swallowing with cerebellar tDCS may provide an opportunity for stroke patients with dysphagia to overcome their swallowing impairments more effectively.

In addition, methodological adaptations of cerebellar tDCS, when used for motor functions like swallowing, were investigated. A methodological study was performed to assess the effects of tDCS on neurophysiological outcome measures using a midline electrode placement over the cerebellum, which is in contrast to commonly used unilateral placements for limb function. This midline placement of the electrode over the cerebellum would also circumvent additional assessments, e.g. assessing motor cortical dominance for swallowing, and provide a more standardised approach for research and possible future clinical applications of tDCS.

Part I of this thesis provides an *introduction* and a detailed *literature review*, starting with the biomechanics and the peripheral and central control of healthy and impaired swallowing, followed by assessments and treatment options for dysphagia. In addition, research on motor learning, cerebellar function and non-invasive brain stimulation are covered. Research on cerebellar tDCS on corticospinal excitability and on motor learning was reviewed. The literature review concludes with presentation of research on tDCS in swallowing rehabilitation, leading to the proposed hypotheses that were tested in this research programme. *Part II* presents, after a short introduction, the methods, results and discussion of the three studies that have been conducted in this research programme. The first two studies evaluated the effects of cerebellar tDCS on motor skill learning in swallowing. *Behavioural study I* on healthy individuals and *Behavioural study II* on patients with dysphagia following stroke. The third study, *Methodological study*, tested the effects of cerebellar tDCS using a midline placement

over the cerebellum using neurophysiological outcome measures. *Part III* provides *conclusions, critique* and *future directions. Part IV* includes *references* and *Part V* is the *appendix.*

2. Biomechanics of swallowing

Swallowing, or deglutition, is an essential and complex motor activity that requires precise organisation of neural and muscular activity. More than 30 pairs of muscles are simultaneously or sequentially active during swallowing (Dodds, Stewart, & Logeman, 1990). These muscles are responsible for voluntary and involuntary movements of soft tissues, cartilages and bones that are needed to breakdown and transfer nutrients. Starting at oral intake and ending with the arrival of food in the stomach, the main anatomical structures needed for swallowing include the lips, jaw, tongue, soft palate, larynx, pharynx and upper oesophageal sphincter. All components must work flawlessly within a short amount of time (0.6 – 1.0 s) to ensure safe ingestion of food and liquids (Jean, 2001; Lang, 2009). Understanding the biomechanics and neurophysiology of deglutition is key to diagnosing and managing patients with dysphagia. The complexity of swallowing processes is often artificially partitioned into stages to conceptualise this uninterruped chain of biomechanical events. The three core components are the oral, pharyngeal and esophageal phases (Perlman & Christensen, 1997). More detailed frameworks distinguish between the oral preparatory and the oral transit or lingual phase within the oral phase (Leopold & Kagel, 1997a; Logemann, 1983). And others again incorporate processes prior to the introduction of the bolus into the oral cavity as an important part of their description of deglutition, referred to as pre-oral phase (Daniels & Huckabee, 2014; Leopold & Kagel, 1997a). A four-phase segmentation will be used to reflect on the processes within each phase more closely.

Pre-oral Phase: The pre-oral phase refers to intrinsic and extrinsic mechanisms that occur prior to bolus ingestion. This includes recognition of the bolus through sight and smell (Daniels & Huckabee, 2014). The anticipation of ingestion in combination with olfactory and visual inputs activates corresponding cortical areas, such as the olfactory cortex, visual cortex, thalamus and the primary and association sensory cortices, which are known to influence swallowing behaviours (Huckabee & Doeltgen, 2012). Preingestive visual and olfactory input resulted in increased salivary production and early airway protection (Ebihara et al., 2006; Ushioda et al., 2012).

Oral Phase: Most motor tasks in the oral phase are executed semiautomatically but can be volitionally controlled at any stage (Miller, 2013). They include oral delivery of food

and liquid, its manipulation and formation into a bolus, and transport of the bolus into the pharynx (Ertekin & Aydogdu, 2003; Perlman & Christensen, 1997). The base of tongue elevates during bolus manipulation, restricting entry of the bolus to the pharynx, which is important for airway protection. Furthermore, labial closure supports containment of the bolus anteriorly and is required for the build-up of pressure which is essential for bolus transfer (Perlman & Christensen, 1997). Once prepared, the bolus is moved through intrinsic and extrinsic tongue muscle contractions in an anterior to posterior motion against the hard palate and towards the pharynx (Taniguchi et al., 2013). The overall duration of the oral phase adapts in response to bolus characteristics and increases from approximately 0.5 s for liquids (Dodds et al., 1990) to approximately 20 s for a solid bolus (Palmer, 1998). Oral transit in isolation, meaning only the time the bolus is propelled out of the oral cavity after it has been prepared, takes less than 0.5 s for liquids but prolongs with increased bolus consistency (Dantas et al., 1990).

Pharyngeal Phase: Onset of the pharyngeal response is the start of the pharyngeal phase and marked by onset of hyolaryngeal excursion (Daniels & Huckabee, 2014). Based on videofluoroscopic imaging in healthy adults, pharyngeal swallowing is generally expected to begin when the bolus head reaches the inferior aspect of the ramus of the mandible (Martin-Harris, Brodsky, Michel, Lee, & Walters, 2007; Stephen, Taves, Smith, & Martin, 2005). However, there is considerable variability in the relationship between the onset of pharyngeal swallowing and anatomical location of the bolus within and across individuals (Stephen et al., 2005). This relationship is influenced by bolus characteristics (Hamdy et al., 2003) and instruction (Daniels, Schroeder, DeGeorge, Corey, & Rosenbek, 2007). For example, a sour taste increases sensory input to the cortex and hence lowers the threshold to initiate swallowing (Kajii et al., 2002). Once initiated, the pharyngeal response is characterised by a rapid sequence of overlapping events (Daniels & Huckabee, 2014).

Following the drop of the tongue base and entrance into the pharynx, the bolus is squeezed towards the cricophagyngeal area by approximation of the base of the tongue to the posterior pharyngeal wall as a result of stylohyoid, posterior belly of the digastric, styloglossus and glossopharyngeus muscle activation (Jean, 2001). Simultaneously, velopharyngeal closure is achieved through contraction of the palatopharyngeus and

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levator veli palatine muscles (Kahrilas, 1993). This approximation contributes to an increase in pharyngeal pressure during in this phase of swallowing (Perlman & Christensen, 1997). At this stage of the pharyngeal phase, airway protection is crucial. Hyolaryngeal excursion describes the movement of the hyoid and the larynx in a superior and anterior direction which facilitates airway protection through pharyngeal shortening and epiglottic deflection (Daniels & Huckabee, 2014). This excursion is achieved through contraction of the suprahyoid muscles and thyrohyoid muscles (Pearson, Langmore, Louis, & Zumwalt, 2012). The mylohyoid presents with most potential for superior hyoid movement; whereas geniohyoid followed by the anterior belly of digastric are most likely responsible for the anterior movement of the hyoid during swallowing (Pearson, Langmore, & Zumwalt, 2011). The geniohyoid demonstrated the highest peak-adjusted electromyographic amplitude compared to the other muscles during hyolaryngeal excursion, confirming its dominant role in hyoid movement (Inokuchi et al., 2014). Contraction of paired submental muscles (mylohyoid, geniohyoid and anterior belly of digastric; Figure 1) are not only important for hyolaryngeal excursion but also for stabilising the floor-of-mouth during the rapid movement of the posterior tongue towards the posterior pharyngeal wall that helps to drive the bolus from the oropharynx into the hypopharynx (Pearson et al., 2012). Other suprahyoid muscles, such as the stylohyoid and posterior belly of digastric, are also suggested to be involved in the elevation of the hyolaryngeal complex during the pharyngeal phase of swallowing, but with a less prominent role (Pearson et al., 2012). Research on temporal muscle activation demonstrated simultaneous contraction of the submental muscles leading to the pharyngeal swallowing response (Doty & Bosma, 1956; Inokuchi et al., 2014; Jean, 2001; Figure 2).
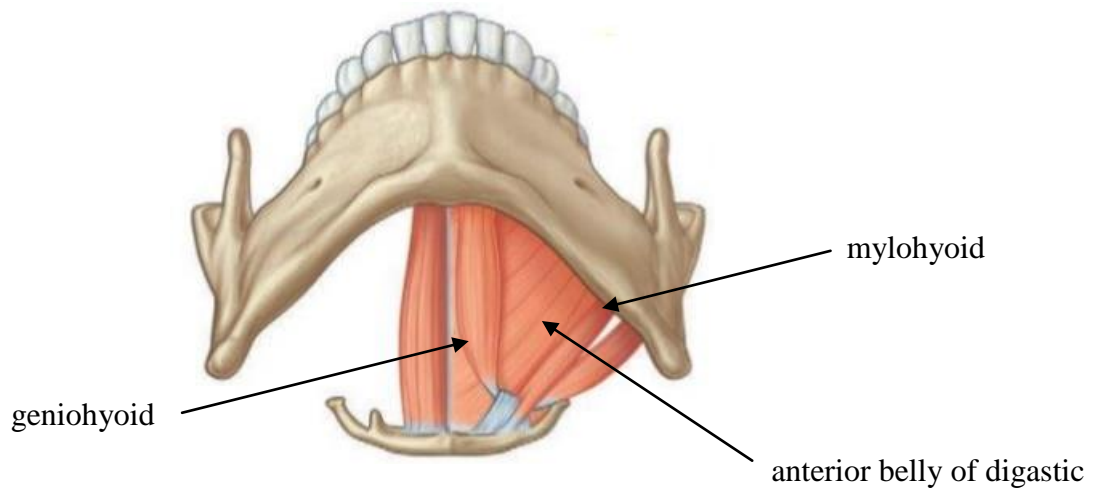
Figure 1. Illustration of the submental muscle group. Anterior belly of digastric is the most superficial muscle of the three submental muscle group; it attaches at the hyoid bone and the inner side of the lower border of the mandible. Mylohyoid forms the floor of the oral cavity and are joined along the midline extending from the mental symphysis to the hyoid bone; laterally, it attaches around the internal margin of the mandible. Geniohyoid sits at midline with attachments at the mental symphysis and the hyoid bone.[1]

In addition to hyolaryngeal excursion, sequential top down contraction of the pharyngeal constrictor muscles occurs (Matsuo & Palmer, 2009). These mechanisms lead to pharyngeal shortening vertically and volume reduction of the pharyngeal space, resulting in additional pressure build-up to drive the bolus inferiorly (Matsuo & Palmer, 2009). Concurrently, the airway is protected through laryngeal closure and compression at multiple levels, including closure of the vocal folds, false vocal folds, arytenoid cartilages, aryepiglottic folds and epiglottis, to prevent food or liquids from entering the trachea (Daniels & Huckabee, 2014). Lastly, the tonic contraction of the cricopharyngeus muscle is terminated through neurogenic input from the brainstem (Ertekin & Aydogdu, 2003). This relaxation is permitting biomechanical opening of the UES through the superior-anterior motion of the hyolaryngeal complex, which allows the bolus to enter the oesophagus (Belafsky & Lintzenich, 2013). Although the basic pharyngeal motor pattern is mainly involuntary, it may be somewhat modified with

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volition (Gay, Rendell, Spiro, Mosier, & Lurie, 1994; Lamvik, Jones, Sauer, Erfmann, & Huckabee, 2015).
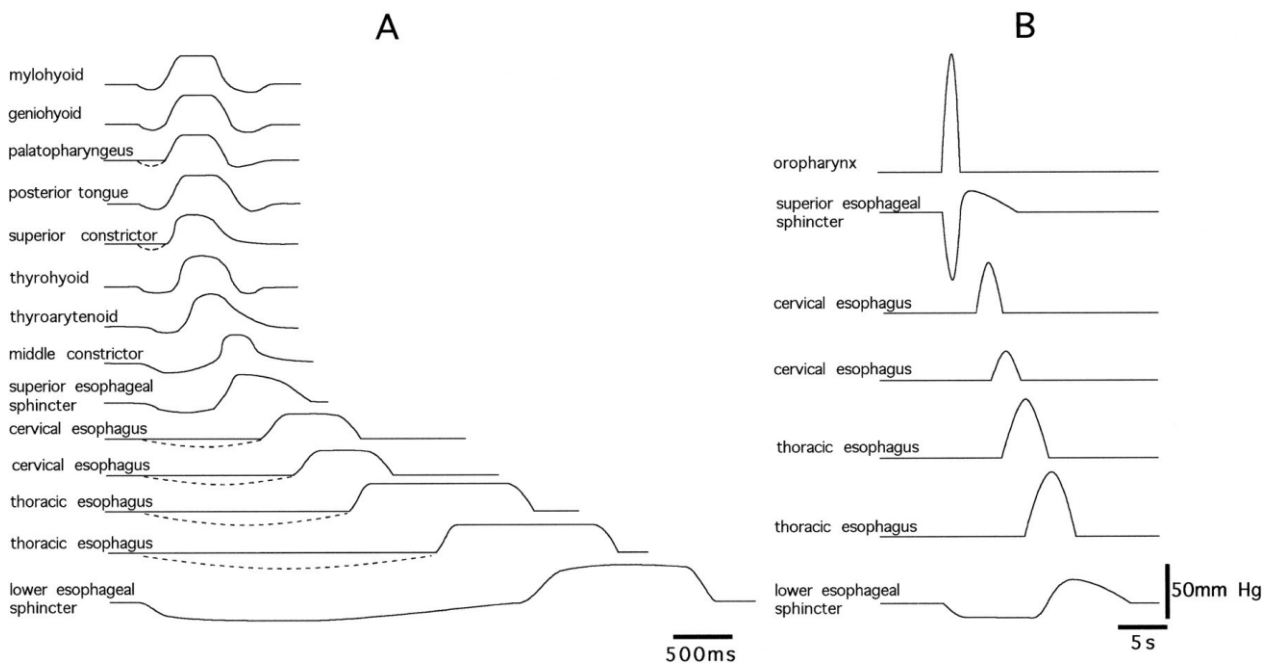
Figure 2. Electromyographic waveforms of sequential muscle activation during the pharyngeal and oesophageal phase of swallowing (Jean et al., 2001 adapted from Doty and Bosma, 1956).[2]

Oesophageal Phase: As the bolus passes through the UES, the oesophageal stage of swallowing is initiated and the bolus is transferred through peristaltic muscle contraction into the stomach (Daniels & Huckabee, 2014). This stage is under involuntary neuromuscular control and varies in duration, between 8 – 20 s, depending on bolus type (Dodds et al., 1973). Once the bolus reaches the caudal end of the oesophagus, the lower oesophageal sphincter relaxes and allows transfer of the bolus into the stomach.

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3. Neural control of swallowing

Swallowing is a complex motor event characterised by systematic execution of numerous biomechanical events. These events are controlled by two parts of the nervous system: the peripheral nervous system (PNS) and the central nervous system (CNS). Within the PNS, cranial nerves (CNs) transmit afferent sensory information and efferent motor signals essential for swallowing. These signals are mediated by an interplay of subcortical and cortical structures within the CNS. The basic motor plan for swallowing is generated within the brainstem but can be modulated by other cortical and subcortical structures of the CNS (Jean, 2001). Understanding the neural control of healthy and impaired swallowing can aid to identify ways to treat inaccurate movements during swallowing.

3.1 Peripheral control

Sensorimotor control of swallowing is driven by eight of the twelve cranial nerves (CN I, II, V, VII, IX, X, XI, XII). Some of these CNs have predominantly sensory (afferent) or motor (efferent) components; however, the majority transmit both sensory and motor information (Wilson-Pauwels, Akesson, Stewart, & Spacey, 2002).

Olfactory (CN I) and optic (CN II) nerves: These nerves carry sensory information about the smell and the sight of the food or drink. This information is mainly received during the pre-oral phase of swallowing. In contrast to all other CNs, the nerve nuclei of CN I and CN II are located in the cerebrum and not in the brainstem (ten Donekelaar, 2011).

Trigeminal nerve (CN V): The trigeminal nerve is the largest CN and splits into three branches: ophthalmic, maxillary and mandibular (Wilson-Pauwels et al., 2002). The latter two are involved in swallowing. The maxillary branch exclusively carries sensory information from the region of the maxilla, cheeks, upper lip, upper teeth, hard palate, gums and mucous membranes of the mouth (ten Donekelaar, 2011). The mandibular branch transmits sensory information from the mandibular region, including the chin,

lower lip, lower teeth, and the anterior two-thirds of the tongue, gums and mucous membranes of the mouth (ten Donekelaar, 2011). The motor component of the mandibular branch supplies the tensor veli palatini and the muscles of mastication (jaw closers: masseter, temporal and medial pterygoid; jaw openers: mylohyoid, anterior belly of the digastric, lateral pterygoid) (Sasegbon & Hamdy, 2017). With innervation supplied to two of the three submental muscles, this nerve is crucial for hyolaryngeal excursion.

Facial nerve (CN VII): The facial nerve has motor and sensory components divided into five branches: temporal, zygomatic, buccal, marginal mandibular and cervical (Wilson-Pauwels et al., 2002). The muscles for facial expression are innervated by all five branches of the facial nerve. Impairments of this nerve can lead to paresis or paralysis that predominantly affect the lower face, as the upper face is bilaterally innervated (Wilson-Pauwels et al., 2002). Furthermore, CN VII innervates the buccinators, zygomaticus, orbicularis oris, risorius, stylohyoid and posterior belly of the digastric (Sasegbon & Hamdy, 2017). Relaxation of the orbicularis oris and activation of accessory facial muscles, if required, allow for oral intake of various bolus sizes. Activation of the orbicularis oris throughout the oral phase creates a lip seal preventing anterior spillage of fluids or food (Matsuo & Palmer, 2015). Contraction of the stylohyoid and posterior belly of the digastric support movements of the tongue base superiorly and posteriorly and the superior hyoid movement, as it is required for oral bolus containment and subsequent pharyngeal transfer (Pearson et al., 2012). Sensory fibres of CN VII receive information (taste) from the anterior two-thirds of the tongue (Wilson-Pauwels et al., 2002). In addition, CN VII also has a parasympathetic role and is responsible for controlling saliva production of the submandibular and sublingual glands (Wilson-Pauwels et al., 2002).

Glossopharyngeal nerve (CN IX): The glossopharyngeal nerve supplies innervation to few motor and sensory regions in isolation; its more substantial contribution is as a component of the pharyngeal plexus when paired with CN X. In isolation, CN IX carries sensory information from the entrance to the pharynx, including soft palate and tonsil, and sensation and taste from the posterior one-third of the tongue (Wilson-Pauwels et al., 2002). The motor component of this nerve innervates only the stylopharyngeus muscle (Finsterer & Grisold, 2015). Contraction of this muscle

elevates the larynx and shortens and dilates the pharynx. Furthermore, CN IX parasympathetically innervates the parotid salivary gland (Finsterer & Grisold, 2015).

Vagus nerve (CN X): The vagus nerve is the longest out of the cranial nerves and consists of many branches. There are three branches in the neck that contribute to swallowing. The pharyngeal branch joins together with the glossopharyngeal nerve as the pharyngeal plexus. The other two branches constitute the superior laryngeal nerve (SLN) and the recurrent laryngeal nerve (RLN). The SLN carries sensory information from the superior regions of the larynx including the vocal folds (Finsterer & Grisold, 2015). Function of this nerve is especially important to detect potential invasion of food or liquids before and during swallowing. Furthermore, the SLN supplies the cricothyroid muscles which is important for vocal fold abduction, allowing for respiration before and after swallowing. Together with CN IX, the SLN also innervates the cricopharyngeus muscle, resulting in a tonic contraction at rest and relaxation during the pharyngeal response (Sasegbon & Hamdy, 2017). Sensory fibres of the RLN provide perceptual information of the larynx below the level of the vocal folds, including the cervical segments of oesophagus and trachea. Food or residues that enter the airway will be detected by this nerve and can be removed through a clearing response, e.g. reflexive cough response. The RLN innervates the intrinsic laryngeal muscles, the interarytenoids, thyroarytenoids and cricoarytenoids, for vocal fold adduction during swallowing (i.e. airway protection) or voice production (Perlman & Christensen, 1997).

Pharyngeal plexus (CN IX and CN X): The pharyngeal plexus (PP) is formed by afferent and efferent vagal and glossopharyngeal nerves (Daniels & Huckabee, 2014). The nerve rootlets emerge from the CN nuclei in the lateral medulla (Wilson-Pauwels et al., 2002). The PP plays a crucial role for many swallowing mechanisms in the pharyngeal phase. It carries sensory information from the entire oropharynx and hypopharynx which is important for perceiving the bolus within the pharynx and preventing aspiration through clearing mechanisms. The motor component of this plexus innervates the levator veli palatine muscles that are partly responsible for velopharyngeal closure, an important contributor to the pharyngeal pressure system (Perlman & Christensen, 1997). In addition, it activates the palatoglossus muscle preventing premature spillage into the pharynx and the glossopharyngeus muscle that

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enables base of the tongue to pharyngeal wall approximation generating pressure on the bolus during the pharyngeal response (Daniels & Huckabee, 2014). Lastly, the PP innervates salpingopharyngeus, palatopharyngeus and the three pharyngeal constrictor muscles (superior, middle and inferior), enabling supraglottic shortening and superior to inferior squeeze of the bolus (Daniels & Huckabee, 2014).

Hypoglossal nerve (CN XII): CN XII has only motor connections to all intrinsic (transversus, longitudinalis, and verticalis) and most extrinsic tongue muscles (genioglossus, styloglossus, hyoglossus) (Wilson-Pauwels et al., 2002). The intrinsic muscles shape the tongue into different contours and therefore assist with formation and propulsion of the bolus into the pharynx (Felton et al., 2007). The extrinsic tongue muscles move the tongue in different directions within the oral cavity via elevation, protrusion and retraction of the tongue and tongue base (Felton et al., 2007).

Ansa cervicalis (CN XII, C1, C2): Ansa cervicalis is a combination of fibres from the hypoglossal nerve and the first two cervical spinal nerves (Dodds et al., 1990). It describes a thin nerve loop in the anterior wall of the carotid sheath that connects the superior root (continuation of CN XII) with the inferior root (arising from the cervical spinal nerves). The fibers of the ansa cervicalis extend towards the lower part of the larynx and innervate the infrahyoid muscles (omohyoid, sternohyoid, sternothyroid, thyrohyoid) and the geniohyoid (Daniels & Huckabee, 2014). Therefore, ansa cervicalis contributes to hyolaryngeal excursion and stabilises the hyoid through innervation of the strap muscles during jaw opening.

3.2 Central control

Subcortical control: Afferent and efferent fibres of the CN V, VII, IX, X, XI and XII either terminate or arise from bilateral representation of their nuclei in the brainstem (Daniels & Huckabee, 2014; Figure 3). The majority of these nuclei is organised in two regions within the medulla, namely the dorsal and ventral swallowing group (Sasegbon & Hamdy, 2017). This construct of interconnected regions operate in synchrony as the central swallowing centre, also known as the swallowing central pattern generator

(CPG) (Jean, 2001). The CPG generates a basic swallowing response which can be modified by inputs from other subcortical or cortical areas of the brain (Hamdy, Rothwell, et al., 1999; Jean, 2001).

In detail, afferent fibers from CN VII, IX and X transmitting sensory information from the oral cavity, larynx and pharynx converge in the nucleus tractus solitarius (NTS), in the medulla (Sasegbon & Hamdy, 2017). In addition, NTS receives visceral sensory information from CN V in the pons (Bieger & Neuhuber, 2006). The NTS and adjacent reticular formation are part of the dorsal swallowing group in the medulla (Jean, 2001). Being the primary sensory nucleus, the NTS is critical for the initiation and organisation of swallowing (Jean, 2001). The NTS communicates with another major component of the brainstem swallowing centre - the nucleus ambiguous (NA) (Jean & Dallaporta, 2013). The NA contains the primary motor nuclei for CN IX, CN X and XI, with associated efferent connections to CN VII, CN V and CN XII (Jean, Amri, & Calas, 1983). These neurons drive the motor activation of the sequential pattern of muscle contractions for swallowing (Sasegbon & Hamdy, 2017). The NA and the surrounding region build the ventral swallowing group (Jean, 2001).

Motor nuclei

Edinger–Westphal nucleus (GVE: CN III)
Oculomotor nucleus (GSE: CN III)
Trochlear nucleus (GSE: CN IV)

Trigeminal motor nucleus (SVE: CN V)
Superior salivatory nucleus (GVE: CN VII)
Facial nucleus (SVE: CN VII)
Abducens nucleus (GSE: CN VI)
Inferior salivatory nucleus (GVE: CN IX)
Nucleus ambiguus (SVE: CN IX, X)
Hypoglossal nucleus (GSE: CN XII)
Dorsal motor nucleus of CN X (GVE: CN X)
Spinal accessory nucleus (SVE: CN XI)

Sensory nuclei

Trigeminal nuclei (GSA: CN V, VII, IX, X):
Mesencephalic nucleus of CN V
Chief sensory nucleus of CN V
Spinal trigeminal nucleus
Vestibular nuclei (SSA: CN VIII)
Dorsal and ventral cochlear nuclei (SSA: CN VIII)

Nucleus solitarius, rostral portion (SVA: CN VII, IX, X)

Nucleus solitarius, caudal portion (GVA: CN IX, X)

Figure 3. Location of the cranial nerve motor and sensory nuclei in the brainstem.[3]

The cerebellum: One subcortical structure that is highly relevant in the modulation of swallowing, and is of particular relevance to this thesis, is the cerebellum (Rangarathnam, Kamarunas, & McCullough, 2014). Numerous studies suggest a relationship between cerebellar damage and impaired swallowing. For example, Alberts, Horner, Gray, and Brazer (1992) analysed the risk for aspiration in regards to the lesion location, including the cerebellum, following stroke. Thirty-eight stroke patients that had undergone a swallowing evaluation including a VFSS were recruited for the study. Twelve of these patients presented with cerebellar damage (small and large vessels, posterior inferior cerebellar arteria) on magnetic resonance imaging (MRI) and more than 60% of the patients with cerebellar damage aspirated on VFSS. Unfortunately, the researchers did not describe how many of the patients with cerebellar strokes presented with cerebellar damage in isolation. Additional damage in other brain

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areas may have contributed to dysphagic symptoms. In addition to the findings from Alberts et al. (1992), several single case studies also reported swallowing impairments in patients with damage to the posterior inferior cerebellar artery (PICA), which is one of the main arteries supplying the cerebellum (Massey, El Gammal, & Brooks, 1984; Nagahiro, Goto, Yoshioka, & Ushio, 1993). Nevertheless, the PICA and the anterior inferior cerebellar artery (AICA) also supply important brainstem structures for swallowing. The PICA supplies the inferior posterior surface of the cerebellar hemisphere, the ipsilateral part of the inferior vermis, and the NA, which is crucial for motor output via CN IX, X and XII in swallowing (Savoiardo, Bracchi, Passerini, & Visciani, 1987). The AICA supplies the middle cerebellar peduncle and the lateral inferior part of the pons (including CN VII nucleus and nerve) (Savoiardo et al., 1987). Damage to the AICA often leads clinically to lateral inferior pontine syndrome, causing dysphagic symptoms (Savoiardo et al., 1987). It is therefore difficult to determine the definite neural substrates underlying swallowing impairment in patients with damage to the PICA or AICA.

A meta-analysis investigating MRI-based neuroanatomical predictors of dysphagia after stroke reported no presentation of dysphagia in 36 patients after an isolated cerebellar lesion, in a total of 656 acute ischemic stroke patients (Flowers, Skoretz, Streiner, Silver, & Martino, 2011). Furthermore, a study by Moon, Pyun, and Kwon (2012) compared stroke lesion location in 76 patients with findings on videofluoroscopic swallowing studies (VFSS). The authors reported two patients with isolated cerebellar lesion infarcts that presented with aspiration, however, with such a small sample, no statistically significant relationship between dysphagia and cerebellar lesion could be determined. Most recently, Isono et al. (2013) retrospectively analysed VFSS of swallowing patterns from seven individuals with spinocerebellar ataxia type three (SCA3) affecting the cerebellum, pyramidal, extrapyramidal, and autonomic systems, and 13 individuals with spinocerebellar ataxia type six (SCA6) primarily affecting the cerebellum. Dysphagia was severe in SCA3 and mild but significant in SCA6, as assessed by a regionally used Japanese swallowing scale and the dysphagia outcome severity scale (DOSS). Although SCA6 mainly affects the cerebellum, it occasionally also affects the brainstem nuclei which then could also be a potential reason for swallowing impairments.

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In summary, damage to the cerebellum may contribute to dysphagic symptoms, and possibly cause dysphagia in isolation, but this has not been systematically evaluated. The shared vascular blood supply and anatomical proximity might predispose patients to brainstem injury in the presence of cerebellar injury. Detailed description of lesion location is required for analysing and reporting on findings that involve the cerebellum in swallowing.

Early research on animals using electrical stimulation was also influenced by the close proximity of brain structures when exploring the representation of swallowing and the oropharyngeal musculature within the cerebellum. In 1930, Mussen applied electrical stimulation to the cerebellar vermis of cats and monkeys, and elicited swallowing and contraction in the pharyngeal musculature. He summarised reports of cerebellar stimulation responses in different muscle groups. Unfortunately, the stimulation methods of the reviewed studies are not further described. Given the close proximity and direct connections between the cerebellum and the medulla (inferior cerebellar peduncle) and the pons (middle cerebellar peduncle), it might be possible that the stimulation of the vermis (especially if a high stimulation intensity was used) was strong enough to evoke a direct response from the brainstem swallowing centres. In later years, Berntson, Potolicchio, and Miller (1973) investigated if electrical stimulation, using neurosurgically implanted cerebellar electrodes, of cerebellar regions, influenced eating and grooming behaviour in fifteen cats. Stimulation facilitated chewing and swallowing of nutritive and even non-nutritive objects by stimulating the basal vermis, the fastigial nucleus and the superior cerebellar peduncle. However, the assessment of general eating and grooming behaviours does not provide specific information on the role of the cerebellum in swallowing. In contrast, research by Gibbs (1992) demonstrated impaired bilateral eyeblink responses but unimpaired jaw movement responses in rabbits (n = 26) with lesions of the anterior interpositus nucleus of the cerebellum or the superior cerebellar peduncle as compared to a control group. The researcher concluded that the lesioned cerebellar region was not essential for the development of a conditioned masticatory response. However, interpreting the tested anticipatory jaw opening as mastication should be considered with caution. There is no evidence that mastication necessarily follows jaw opening. However, jaw movements are only one aspect of swallowing, and may well not be primarily affected when cerebellar lesions occur. The results of these animal studies may provide early

indications of cerebellar involvement in swallowing-related behaviours and pharyngeal muscle activation, especially when stimulated around the vermis. Mottolese et al. (2013) investigated cerebellar motor representations via electrical stimulation in human subjects undergoing tumour surgery outside the cerebellum. They provided evidence of representation of the face and mouth in the posterior lobe (lobule VI) by recording EMG signals from the zygomaticus and orbicularis oris muscles. While direct electrical stimulation offers specific insights into cerebellar function, it is an invasive procedure that requires general anaesthetics.

An alternative method to assessing cerebellar activation or functional connections to surrounding brain structures during swallowing or swallowing-related activities in humans is brain imaging, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Zald and Pardo (1999) analysed regional cerebral blood flow (rCBF) using PET for three conditions: voluntary swallowing of saliva, lateral tongue movements and resting with closed eyes in healthy eight volunteers. Voluntary saliva swallowing induced significant and robust activations in the inferior precentral gyrus bilaterally, the right anterior insula, and the left cerebellum (Crus Ia/VI region) in all participants. This significant increase in unilateral rCBF in the cerebellum was not found during the tongue movement condition indicating that this activation is unique to swallowing. In the same year, Hamdy et al. (1999) published their findings on using an optimised PET method by labelling water to identify rCBF with more advanced resolution. Constant intraoral water infusion at midline allowed the participants to swallow a 5 ml water bolus at different frequencies in supine position. Although activation was observed in many other areas including the insula and brainstem, they found the strongest activation for the sensorimotor cortices and the cerebellum. The signal was particularly strong within the left cerebellar hemisphere, but the activation also spread across the vermis to the right hemisphere. The amplified rCBF within the cerebellum suggests an important role of this structure in swallowing. In 2005, Harris et al. refined the PET methods used by Hamdy et al. (1999) and avoided the main limitation of being in an unnatural supine position during swallowing in eight healthy males. Two conditions - volitional water swallowing and rest – were performed by the participants. During swallowing the participants were sitting in an upright position. The PET scans were then also performed in supine position with a delay of 10 min following execution of the swallowing or resting period. The authors themselves

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raise the point that this method is still controversial in its capacity to accurately identify brain activation for swallowing. Nevertheless, they reported similar brain activation patterns to Hamdy et al. (1999), in particular, unilateral activation in the left cerebellar hemisphere.

In contrast to the PET scan method, fMRI is an another imaging technique that can achieve higher spatial resolution of brain activation but is more sensitive to motion artefacts. This limitation needs to be considered when investigating and interpreting swallowing-related neural activation, as head movements before or during swallowing are highly likely. Suzuki et al. (2003) used a block design of volitional saliva swallowing to reduce motion artefacts on fMRI results when investigating cerebellar and basal ganglia activation in eleven healthy volunteers (Figure 4). Although bilateral cerebellar activation was observed in the vast majority of participants, the group analysis revealed a prominent unilateral cerebellar activation in the left posterior lobe. Further research on cerebellar activation using different consistencies or swallowing-related movements support bilateral cerebellar involvement in swallowing. Shibamoto, Tanaka, Fujishima, Katagiri, and Uematsu (2007) reported bilateral cerebellar activation when healthy participants (n = 21) swallowed a capsule. No activation was revealed while swallowing other consistencies, such as solids (agar) or water. In contrast to the previously reported studies, Shibamoto et al. used a single swallow instead of a repetitive swallowing design. Other studies assessing brain activation following single swallows were also not able to identify cerebellar activation (Hartnick, Rudolph, Willging, & Holland, 2001; Kern et al., 2001; Mosier & Bereznaya, 2001). The strong bilateral cerebellar activation during swallowing of a capsule might be the result of the task complexity and the coordination required to swallow a capsule without water in supine position, especially during the oral transport and pharyngeal phase. In line with previous research, Malandraki and colleagues observed cerebellar activation for pharyngolaryngeal muscle activation during swallowing of 3 ml water in healthy volunteers of different age groups on fMRI (Malandraki, Perlman, Karampinos, & Sutton, 2011; Malandraki, Sutton, Perlman, Karampinos, & Conway, 2009). The authors further reported bilateral cerebellar activation during throat clearing, which is a consistent finding with other reports on cerebellar involvement in supralaryngeal and laryngeal motor control, e.g. during phonation and coughing (Grabski et al., 2012; Simonyan, Saad, Loucks, Poletto, & Ludlow, 2007).
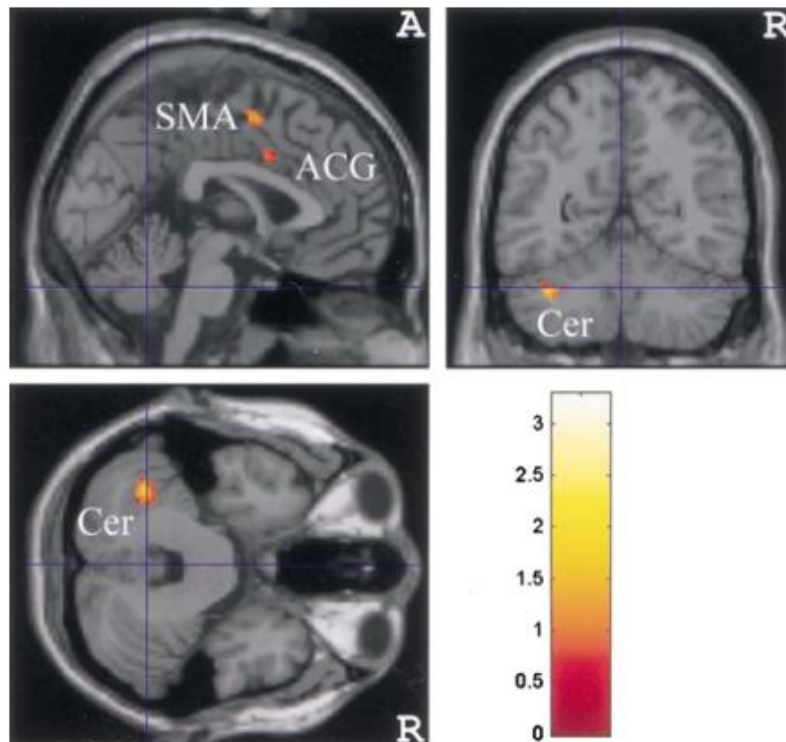
Figure 4. Cerebellar activations in healthy human swallowing demonstrated in the group analysis of Suzuki et al. (2003). ACG = anterior cingulate gyrus; Cer = cerebellum; SMA = supplementary motorarea; A = anterior; R = right.[4]

The higher temporal resolution of fMRI provides stronger evidence for bilateral activation of the cerebellum during swallowing than the more unilateral activation pattern found using PET scans. Nevertheless, the left hemispheric dominance of the cerebellum activation during swallowing in some studies remains an area worthy of further investigation. Current findings suggest greater involvement of the cerebellum during the pharyngeal phase of swallowing than during isolated oral movements. Although these studies confirm cerebellar activation during swallowing, the functional relevance of the cerebellum in swallowing remains unknown.

A recent review by Rangarathnam and colleagues discussed the potential role of the cerebellum in deglutition (Rangarathnam et al., 2014). In addition to the evidence of cerebellar activation during swallowing, the authors also consider anatomical and functional connectivity to other brain areas. They proposed that the cerebellum

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[4] Reprinted by permission from Springer Nature: Dysphagia, 18(2), 71-77, Activation of cerebellum and basal ganglia on volitional swallowing detected by functional magnetic resonance imaging, Suzuki, M., Asada, Y., Ito, J., Hayashi, K., Inoue, H., & Kitano, H., Copyright (2003).

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compliments swallowing motor control with its many connections to cortical sensorimotor regions and subcortical structures. Figure 5 provides a schematic summary of cerebellar connections within the neural network of swallowing (adapted from Rangarathnam et al., 2014). To date, very few of the proposed connections within the figure are supported by evidence. However, two studies have investigated the connections between the cerebellum, the sensorimotor cortices and other subcortical structures for swallowing.
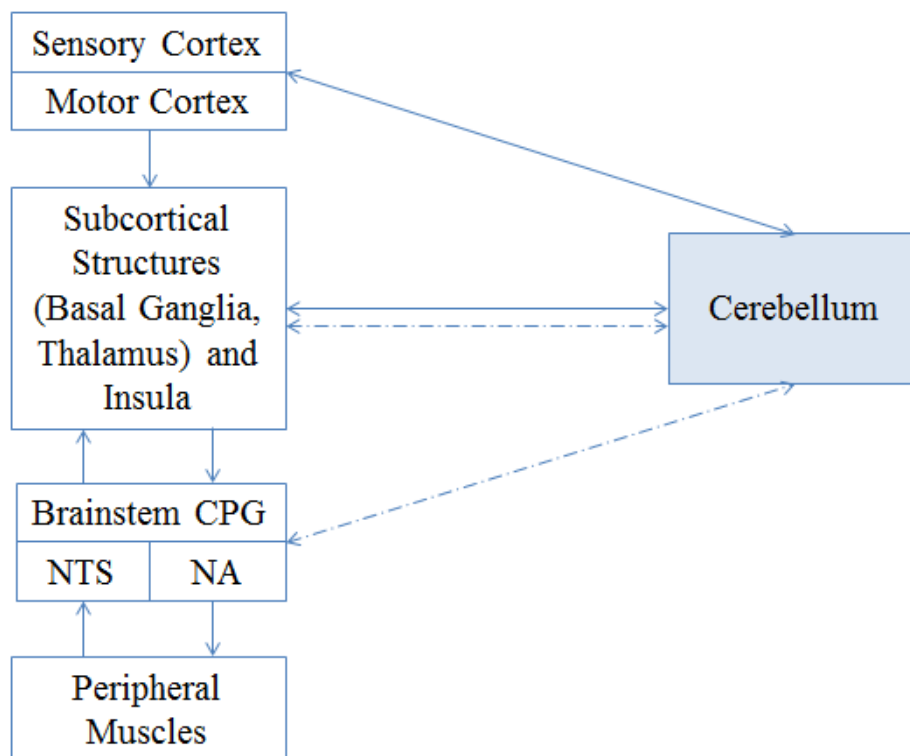
Figure 5. Potential role of the cerebellum in motor and sensory aspects of swallowing. NA = Nucleus ambiguous; NTS = Nucleus tractus solitarius. Solid lines indicate motor and sensory augmentation of swallowing by the cerebellum via a loop system based on early evidence. Dotted lines indicate suspected role of the cerebellum in temporal aspects of motor output and timely initiation of pharyngeal swallowing by Rangarathnam et al. (2014). [5]

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[5] Adapted by permission from Springer Nature: The Cerebellum, 13(6), 767-776, Rangarathnam, B., Kamarunas, E., & McCullough, G. H. (2014). Role of cerebellum in deglutition and deglutition disorders., Copyright (2014).

Mosier and Bereznaya (2001) collected fMRI data from eight healthy volunteers to determine the functional relationship of brain regions in the neural control of swallowing saliva and 3 ml water. They proposed that swallowing occurs by the activation of parallel networks, where sensorimotor cortical areas and subcortical sites, including the cerebellum, are involved. Using path-analysis and structural equation modeling (SEM), the researchers demonstrated functional connectivity in the form of a closed-loop system between the cerebellum and primary motor and sensory cortices, and back to the cerebellum via the thalamus and the basal ganglia. The cerebellum negatively influenced the brain structures within this loop system for swallowing, similar to cortical strategies in voluntary movements (Mosier & Bereznaya, 2001). Based on these similarities, the researchers proposed that the role of the cerebellum is to modulate and coordinate lingual and pharyngeal movements for swallowing. This hypothesis is supported by data from Jayasekeran and colleagues (2011) who demonstrated not only functional but also anatomical connection between the cerebellum and the primary motor cortex in 16 healthy individuals (Jayasekeran, Rothwell, & Hamdy, 2011). Transcranial magnetic stimulation (TMS) applied over the cerebellum prior to cortical motor stimulation evoked a larger pharyngeal motor evoked potentials (PMEPs) response compared to PMEPs evoked from the motor cortex only. This implies that the cerebellum serves the role as a modulator; explicitly said, an 'intensifier', on the cortical impact for swallowing (Jayasekeran et al., 2011). Cortical structures are highly relevant for swallowing, as they can influence the brainstem driven pharyngeal response and are involved in volitional aspects of swallowing (Babaei et al., 2013; Hamdy, Mikulis, et al., 1999; Huckabee, Deecke, Cannito, Gould, & Mayr, 2003). Up-regulation or down-regulation of cortical functions by the cerebellum could, therefore, be important to alter swallowing.

Cortical control: Neuroimaging research has identified multiple cortical regions that are active prior to and during deglutition, including the primary motor cortex (M1), somatosensory cortex (S1), premotor area, supplementary motor area (SMA), anterior and posterior cingulate cortex, prefrontal cortex, insula, temporopolar cortex were demonstrated to be active (e.g. Hamdy et al., 1999a; Malandraki et al., 2009). Unfortunately, most of the studies using brain imaging during swallowing suffer from being unable to differentiate cortical activation for voluntary components of oral control and the pharyngeal swallowing response. The cerebellum has direct efferent pathways

to M1 and receives inputs from M1 and S1 in a cortical-loop system for movement execution (Allen & Tsukahara, 1974). The roles of these two cortical regions (M1 and S1), collectively referred to as the "sensorimotor cortex", are therefore of particular relevance to this thesis. Activations of the sensorimotor cortex are the most consistently active brain regions during volitional swallowing according to two reviews of neuroimaging studies on swallowing (Humbert & Robbins, 2007; Sörös, Inamoto, & Martin, 2009). Within these areas, the greatest activation on fMRI was found in the oropharyngeal somatotopic representations of these cortices (Furlong et al., 2004; Hamdy et al., 1999; Mosier & Bereznaya, 2001).

Sensorimotor control of swallowing is frequently referred to as bilateral, however, there is ongoing debate about the lateralisation of cortical input to swallowing. For example, Mosier, Liu, Maldjian, Shah, and Modi (1999) found healthy subjects with either left or right hemispheric dominance for swallowing using fMRI. Functional MRI on stroke patients revealed that acquired brain lesions in the left hemisphere were primarily responsible for difficulties in the oral phase and right hemispheric lesions were associated with pharyngeal phase difficulties, also leading to more frequent aspiration (Robbins, Levine, Maser, Rosenbek, & Kempster, 1993). The researchers argue that the left hemisphere is responsible for the more voluntary oral aspects of swallowing and the right hemisphere organises the more reflexive pharyngeal swallowing response. In Robbins et al.'s study (1993), patients with left hemispheric lesions demonstrated significant longer oral transition times on the VFSS compared to healthy individuals or patients with right hemispheric lesions. This study provides important insights into cortical control mechanisms of swallowing in a clinical population; however, concepts about the control of normal swallowing can only be inferred with limitations from a neurologically impaired population. Nevertheless, the results of Robbins et al. are supported by Dziewas and colleagues who studied ten healthy individuals using magnetoencephalography (MEG) (Dziewas et al., 2003). This imaging technique provides advantages of a higher temporal resolution (milliseconds) and allows the participants to sit upright during the examination of swallowing. Dziewas and colleagues (2003) reported strong left hemispheric activation during volitional swallowing of orally infused water. On the other hand, the authors reported more bilateral activation during reflexive swallowing, with water being infused transnasally

into the pharynx directly. The results from these studies suggest that hemispheric lateralisation might be a function of volitional involvement in swallowing.

In contrast, Kern, Birn, Jaradeh et al. (2001) reported bilateral sensorimotor cortical activation during swallowing and swallowing-related tasks but greater volume recruitment in the right hemisphere was found. The authors highlight that they enrolled predominantly right-handed participants into the study, except for one left-handed participant. Interestingly, the left-handed participant presented with primarily left hemispheric sensorimotor activations compared to all right-handed participants. Yet, other studies suggest that hemispheric dominance is not related to other dominant cortical functions, including handedness, rather dominance varies for each person (Hamdy, Aziz, Thompson, & Rothwell, 2001; Humbert et al., 2009; Martin, Goodyear, Gati, & Menon, 2001). In the case of Kern et al.'s study (2001), this finding was either a coincidence or more likely the large voxel size that was used in this study might have limited detailed interpretation and comparison of activation locations. Research methodology was improved by Malandraki et al. (2009) performing and analysing high-resolution anatomical fMRI scans of swallowing and swallowing-related tasks. The researchers confirmed bilateral activation of the sensorimotor cortices during volitional water swallowing. However, no further analysis regarding hemispheric lateralisation during swallowing was undertaken.

Using brain mapping procedures from neurostimulation instead of neuroimaging, Hamdy et al. (1996) corroborated findings of bilateral motor cortical projections to the oropharyngeal musculature. In particular, they demonstrated a symmetric contralateral response in the peak-to-peak amplitude of motor evoked potentials (MEPs) from both mylohyoid muscles (left and right side investigated separately) via surface electrodes. Furthermore, data from the pharyngeal and oesophageal musculature was collected by inserting an intraluminal catheter transorally into the pharynx. In contrast to their results from the submental muscles, pharyngeal motor representations were reported to be asymmetric in this and a subsequent study (Hamdy et al., 1996; Mistry et al., 2007). Unfortunately, the significance of their results is weakened because the mean peak-to-peak amplitudes were derived from only three stimuli with large variability of responses [e.g. contralateral MEP responses from the right mylohyoid were 153 µV (+/-120µV) and 103 µV (+/- 70µV) for ipsilateral MEP responses] (Hamdy et al., 1996).

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Furthermore, due to the close proximity of submental muscles, sEMG may not be suitable to detect muscle or side specific differences. Nonetheless, Hamdy et al.'s (1996) findings support previous reports (e.g. Dziewas et al., 2003; Robbins et al., 1993), suggesting different cortical representations during the oral (more volitional) and pharyngeal (more reflexive) phases of swallowing. Although the submental muscles are highly involved in both of these phases, dominant unilateral motor cortical representations of the pharyngeal musculature might be predominantly active during swallowing and therefore result in strong unilateral activation during reflexive swallowing on neuroimaging. However, a neurophysiological rationale for this most likely existing hemispheric lateralisation during swallowing has not been found and remains further investigation.

4. Dysphagia

The term "dysphagia" directly translated means "difficulty eating". It consists of the Greek word segments 'dys' meaning disordered or abnormal and 'phagia' which translates to eating (Cinocco, 2007). Cinocco defines dysphagia as *"a sensation of difficulty in the passage of solids or liquids from the mouth to the stomach. Dysphagia can also be thought of as trouble swallowing, coughing, choking or inability to safely handle food or even one's own saliva."* (Cinocco, 2007, p. 1). This definition points out many signs and symptoms of dysphagia and captures the possibility that difficulties with swallowing can occur without conscious awareness (i.e. silent aspiration). Dysphagia can result from decreased sensorimotor functions associated with ageing and congenital disorders, and/or damage to the CNS or PNS such as with traumatic brain injuries, neurologic conditions, head and neck cancer or (neuro)surgery (Clavé & Shaker, 2015). The degree of impairment and complications can range from mild discomfort during swallowing to severe symptoms such as aspiration pneumonia. This wide range of associated health complications of dysphagia can result in decreased quality of life and increased morbidity rates (Eslick & Talley, 2008; Leow, Huckabee, Anderson, & Beckert, 2010; Martino et al., 2005). Furthermore, it can cause high financial costs to health care systems (Attrill, White, Murray, Hammond, & Doeltgen, 2018). Thus, development of effective dysphagia management strategies is imperative.

4.1 Prevalence, pathophysiology and consequences

The Australian and New Zealand Society for Geriatric Medicine (2011) reports an estimated prevalence of dysphagia in the community of 7 – 22% and an incidence of up to 40 – 50% of the elderly population living in facilities for long-term care (Chan, Phoon, & Yeoh, 2011). Sura, Madhavan, Carnaby, and Crary (2012) reviewed prevalence rates of dysphagia in the elderly population in the United States and reported slightly higher numbers with up to 13% – 38% of elderly who live independently and up to 68% of elderly nursing home residents. Variations of these prevalence estimates can, for example, be explained by different assessment methods in the reviewed studies, e.g. questionnaire versus clinical assessments, or different inclusion criteria used, e.g.

some included less common types of dysphagia (e.g. oesophageal). With age, swallowing anatomy and physiology changes, e.g. reduced muscle strength and mass (sarcopenia), oral dryness (xerostomia) or diminished oropharyngeal sensation (Britton, 2016). Compared to young healthy individuals, these age-related changes result in a weaker, slower and less coordinated swallowing mechanism (Britton, 2016). For example, reduced bolus propulsive force and associated oropharyngeal residue (Rofes et al., 2010), slower oral (Cook et al., 1994; Shaw et al., 1995; Yoshikawa et al., 2006) and oro-pharyngeal transit times (Yokoyama, Mitomi, Tetsuka, & Tayama, 2000) and reduced laryngeal and hyoid elevation leading to pharyngeal residue, and thus increasing the risk for penetration and aspiration have been reported in elderly individuals (Britton, 2016).

Stroke is the leading cause of neurologic dysphagia, occurring in up to 81% of stroke sufferers (Roden & Altman, 2013). The likelihood of stroke increases with age. Approximately 9000 new stroke cases are documented in New Zealand each year and 60.000 stroke survivors are living with the consequences (Stroke Foundation of New Zealand, 2009). A quarter of strokes occur at the age of under 65 years (Stroke Foundation of New Zealand, 2009). The consequences of stroke include difficulties with activities of daily living, cognitive deficits, communication problems and swallowing impairments. The incidence of dysphagia in the stroke population is lowest when identified using screening techniques ($37 - 45\%$), followed by clinical testing ($51 - 55\%$), and the highest when using instrumental testing ($64 - 78\%$) (Martino et al., 2005). Important to note is that with increased specificity of the assessment tool, there is a higher incidence rate of dysphagia.

Swallowing impairments following cortical or subcortical strokes can affect one or multiple stages of the swallowing process (Daniels & Huckabee, 2014; Leopold & Kagel, 1997). Early in the process of swallowing, impaired motor function and/or sensory perception of the bolus during the pre-oral and oral phase can decrease bolus manipulation and control of the food or liquid. This can results in anterior or posterior leakage of the bolus from the oral cavity. The more serious matter is posterior premature spillage into the pharynx, resulting in possible penetration or aspiration of the bolus. An additional concern with sensory deficits is that penetration and aspiration can be caused by delayed initiation of pharyngeal swallowing (Daniels & Huckabee,

2014). Even minor disruptions of swallowing biomechanics during the pharyngeal phase pose a high risk of aspiration to the patient, as the bolus passes the entrance of the airway. Mechanisms to protect the airway, such as hyolaryngeal excursion and/or specific vocal fold closure, are therefore crucial but also prone to impairment (Daniels & Huckabee, 2014). The risk of aspiration increases if a loss of pharyngeal sensation exists, which disables the detection of food or liquid in the pharynx allowing it to enter the airway. As mentioned above, silent aspiration is when food or liquid enters the airway without being recognised and consequently removed (Aviv et al., 1996). Approximately 2% – 25% of acute and 15% - 39% of subacute stroke patients may aspirate silently as identified in videofluoroscopy studies (Ramsey, Smithard, & Kalra, 2005). Aspirate that cannot be cleared out of the airway poses a significant risk for the development of aspiration pneumonia (Ramsey et al., 2005). Furthermore, patients with dysphagia are at risk of malnutrition, dehydration and mortality (Clavé & Shaker, 2015).

4.2 Assessment

Thorough assessment of the swallowing impairments builds the foundation of the dysphagia management practices. Although methods for dysphagia assessment are rapidly expanding, the more commonly used methods within the behavioural swallowing examination include a review and discussion of the medical history, symptom-specific questionnaires, CN examination and tests of oral intake, if appropriate. Additional insights can be gathered using instrumental swallowing assessments, including imaging techniques and physiologic measurements. Furthermore, advances in the understanding of swallowing neural control, and neurophysiological recovery processes post-stroke have led to emerging neurophysiologic assessments.

Clinical swallowing assessment: The standard clinical swallowing examination, as proposed by Daniels and Huckabee (2014), consists of a medical history review as well as collecting information on the current medical status of the patient from records and the patient. This includes gathering information regarding the cognitive status and communication skills (Daniels & Huckabee, 2014). Quantitative data can be collected

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using validated questionnaires regarding dysphagia presence and/or severity and the patient's quality of life, such as the Eating Assessment Tool (Belafsky et al., 2008; EAT-10) or the Swallowing-related Quality of Life Questionnaire (McHorney et al., 2002; SWAL-QOL). In contrast to the more time-consuming SWAL-QOL, the EAT-10 provides a fast and efficient symptom-specific instrument to detect oropharyngeal dysphagia with very good internal consistency, test-retest reliability and criterion-based validity (Belafsky et al., 2008). The performance of a CN examination, such as the one proposed by Daniels & Huckabee (2014), provides valuable information about swallowing function as a result of cranial nerve impairment. Based on the findings of the examination up to this point, the clinician decides if direct evaluation of oral intake is indicated.

Water swallowing protocols, mainly developed to screen for dysphagia, vary in their administration and interpretation (Martino et al., 2005). They used different quantities of water (ranging from 10ml to 150ml) or different methods of ingestion (ranging from taking small sips to drinking the fluid in its entirety) (Daniels et al., 1998; DePippo, Holas, & Reding, 1992; Gottlieb, Kipnis, Sister, Vardi, & Brill, 1996; Hughes & Wiles, 1996; Kidd, Lawson, Nesbitt, & MacMahon, 1993; Lim et al., 2001; Suiter & Leder, 2008). In addition to a dichotomous pass or fail interpretation used by most of these tests, the Timed Water Swallowing Test (TWST) from Hughes and Wiles (1996) is timed and normed to objectively quantify swallowing efficiency of liquids (150 ml of water). It is sensitive to changes in swallowing function resulting from neurological disorders (Hughes & Wiles, 1996; Miller et al., 2009). Wu, Chang, Wang, and Lin (2004) validated a 100 ml TWST protocol against a VFSS in 59 patients with dysphagia following stroke. They found that reduced swallowing speed was an indicator for dysphagia with high sensitivity but reduced specificity. Not surprisingly, the presence of choking was associated with high sensitivity and specificity for identifying dysphagia (Wu, Chang, Wang, & Lin, 2004). The development of the Test of Masticating and Swallowing Solids (TOMASS) was inspired by the TWST and provides a tool to quantify oral ingestion of solid food (Athukorala et al., 2014). Normative data from the healthy population has been collected and reported by Huckabee and colleagues (2017).

Further assessments can be used as adjuncts to the behavioural swallowing exam, such as pulse oximetry (for a review see Britton et al., 2017), cervical auscultation (Borr, Hielscher-Fastabend, & Lücking, 2007; Leslie et al., 2007; Leslie, Drinnan, Finn, Ford, & Wilson, 2004) or cough reflex testing (Addington, Stephens, Gilliland, & Rodriguez, 1999; Miles, Zeng, McLauchlan, & Huckabee, 2013; Sato et al., 2012). Pulse oximetry is a low cost, portable and non-invasive tool which measures the blood oxygen level and has been tested to identify silent aspiration (Collins & Bakheit, 1997; Ramsey, Smithard, & Kalra, 2006; Wang, Chang, Chen, & Hsiao, 2005). Although pulse oximetry would add an objective measure to the subjective clinical exam, the relationship between oxygen level and dysphagia still remains unclear. One cause might be that oximetry readings are highly affected by variables such as breath-holding, posture or compromised pulmonary functioning (Higo, Tayama, Watanabe, & Nito, 2003). Cervical auscultation is used to examine acoustic characteristics of swallowing and to identify possible abnormalities in sounds, hence in swallowing (Bergström, Svensson, & Hartelius, 2014; Borr et al., 2007; Leslie et al., 2004). However, no definitive data correlated with sounds of specific physiologic swallowing events or abnormalities. This technique is subjective and also depends on the experience of the listener (Borr et al., 2007). Cough reflex testing uses tussive agents, e.g. citric acid or capsaicin, to test the integrity of CN X, i.e. sensory perception in the pharynx. Preliminary evidence exists that the cough reflex test, when used as adjunct to the behavioural swallowing assessment, can significantly reduce the pneumonia rates in acute stroke patients (Davies, 2016). However, no clear standard protocol exists and more population-specific data is needed for wide-ranging implementation into clinical praxis.

The clinical examination, with a few exceptions, is primarily limited to subjective analysis with disputable results for reliability and validity of clinical observations (McCullough et al., 2005) and techniques such as pulse oximetry and cervical auscultation (Stroud, Lawrie, & Wiles, 2002; Wang et al., 2005). Future work on validating bolus-related measures such as the TWST and TOMASS in the stroke population is necessary and may provide valuable insights into swallowing performance at bedside. Despite the development of these quantitative measures, the behavioural swallowing examination provides only secondary or indirect information about the

patient's swallowing status. Instrumental assessment is required to characterise swallowing function or dysfunction and to plan rehabilitation.

Instrumental swallowing assessment: Instrumental techniques can be categorised into imaging methods and physiologic methods. The two most frequently used clinical imaging methods are VFSS (Logemann, 1983), and videoendoscopic evaluation of swallowing (Bax, McFarlane, Green, & Miles, 2014; Langmore, Kenneth, & Olsen, 1988; Lim et al., 2001; VEES); ultrasonography has been described but is not widely used (Kuhl, Eicke, Dieterich, & Urban, 2003; Macrae, Jones, Myall, Melzer, & Huckabee, 2013; Peng & Miethke, 2000; Yabunaka et al., 2011).

The VFSS uses ionising radiation to primarily capture the dynamics of oropharyngeal physiology but can also be extended to view oesophageal mechanisms during swallowing (Rugiu, 2007). It is widely considered the be the gold-standard diagnostic test by providing detailed two-dimensional, dynamic, radiographic images of swallowing. In contrast, VEES utilises a fibreoptic endoscope positioned in the hypopharynx to provide an intraluminal view before and after swallowing (Bax et al., 2014; Lim et al., 2001; Tohara et al., 2010). This technique is superior to VFSS in that it can be performed at bedside, does not require ionising radiation exposure and allows inspection of the patients mucosal status. However, VEES is limited in that it provides only indirect information regarding oral and oesophageal phases of swallowing. Furthermore, a 'white-out' period during the pharyngeal phase limits the direct assessment of intra-swallow aspiration and pharyngeal dynamics. Research studies comparing VEES and VFSS have identified significantly higher ratings of aspiration (Kelly, Drinnan, & Leslie, 2007) and pharyngeal residue (Kelly, Leslie, Beale, Payten, & Drinnan, 2006) for the VEES evaluation. Although the researchers present these findings based on a well-designed study, they do not provide a sound explanation of these phenomena. The more coloured representation of residues and structures on VEES compared to the black-and-white picture on videofluoroscopy (VFS) might explain the higher residue ratings for the VEES. However, these speculations would need to be confirmed in future studies. Taken together, both techniques have been proven to be superior to behavioural swallowing examinations (Wilson & Howe, 2012). Importantly, even though both techniques enable visualisation of impaired

biomechanics they do not provide information about the underlying pathophysiology, e.g. spasticity, weakness, apraxia, or other neuromuscular changes.

Physiologic measurements of swallowing may include pharyngo-oesophageal manometric analysis. Although historically more commonly used for assessments of oesophageal peristalsis, manometry is slowly emerging into clinical practice to gain quantitative information about physiological functions in the pharyngeal phase of swallowing such as UES integrity, pharyngeal peristalsis and intrabolus pressures (e.g. Bhatia & Shah, 2013; Brasseur, Wylie, & Dodds, 1991; Dantas, Cook, et al., 1990; Hila, Castell, & Castell, 2001; Omari et al., 2012, 2014; Taher I. Omari et al., 2016; Ravich, 1995). Moreover, pharyngeal manometry can be used as a biofeedback tool to retrain control over sequential pharyngeal muscle activation in patients with dysphagia following stroke (Huckabee et al., 2014).

4.4 Treatment

The spectrum of treatment for dysphagia following stroke includes a variety of compensatory strategies and rehabilitative interventions. Compensatory strategies, such as alterations of bolus characteristics, postural adjustments or bolus control techniques, are used to improve the patient's swallowing function in the short-term by changing bolus flow (Daniels & Huckabee, 2014). Furthermore, specific swallowing manoeuvres that facilitate airway protection or the conscious clearance of pharyngeal residuals can be used to reduce the risk of penetration and aspiration. Although compensatory strategies are valuable to immediately improve swallowing safety, they are only short-term solutions. Dysphagia rehabilitation approaches, on the other hand, have been developed to alter pathophysiologic swallowing mechanisms through repetition over time (Daniels & Huckabee, 2014). There is only a limited amount of evidence-based studies investigating dysphagia rehabilitation approaches and most of them have methodological problems (Macrae & Humbert, 2013; Speyer, Baijens, Heijnen, & Zwijnenberg, 2010).

Commonly used dysphagia rehabilitation approaches, such as effortful swallowing, lingual strengthening or the tongue hold manoeuvre, aim to strengthen the oropharyngeal musculature to restore safe and effective swallowing (Burkhead, Sapienza, & Rosenbek, 2007). Strength training is used based on the assumption that muscle weakness is the underlying cause for the swallowing disorder and the consequence of neurological injuries (Huckabee & Macrae, 2014). However, neurological damage can lead to a variety of causes for motor dysfunction, such as spasticity (O'Dwyer, Ada, & Neilson, 1996) or apraxia (Ziegler, 2008). Strengthening approaches may therefore only be indicated in swallowing rehabilitation when a specific diagnosis of muscle weakness exists and not as an overall approach for all swallowing impairments. Adverse effects of effortful swallowing have been identified, including mistiming of tongue base movements (Garcia, Hakel, & Lazarus, 2004) or deeper penetration of retentions in a patient with severe pharyngeal dysfunction (Bülow, Olsson, & Ekberg, 2001). In addition, many repetitions are required to achieve the presumed goals of improved force production, coordination, and precision of movement through muscle strengthening (Burkhead et al., 2007). Intensive muscle strength training can result in muscle fatigue or detraining of lingual strength and cheek strength (Clark, O'Brien, Calleja, & Newcomb Corrie, 2009).

In addition to behavioural treatment approaches, advances in medical technology have led to the development of non-invasive brain stimulation (NIBS) techniques that can be used to facilitate motor recovery following stroke. The considerably more advanced knowledge of the effects of NIBS in limb rehabilitation and better understanding of swallowing neurophysiology guided the use of NIBS for swallowing rehabilitation. For instance, Hamdy and colleagues demonstrated that recovery of dysphagia was associated with an increase in cortical excitability of pharyngeal representations in the unaffected cortex (Hamdy et al., 1998). This has led to the development of NIBS protocols using repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) targeting cortical reorganisation after stroke. The development of NIBS protocols for swallowing have gained increasing attention over recent years and their usage and effects on dysphagia rehabilitation have been evaluated in several reviews (Doeltgen, Bradnam, Young, & Fong, 2015; Mistry et al., 2014; Pisegna et al., 2015; Simons & Hamdy, 2017; Yang, Pyun, Kim, Ahn, & Rhyu, 2015).

First, the effects of rTMS over the pharyngeal motor cortex were explored on the swallowing function in healthy volunteers (Gow, Hobson, Furlong, & Hamdy, 2004; Jayasekeran et al., 2010; Jefferson, Mistry, Michou, et al., 2009; Michou et al., 2012; Mistry et al., 2007). This series of studies demonstrated that 1 Hz rTMS decreases and 5 Hz rTMS increases motor cortical excitability of pharyngeal projections, resulting in faster or slower swallowing reaction times compared to controls respectively. Although it remains unknown if a change in swallowing reaction times implies a change in swallowing function, rTMS has been demonstrated to be a valuable tool to test the effects of rTMS on healthy brain function and/or to imitate a brain lesion. For instance, low-frequency 1 Hz rTMS can be used to generate a virtual lesion in a healthy brain (Mistry et al., 2007). This generated lesion can be reversed in a subsequent intervention using 5 Hz rTMS over the contralesional hemisphere (Jefferson et al., 2009). Although the neurophysiologic assessment and interpretation of pharyngeal MEP recordings using an intraluminal catheter is problematic due to unspecific muscle recording as a result of catheter movements (Macrae, Jones, & Huckabee, 2014), findings appear to be replicable. In addition, the results of this research series using pharyngeal MEP recordings are supported by a small pilot study measuring MEPs via surface electrodes from the submental muscle group (Verin, Michou, Leroi, Hamdy, & Marie, 2012). Most recently, Vasant and colleagues demonstrated that high-frequency rTMS (10 Hz) over the cerebellum increases pharyngeal motor cortical excitability and produced lasting effects in PMEPs of up to 30 min post-intervention compared to controls (Vasant, Michou, Mistry, Rothwell, & Hamdy, 2015). Interestingly, the strongest cerebellar-pharyngeal representation was ipsilateral to the strongest pharyngeal cortical representation in the majority of subjects (14/17) (Vasant et al., 2015). This study underlines functional involvement of the cerebellum in swallowing and presents the cerebellum as an alternative target to cortical stimulation to affect swallowing neuropathways. Nevertheless, a measure of swallowing function is required to determine whether an increase or decrease in PMEPs for either of these inputs is positive to swallowing, or to the relationship between these structures. This is also of clinical relevance, as cerebellar rTMS poses a lower risk of adverse events, in particular seizures, compared to motor areas (Machii, Cohen, Ramos-Estebanez, & Pascual-Leone, 2006; Rossi, Hallett, Rossini, & Pascual-Leone, 2009). However, cerebellar stimulation protocols have not yet been investigated for rehabilitation in patients with dysphagia post-stroke.

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Current rTMS protocols in stroke patients aim to counteract hemispheric imbalance caused by the lesion either by increasing activity of the impaired hemisphere or inhibiting function of the contralesional hemisphere (Michou, Raginis-Zborowska, Watanabe, Lodhi, & Hamdy, 2016). In a recent meta-analysis, a moderate significance of the overall effect size for four randomised controlled trials (RCT) utilising rTMS as post-stroke rehabilitation for dysphagia was reported (Pisegna, Kaneoka, Pearson, Kumar, & Langmore, 2015; Figure 6). A comparison of hemispheric rTMS application with respect to the lesion suggest stronger effectiveness of stimulation over the unaffected hemisphere with a significant combined effect size of 0.65 (95% CI 0.14, 1.16; $p = 0.01$) compared to an effect size of 0.46 (95% CI -0.18, 1.11; $p = 0.16$) for rTMS over the affected hemisphere (Pisegna et al., 2015). However, the overall interpretation of these studies with patients is limited by differences in the methodology (e.g. targeting different cortical representations, stimulation protocols), outcome measures (e.g. VFSS, self-evaluation of dysphagia) and patients characteristics (e.g. time post-onset).

Similar to rTMS, tDCS is another NIBS technique that enables change in cortical excitability; and it does this in a polarity dependent manner. It was demonstrated that anodal tDCS increases and cathodal tDCS decreases motor cortical excitability when MEPs were measured from the abductor minimi muscle in healthy volunteers (Nitsche & Paulus, 2001). The research on tDCS for swallowing and dysphagia rehabilitation builds the main focus of this research programme and is therefore reported and discussed in greater detail in section 8.1. In introduction, however, it bears mentioning that the application of tDCS follows the same approach as used for rTMS interventions by modulating hemispheric imbalance following acquired brain damage. Although tDCS in swallowing rehabilitation has a slightly smaller effect size compared to rTMS, the data was pooled from fewer studies in the meta-analysis by Pisegna et al. (2015). TDCS has several advantages over rTMS applications such as cost efficiency and fewer side effects (Simons & Hamdy, 2017; Table 1). In contrast to rTMS, tDCS has most effectively been used when applied in combination with various types of behavioural rehabilitation in limb function (Hummel & Cohen, 2006; Schlaug, Renga, & Nair, 2008).
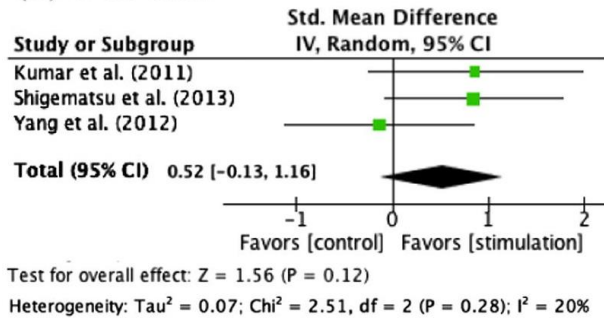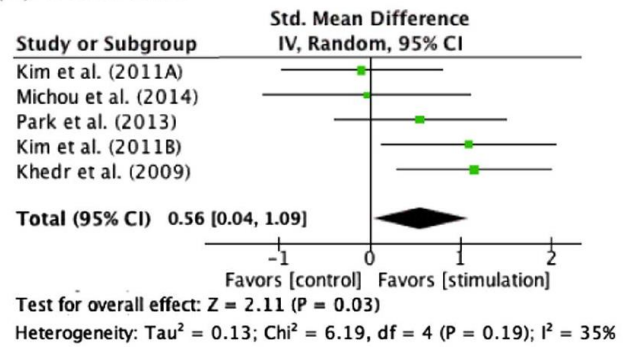
Figure 6. Meta-analysis results from current studies using transcranial direct current stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS) in post-stroke dysphagia rehabilitation.[6]

Table 1. Comparison of rTMS and tDCS for clinical use.[7]

	RTMS	TDCS
Equipment	Pulse generator, stimulation coils	Current generator, electrodes, sponge soaked in saline
Costs	High	Low
Safety aspects/side effects	Risk of fainting and seizures (low) [41]	Skin irritation under electrode, phosphine, nausea, headache, dizziness [42]
Physiological effects	Magnetic field generates action potential in neuron	Direct current increases neurone spontaneous firing rate
Ease of delivery	Relatively difficult requires trained coil holder, large bulky equipment	Relatively easy to apply, equipment is portable

Studies utilising cortical tDCS in dysphagia rehabilitation used it as an adjunct to compensatory strategies or strengthening exercises (Kumar et al., 2011; Shigematsu, Fujishima, & Ohno, 2013). However, the practice of strength training exercises as the default approach for all dysphagic symptoms has recently been questioned (Huckabee & Macrae, 2014). Specifically, unspecific muscle strengthening exercises (e.g. effortful swallowing) might be contraindicated in some cases of dysphagia where the neurological cause for the motor impairment is unknown (Huckabee & Macrae, 2014). Enhancing the effects of these unspecific treatment approaches with tDCS might, therefore, be inappropriate.

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[6] Reprinted with permission by Elsevier: Pisegna, J. M., Kaneoka, A., Pearson, W. G., Kumar, S., & Langmore, S. E. (2016). Effects of non-invasive brain stimulation on post-stroke dysphagia: a systematic review and meta-analysis of randomized controlled trials. Clinical Neurophysiology, 127(1), 956-968. Copyright (2016).
[7] Published in Simons, A., & Hamdy, S. (2017). The use of brain stimulation in dysphagia management. Dysphagia, 32(2), 209-215. Open access source by Creative Commons Attribution 4.0 International License http://creativecommons.org/licenses/by/4.0/.

Skill training is an alternative to strength training and is commonly used in other domains such as post-stroke motor recovery from hemiparesis in the upper and/or lower extremities. Recovery from hemiparesis requires recruitment of neuronal resources from the damaged and undamaged cortical areas to optimise the planning, execution and control of lost motor functions (Matthews, Johansen-Berg, & Reddy, 2004). Stroke patients can facilitate this cortical reorganisation of motor networks through skill training using motor skill learning (Lefebvre et al., 2014). Motor skill learning as a concept of neurorehabilitation has received considerable attention in the physical therapy literature (Kitago & Krakauer, 2013; Krakauer, 2006; Matthews, Johansen-Berg, et al., 2004) and is slowly emerging into practices for dysphagia rehabilitation.

5. Motor skill learning

In the corticospinal literature, motor skills are commonly defined as gradually acquired movements through practice until they can be performed effortlessly (Ungerleider, Doyon, & Karni, 2002). Examples of motor skills are fine finger movements when playing an instrument or multijoint actions when grasping an object (Ungerleider et al., 2002). Although swallowing requires a highly coordinated sequence of muscle activations like these motor skills, it does not entirely fit into the classic definition of a motor skill. Swallowing is innate and functionally present pre-natally. Therefore, it does not have to be acquired through repeated practice. Another main difference between a motor skill, as defined, and swallowing lies in the neural control mechanisms. Acquired motor skills are usually voluntary, with the primary motor cortex innervating the limb to execute the movement (Kim et al., 1993). Swallowing, on the other hand, is a semi-reflexive response of the corticobulbar system, and although it can be volitionally initiated, it is primarily brainstem driven (Jean, 2001). Nevertheless, relearning a previously automatic and subconscious task after brain injury, such as swallowing, is akin to the acquisition of a motor skill. Patients have to bring this previous subconscious task into the realm of consciousness and modify aspects of complex muscle activations. Swallowing skill training using biofeedback may be useful to regain swallowing function. In motor rehabilitation, swallowing skill may be defined as the ability to voluntarily modulate timing, strength (force) and coordination of multiple muscles that are involved in swallowing, in the performance of a complex, goal-directed spatiotemporal task (Huckabee, personal communication, August 19, 2017).

Practice is required to achieve a higher level in the performance of skilled behaviour. Hereby long-term improvements of motor skills are desired, especially in the context of neurorehabilitation. When determining the effects of skill training, it is crucial to distinguish between short-term gains during or immediately following the practice, i.e. *motor performance*, and more permanent changes as a result of *motor learning* (Kantak & Winstein, 2012; Kitago & Krakauer, 2013; Salmoni, Schmidt, & Walter, 1984).

Retention and transfer tests are common tools to assess motor performance and motor learning. *Retention tests* determine the level of motor performance following skill training, using the same conditions that have been used during skill training (Kantak & Winstein, 2012). *Transfer tests* assess motor skill learning and the generalisation of the

learned skill to untrained conditions (Kantak & Winstein, 2012). They require "a new variation of the practiced skill, or on a different, but related skill that was not practiced before, or in a different testing situation or context." (Kantak & Winstein, 2012, p. 221). A common variation in the assessment of motor skill learning is the type of feedback that is provided during the transfer test compared to the training.

Feedback during motor skill practice is essential for motor skill learning to occur (Sharma, Chevidikunnan, Khan, & Gaowgzeh, 2016). The feedback is most commonly provided as knowledge of performance (KP) and knowledge of results (KR) (Lauber & Keller, 2014; van Vliet & Wulf, 2006). KP refers to feedback that the practitioner receives about the quality of the movement or the movement pattern through intrinsic sensory perceptual information and kinematic feedback, or extrinsic feedback, e.g. verbal feedback during the execution of a movement (McGill, 2001). KR refers to extrinsic feedback that the practitioner receives after completion of the task, i.e. the performance outcome (Salmoni et al., 1984). When testing for motor skill learning, a no-feedback condition, i.e. no KR and KP, is required to assess the participants level of independence in skill performance (Muratori, Lamberg, Quinn, & Duff, 2013; Wulf, Shea, & Lewthwaite, 2010). In other words, if KP and KR is available to the participant during the assessment of motor skill learning, both feedback conditions can influence motor performance and limit the interpretability of motor learning effects (Schmidt & Lee, 2005).

Retention and transfer tests can be performed immediately after skill practice or delayed. Immediate retention or transfer is tested directly and up to two hours following the training, whereas delayed tests are generally conducted 24 h or more post training (Kantak & Winstein, 2012). Delayed transfer tests have been demonstrated to be a better predictor of changes in motor skill learning, compared to immediate testing (Kantak & Winstein, 2012). These results are supported by other definitions of motor skill learning which are proposing that multiple training sessions are required for motor learning to take place (Wulf et al., 2010). Only delayed assessments, and not immediate assessments after one training session, could therefore truly reflect achievements in motor skill learning.

5.1 Neurophysiological basics of motor skill learning

Behavioural, electrophysiological, functional, and cellular/molecular studies help to understand motor skill learning in greater detail (Luft & Buitrago, 2005). Motor skill learning is characterised by rapid changes in behaviour in the first few sessions and by slower gains in subsequent sessions (Dayan & Cohen, 2011; Figure 7). Similarly, changes within a single training session follow the same pattern of initial fast skill acquisition followed by a slower acquisition phase (Dayan & Cohen, 2011). This pattern is more distinct within the first few sessions and incrementally decreases in later sessions (Luft & Buitrago, 2005). The acquired level of motor performance achieved in one session (online learning) can be retained following a break period and might even demonstrate small improvements at the beginning of the next session compared to the level of performance at the end of the previous session (offline learning) (Robertson, Pascual-Leone, & Miall, 2004). The term consolidation describes both behavioural improvements that occur between skill training sessions as well as the stabilisation of the learned skill in the long-term motor memory (Krakauer & Shadmehr, 2006; Robertson et al., 2004). Even a longer break of several days or weeks without practice following several training sessions can result in long-term retention of the learned skill (Romano, Howard, & Howard, 2010).

Behavioural changes through motor skill learning are accompanied by neural reorganisation and structural neuroplastic changes (Kleim et al., 2006). Several investigations explored neural activation and neuroplastic changes through motor skill learning in the corticospinal system using fMRI, NIBS or electroencephalography (EEG) (Dayan & Cohen, 2011; Mehrkanoon, Boonstra, Breakspear, Hinder, & Summers, 2016). They reported that among other neural structures involved in the complex process of motor skill learning, the cerebellum and the M1 are the key brain regions for learning-dependent plasticity. Two important mechanisms of learning-induced plasticity have been identified at these structures and their connecting network - the synaptic strengthening or weakening of neural connections through long-term potentiation (LTP) and long-term depression (LTD) (Sanes & Donoghue, 2000). LTP and LTD facilitate or inhibit neural transmission respectively.
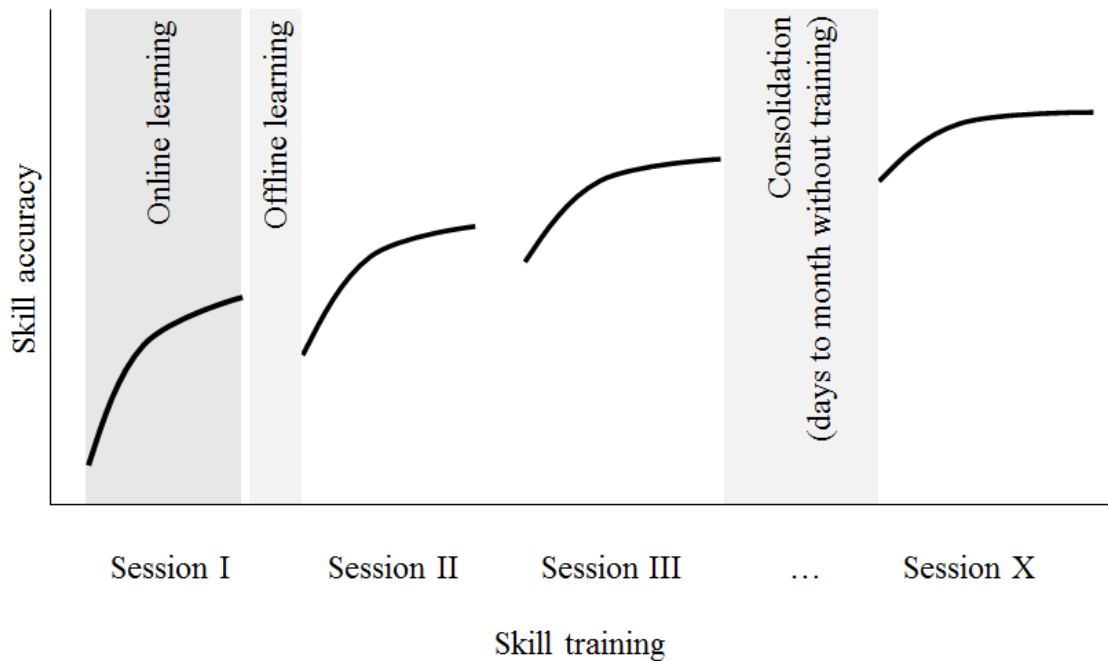
Figure 7. Motor skill learning curve and conceptual components of motor learning.[8]

The fast phase of motor skill learning is primarily characterised by rapid functional changes within these brain regions and networks at the synaptic level, e.g. changes in the amount and/or frequency of released neurotransmitters and in the number and/or type of neurotransmitter receptors on the post-synaptic membrane (Dayan & Cohen, 2011). In particular, cerebellar output was proposed to be primarily mediated by LTD of the synapses between parallel fibers and Purkinje cells (Hirano, 2013; Ito, 2001). With the repeated use of the same neural substrates through practice, the connection between neural structures strengthen and new neural networks are built to support learning; known as Hebbian learning (Hebb, 1949). The slow phase of motor learning and the retention of motor skills are therefore characterised by structural long-term changes and primarily attributed to changes in gray and white matter of M1 (Dayan & Cohen, 2011). Recent research, exploring the temporal dynamics of neurophysiological changes in the cerebellum and M1, demonstrated that changes in cerebellar output occur primarily in the early phase of motor skill learning whereas LTP-like plasticity of M1 was demonstrated in the retention phase of motor skill learning (Kida & Mitsushima, 2017; Spampinato & Celnik, 2017). In synchrony with behavioural

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change, functional and structural changes plateau after several repetitions within one training session or following multiple training sessions when the maximum capacity for neuroplastic change is reached (Karni et al., 1998).

Neuroplastic changes evoked through behaviourally driven motor learning in the intact brain can similarly be found in the impaired brain following stroke (Matthews, Johansenberg, & Reddy, 2004; Nudo, 2013). During this reorganisation of cortical networks, mechanisms of plasticity include a shift or expansion in motor representations, synaptic sprouting, dendritic plasticity or formation of new axon terminals occur (Hallett, 2001; Matthews, Johansen-Berg, et al., 2004). Additionally, mechanisms such as inflammatory mediators following brain injury may alter cortical reorganisation after injury (Comelli et al., 1993; Schäbitz, Schwab, Spranger, & Hacke, 1997). The use of motor skill learning in neurorehabilitation has, therefore, become a popular approach to facilitate this reorganisation to enable fast and efficient motor recovery.

The majority of research on neuroplasticity and motor skill learning is based on animal studies and human studies of limb function. Only a few studies have investigated neuroplastic changes related to motor skill learning in oral motor tasks, similar to swallowing. Svensson and colleagues were one of the first to investigate plastic changes in corticomotor control of the human tongue induced by a daily one-hour, tongue-protrusion training task in healthy young volunteers (Svensson et al., 2003, 2006). They used TMS to assess MEPs from the right dorsal surface of the tongue and the first dorsal interosseous (FDI) muscle of the hand at baseline and at different follow-up timepoints between the two studies, ranging from immediately after tongue training and 30 min post, one day, seven days and two weeks following the training. Svensson et al. (2003, 2006) documented decreased stimulation thresholds, increased MEP amplitude and an increased size of the corticomotor topographic maps at different timepoints post training. This reflects greater cortical excitability and a larger cortical area dedicated to control oral movements. In particular, significant changes of cortical plasticity immediately after the training that lasted up to seven days post training when compared to baseline were demonstrated. However, this effect was not maintained at two weeks following the training. As hypothesised, no changes of cortical plasticity were found for the FDI muscle, suggesting the training was specific to corticomotor control of the

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tongue. In contrast to swallowing, protrusion of the tongue is entirely voluntary motor behaviour. It, therefore, needs to be determined if these findings also translate to a semi-reflexive behaviour such as swallowing. Furthermore, plasticity changes in this study were assessed in the area of M1 exclusively. However, the same research group showed a significant increase of fMRI-BOLD activity not only in the precentral gyrus (area of cortical tongue representation) but also in the SMA, putamen and cerebellum one day after tongue protrusion training using the same protocol (Arima et al., 2011). Changes in other cortical and subcortical brain structures involved in motor skill learning would need to be addressed in future studies.

A potential problem for studies utilising MEPs to document cortical changes are the highly variable response obtained, and the requirement for stable electrode placement to assess one specific muscle or a group of muscles. The reliability of the electrode to mucosa contact at the dorsal surface of the tongue in the studies by Svensson and colleagues (2003, 2006) might be problematic, given that the electrode was placed inside the oral cavity which is a moist environment for adhesive electrodes. Nevertheless, findings of neuroplastic changes following tongue motor control were confirmed in subsequent TMS studies (Baad-Hansen, Blicher, Lapitskaya, Nielsen, & Svensson, 2009; Komoda et al., 2015; Kothari et al., 2013).

Kothari et al. (2013) compared three different tongue training paradigms, by randomly assigning 48 healthy participants into one of the three groups. They reported increased MEP amplitudes and decreased resting motor thresholds, both indicating increased motor cortical excitability, following tongue protrusion training and gaming-based tongue training. In contrast, training with therapeutic tongue exercises, consisting of sensory stimulation and strength-based tongue mobilisation training, did not lead to changes in corticomotor excitability. This may be explained by the nature of the therapeutic exercises, which did not require an active neural recruitment since the participant was mainly passive. The results, therefore, demonstrate the advantages of skill-based training to evoke neuroplastic changes. Furthermore, it was demonstrated that engagement and motivation of the participant are prerequisite for cortical changes (Kothari et al., 2013). Unfortunately, it remains unclear if the training was instructed by the same person performing the assessment or not. Potential bias could exist if no blinding was used for the assessments.

Taken together, the results of these studies demonstrated an increase in corticomotor plasticity following volitional skill-based orofacial motor training, which may indicate that changes for more reflexive oropharyngeal motor functions such as swallowing are possible.

5.2 Motor skill training in swallowing

A limited number of skill-based training approaches for swallowing have been developed in recent years with the premise to become neurorehabilitative tools. For example, pharyngeal manometry has successfully been used for biofeedback, to modulate the sequencing of pharyngeal muscle contraction (Huckabee et al., 2014; Lamvik, Jones, Sauer, Erfmann, & Huckabee, 2015). However, this approach requires transnasal insertion of a catheter into the pharynx which may lead to patient discomfort. Carnaby-Mann & Crary (2008) introduced the McNeill Dysphagia Therapy Program (MDTP) which is a systematic exercise and skill-based therapy framework in which the level of difficulty increases through modification of the bolus consistency, volume and rate of intake. The researchers confirmed the effectiveness of the MDTP in subsequent studies (Carnaby-Mann & Crary, 2010; Crary, Carnaby, Lagorio, & Carvajal, 2012); however, the exact skill-training protocol remains largely unknown.

Surface EMG for skill training in swallowing: The majority of skill-based trainings utilises sEMG to measure swallowing related muscle activity (Huckabee & Macrae, 2014). Although biofeedback has been used for more than two decades in the context of muscle strengthening in dysphagia rehabilitation (Bryant, 1991; Crary, 1995; Huckabee & Cannito, 1999), its use for skill-based training is more recent (Athukorala et al., 2014; Stepp et al., 2011). In contrast to intramuscular electrodes that assess muscle activity from a specific muscle, sEMG assesses surface recorded muscle activity of collective underlying muscles (Crary & Groher, 2000). Although intramuscular electrodes have better spatial and temporal resolution, invasiveness limits their clinical use as a biofeedback tool (Stepp, 2012). Therefore, surface electrodes have primarily been used to monitor muscle activation during swallowing with infrahyoid or submental placement of electrodes (Crary & Groher, 2000; Stepp, 2012). Given the anatomy of

head and neck consists of small overlapping muscle fibres, sEMG detects signals from all muscles underlying the area of the electrodes. For example, submental sEMG records activity from the anterior belly of digastric, mylohyoid and geniohyoid, with minimal input from genioglossus and platysma (Palmer, Luschei, Jaffe, & McCulloch, 1999).

Several studies investigated the relationship between submental muscles activation during swallowing and the displayed sEMG waveform. It was documented that effortful swallowing results in increased submental muscle activity, hence higher amplitude sEMG waveforms, compared to normal swallowing (Huckabee, Butler, Barclay, & Jit, 2005; Wheeler-Hegland, Rosenbek, & Sapienza, 2008). However, the amplitude of the sEMG signal simply reflects changes in neuro-electrical potentials occurring in the communication process between motor neuron and muscle, including the resulting muscle response, and not muscle strength (Kuriki et al., 2012). For example, the neuro-electrical exchange can be increased which equals a higher sEMG magnitude, if a large muscle response is needed; i.e. the muscle needs to contract (or relax) more (Kuriki et al., 2012). This principle also needs to be considered for the stroke population, who have difficulties to produce high EMG amplitudes during swallowing due to weakness or fatigue of the muscles (Azola et al., 2015). Although submental sEMG can be used to differentiate between normal and effortful swallowing, there was no (Wheeler-Hegland et al., 2008) or only a weak negative correlation (Huckabee et al., 2005) between the magnitude of submental sEMG and the magnitude of swallowing biomechanics and pharyngeal pressures, respectively. In other words, submental sEMG lacks specificity to draw conclusions about the pharyngeal phase of swallowing, such as the degree of hyolaryngeal excursion or pharyngeal contraction. The researchers of both studies acknowledged the main disadvantage of submental sEMG, which is the differentiation of muscles in the sEMG signal. Although submental sEMG is assumed to primarily measure the activity of the anterior belly of the digastrics, mylohyoid and geniohyoid, registration of activity from other muscles, e.g. hyoglossus and genioglossus, cannot be ruled out.

In contrast to sEMG magnitude, significant correlations were found for temporal measures of submental sEMG and swallowing biomechanics, such as for maximum hyoid displacement and maximum submental sEMG activity (Wheeler-Hegland et al., 2008). Consequently, the sEMG signal from the submental muscle group provides information about the timing and magnitude of muscle activation during swallowing. This information can be used as biofeedback tool for patients with dysphagia, to help them understand and control the timing and magnitude of their swallowing.

Combining skill-based training and sEMG biofeedback, Stepp et al. evaluated the feasibility of game-based therapy for dysphagia rehabilitation (Stepp et al., 2011). Six unimpaired participants and one patient with severe oropharyngeal dysphagia following brainstem stroke participated in this study. Surface EMG recordings were taken using a bilateral electrode placement on the anterior neck surface, measuring activations of the thyrohyoid, sternohyoid, and possibly omohyoid muscles (Stepp et al., 2011). The participants received real-time visual feedback of their neck muscle activity on a laptop computer screen placed in front of them. An object (big fish) moved vertically on the left side of the computer screen based on the magnitude of sEMG amplitude of the user. The object moved up during muscle contraction, and down when the muscles relaxed to a resting state. The participants were asked to control the big fish such that a target object (smaller fish), moving at constant velocity vertically from the right side of the screen to the left, is at the same height as the big fish at the right point in time (Figure 8).
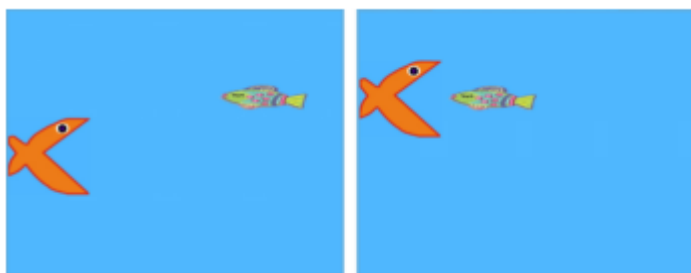
Figure 8. Screenshots of game-based swallowing therapy approach using sEMG biofeedback of the anterior neck musculature by Stepp et al. (2011).[9]

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[9] Reprinted, with permission from IEEE: Stepp, Britton, Chang, Merati, and Matsuoka. Feasibility of game-based electromyographic biofeedback for dysphagia rehabilitation, Copyright © 2011, IEEE.

The participants performed 10 trials, with seven small target fish presented during each trial. Breaks of 1 – 2 min between trials were mandatory and a slightly longer break was held after five trials. Successful trials were rewarded through visual feedback on the computer screen ("You ate the fish!") and verbal feedback by the investigator. The healthy volunteers, who took part in one session, acquired a higher average of 3.3 out of seven fish targets (STD = 0.9) compared to the participant with dysphagia [average of 0.9 targets (STD = 0.6)] during the first session. Throughout five subsequent sessions, the patient was able to double this result to 1.8 targets (STD = 0.4) in the final session. Following this intervention, the patient reported more sufficient management of secretions. Further qualitative clinical outcomes, such as faster initiation of voluntary laryngeal elevation, were noted by the therapist. Faster initiation of laryngeal elevation may indicate increased volitional control of the suprahyoid and thyrohyoid muscles, which are responsible for laryngeal elevation during swallowing. However, since active swallowing was not required to move the sEMG controlled object, further investigations how these findings translate to swallowing are required. All participants and especially the patient practised this game-based sEMG biofeedback approach without frustration and were highly motivated (Stepp et al., 2011). These motivational effects and potential benefits for swallowing require further investigation of this approach. Furthermore, its effects on swallowing using more objective measurements may be warranted in a larger sample.

Another skill-based training approach for swallowing is contained as a protocol in the Biofeedback in Strength and Skill Training (BiSSkiT[CE]) software developed by Huckabee and colleagues. In addition to skill training, a skill assessment and traditional strength training and assessment protocols are also incorporated in this software. In contrast to Stepp et al.'s approach, this skill training protocol uses the sEMG signal from the submental muscles during swallowing as biofeedback. For both skill-related settings, the software user is required to manipulate submental muscle activity during swallowing in order to place the peak of the continuous real-time sEMG waveform inside a visual target (Figure 9). Compared to the skill assessment function, where the same-sized target box is always in a fixed centred position, the skill training function uses a target box that varies in size and position on each screen. Thus, the user must control timing and magnitude of swallowing in a slightly different way each trial.
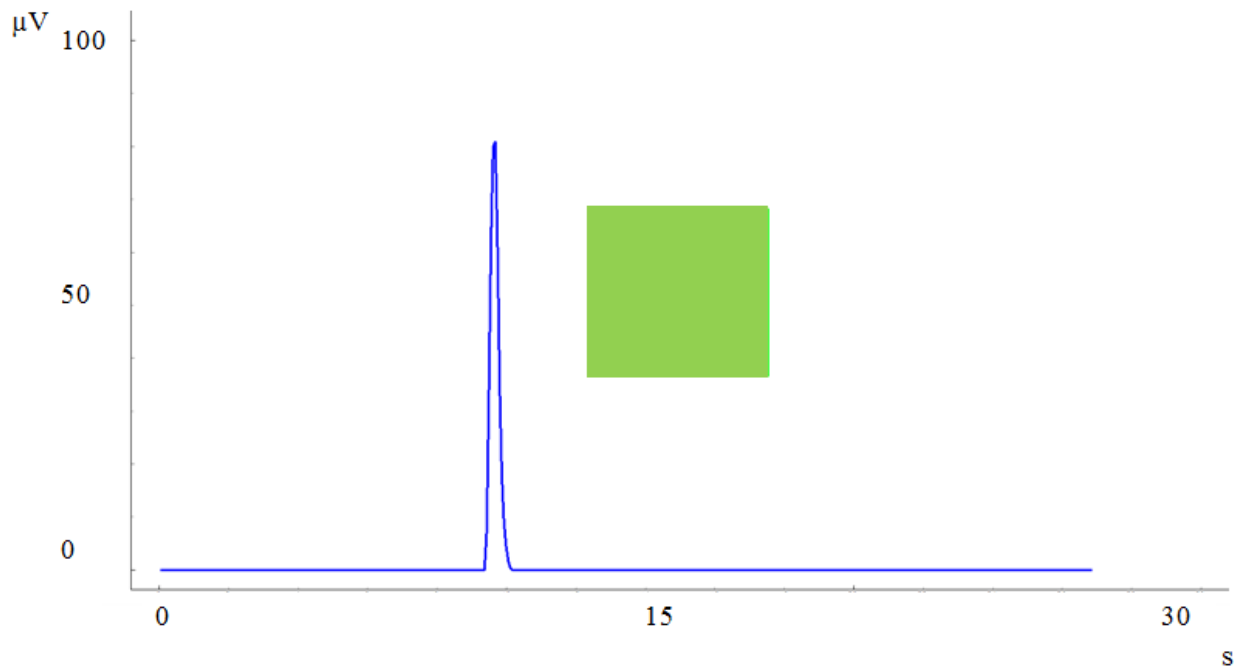
Figure 9. Screenshot of the one skill training trail on the Biofeedback in Strength and Skill Training (BiSSKiT^CE). The y-axis displays submental muscle activation during swallowing in microvolts (μV) and the x-axis time in seconds (s).

The skill training in BiSSKiT^CE is based on the principles of motor learning (Huckabee & Macrae, 2014) and neuroplasticity (Kleim & Jones, 2008). Huckabee and Macrae (2014) summarised and discussed three fundamentals of motor learning for swallowing rehabilitation, which are specificity of practice, task challenge, and feedback. Specificity of practice, which requires to exercise the motor behaviour that is desired to be improve or changed, is achieved by using swallowing as the training task. Many repetitions of a motor behaviour are required to achieve lasting neuroplastic changes (Kleim & Jones, 2008). The software is pre-set to 10 repetitions per trainings block. However, more or less repetitions can be required to achieve the best possible motor learning experience for the individual. The software allows the generation of individualised skill training protocols which can be adjusted based on the user's swallowing abilities. This is also the case for other features of the skill training in BiSSKiT^CE, such as the target size or different feedback options.

The second motor learning principle, task challenge, is achieved by using different positions of the target on the computer screen so the person performing the swallowing skill training is required to adapted the motor behaviour accordingly. In addition, the

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software is pre-set to automatically augment task complexity by changing the target size. For example, following three successful trials of placing the peak of the waveform inside the target box ("hit"), the size of the target decreases by 10% and vice versa, the target size increases following three consecutive misses. This was also designed to increase motivation and requires the individual to plan motor execution in advance. In other words, each trial requires execution of a new motor task, rather than memorizing and replaying the same pattern of muscle activation (Conditt, Gandolfo, & Mussa-Ivaldi, 1997). Neuroscience research also found that increased task variability lead to improved retention (Lee & Genovese, 1988; Shea & Kohl, 1991) and generalization of learning to new tasks (Catalano & Kleiner, 1984) when compared to practising the same task repetitively.

The last principle of motor learning, feedback, is achieved by the real-time presentation of the sEMG signal in relation to the target on the computer screen. In addition, the words "hit" or "miss" can be displayed next to the target or audio cues can be played following task completion to easily identify if the trial was successful or not.

Sella (2012) compared the effects of swallowing skill training and strength training using submental sEMG and the BiSSkiT[CE] software. Forty healthy volunteers were randomised to perform either the skill or strength training task. Participants completed the training over a two week period. The training consisted of one session a day on five workdays per week, consisting of 100 swallowing trials per session (five blocks with 20 trials each). To test the effects of the dosage of training, six of these participants volunteered for a pilot study, which involved an additional two weeks of training, i.e. four consecutive weeks of training. Participants were tested for biomechanical (pharyngeal pressures, hyoid displacement, submental muscle activity), structural (cross-sectional area of submental muscles) and neurophysiological (submental MEP magnitude) changes from pre to post training.

As hypothesised, they reported increased sEMG activity following strength training but not skill training in an effortful swallowing task. However, they did not identify any differences between skill and strength training in any other outcome measure. One reason for the lack of difference between the groups may have been that the data analysis was based on a small sample size and with inadequate statistical power due to

the division of participants into many subgroups (two vs four weeks, strength vs skill, and immediate and delayed feedback group within the skill condition). An effect in the assessment of neurophysiological parameters may have been missed since the assessment of change in M1 excitability was only performed unilaterally. Given that M1 is most likely involved in swallowing bilaterally (Malandraki et al., 2009), an assessment from both hemispheres might have allowed identification of possible changes. Another reason may have been that the two training-conditions were too similar. Although the strength training task focused on strengthening, this training still had a skill component to it through the use of biofeedback. This demonstrates the difficulty in differentiating between purely strength and skill tasks or outcome measures for swallowing. As the assessment of swallowing skill, the researchers used the accuracy of submental muscle contraction defined as increased target hit rate after ten or twenty practice sessions. Neither of the two skill training protocols (two or four weeks) resulted in significant changes of motor performance. This assessment of swallowing skill may have been inadequately sensitive in healthy individuals that are already swallowing at their maximum capacity, i.e. the outcome measure "hit rate" did not take the changes of the box size into account and stayed therefore stable at approximately 70% success rate. Although the participant might have gained accuracy in submental activation during swallowing, as measured by decreased target size, target hit rate was not suitable to detect this improvement. Refinement of the swallowing skill measure using this or similar skill training methods is necessary, especially when studying unimpaired swallowing mechanisms.

Athukorala et al. (2014) evaluated the skill training approach described by Sella (2012) in patients with dysphagia secondary to Parkinson's disease (PD) using more clinically oriented outcome measures. The researchers reported significant effects of the BiSSKiT^{CE} skill treatment in swallowing efficiency for liquids (assessed by the TWST) and timing parameters on sEMG such as pre-motor time, pre-swallow time, and duration of submental muscle contraction. Furthermore, improvements in quality of life were demonstrated using the SWAL-QOL. The results of this study demonstrated that this patient population was able to improve their functional and behavioural outcome measures of swallowing. Unfortunately, these findings are based on a small sample size (n = 10) within a heterogeneous patient population. Furthermore, no instrumental

swallowing assessment or neurophysiological documentation of change following swallowing skill training took place in this study.

In conclusion, exciting developments and early investigations of sEMG biofeedback for swallowing skill training have demonstrated the potential of this application for dysphagia rehabilitation in patients with neurological disorders (Athukorala et al., 2014; Stepp et al., 2011). However, improved methodologies are required, e.g. better differentiation between strength and skill components of swallowing and a refined measure of swallowing skill, particularly, when this approach is used to assess swallowing skill learning in participants with unimpaired swallowing function.

6. Cerebellum

The cerebellum is a crucial part for sensorimotor learning within a cerebro-cerebello-cortical loop system (Ito, 2000). The cerebellum monitors, corrects for errors and refines behaviours when learning or relearning a motor skill (Sokolov, Miall, & Ivry, 2017). In addition to motor functions, it is also involved in many tasks such as attention, language, cognition and executive function (Sokolov et al., 2017). Given the diverse functionality of the cerebellum and in particular the role in motor skill learning, it is an area of great interest for neurorehabilitation. The goal of neurorehabilitative interventions is to enhance its function or to influence the networks with other cortical or brainstem centres to facilitate motor recovery (Grimaldi, Argyropoulos, Boehringer, et al., 2014; van Dun et al., 2016). A detailed understanding of the neuroanatomical organisation of the cerebellum and its role in sensorimotor control and learning is required for the development of such interventions.

6.1 Neuroanatomy and physiology

The cerebellar cortex is located posterior of the brainstem and consists of two hemispheres which are separated by a midline portion called the vermis (Voogd & Glickstein, 1998; Figure 10). Functionally, it can be divided into three parts: the vestibulocerebellum, spinocerebellum and cerebrocerebellum (Purves et al., 2004). The phylogenetically most primitive structure of the cerebellum is the vestibulocerebellum (archicerebellum) (Purves et al., 2004). It consists of the flocculonodular lobe, which receives input mainly from the vestibular system, and the immediately adjacent part of the vermis and is important for posture, balance and vestibular reflexes (Purves et al., 2012). It is separated from the posterior lobe and the spinocerebellum (paleocerebellum) by the posterolateral fissure (Purves et al., 2004). This region is responsible for axial body and limb movements as it primarily receives inputs from the spinal cord (Purves et al., 2004). The anterior lobe or cerebrocerebellum (neocerebellum, pontocerebellum) makes up the biggest part of the cerebellum and consists of the lateral cerebellar hemispheres (Purves et al., 2004). It is separated from the spinocerebellum by the primary fissure and generates outputs to the premotor and

primary motor cortex (Purves et al., 2004). The cerebrocerebellum is highly involved in the coordination and planning of skilled movements (Purves et al., 2004).

The cerebellum receives vascular supply from the basilar artery and vertebral artery branching into three main cerebellar arteries: the superior cerebellar artery (SCA), the anterior inferior cerebellar artery (AICA) and the posterior inferior cerebellar artery (PICA), supplying the distal, middle and proximal cerebellum respectively (Tatu, Moulin, Bogousslavsky, & Duvernoy, 1996). Importantly, these arteries are also responsible for the blood supply to the medulla, pons and midbrain (Tatu et al., 1996). Hence, a disruption of blood supply in these arteries not only affects the cerebellum, but also important brain regions for swallowing, such as the nucleus tractus solitary or the trigeminal nucleus (Tatu et al., 1996).
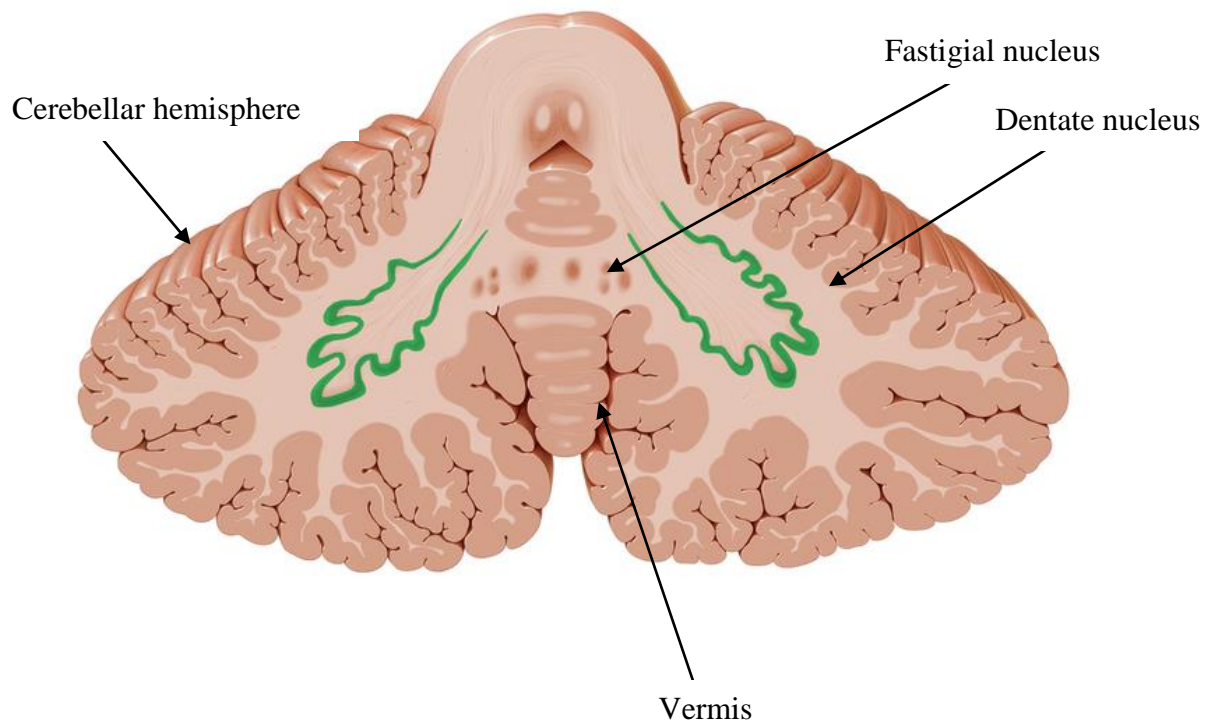
Figure 10. Anatomy of the cerebellum with the dentate nucleus marked in green.[10]

The cerebellar cortex is made up of an internal core of white matter surrounded grey matter (Perrini, Tiezzi, Castagna, & Vannozzi, 2013). Several pairs of intrinsic nuclei, such as the fastigial, interposed and dentate nuclei are situated within the white matter of the cerebellar cortex (Perrini et al., 2013). The dentate nuclei are the largest of these

nuclei. The grey matter can neuroanatomically be divided into three layers which are named after the neurons that have their cell bodies in that particular layer (Apps & Garwicz, 2005; Figure 11). The innermost layer is the granule cell layer, followed by the Purkinje cell layer and the molecular layer on the surface (Apps & Garwicz, 2005). Granule cells receive excitatory input from various sources, such as the pontine nuclei or the spinal cord via glutamatergic (excitatory) mossy fibres (Apps & Garwicz, 2005). Furthermore, they receive inhibitory input from other interneurons, mainly Golgi cells (Purves et al., 2004). The axons of the granule cells rise up into the molecular layer where they run horizontally as parallel fibres and synapse onto all other cell types within the molecular layer, including Purkinje cells (Jörntell & Ekerot, 2002).

Purkinje cells have their cell bodies located in the Purkinje cell layer and are GABA-ergic cells (inhibitory). In addition to the excitatory input from the parallel fibres, they also receive excitatory input from the inferior olive via climbing fibres (Apps & Garwicz, 2005). On the other hand, Purkinje cells receive inhibitory input from two interneurons: stellate cells and basket cells (Linas, Walton, & Lang, 2004). In an internal cerebellar loop system, input of all excitatory and inhibitory fibres influence the activity of the Purkinje cells. Purkinje cells compare and weigh the signals of the different inputs and then influence the internal loop system to minimise errors via plasticity in their synapses (Ito, 2001). Most importantly, Purkinje cells are the only output source of the cerebellum (Bostan, Dum, & Strick, 2013). The inhibitory nature of the Purkinje cells is a main factor in this loop system, as it is able to modulate deep cerebellar nuclei output, primarily of the dentate nuclei. Projections from dorsal proportions of these nuclei are closely associated with motor functional domains (Strick, Dum, & Fiez, 2009). Purkinje cells have, therefore, profound influence on how much output leaves the cerebellum, i.e. how much the cerebellum is involved in the error correction process of a movement.
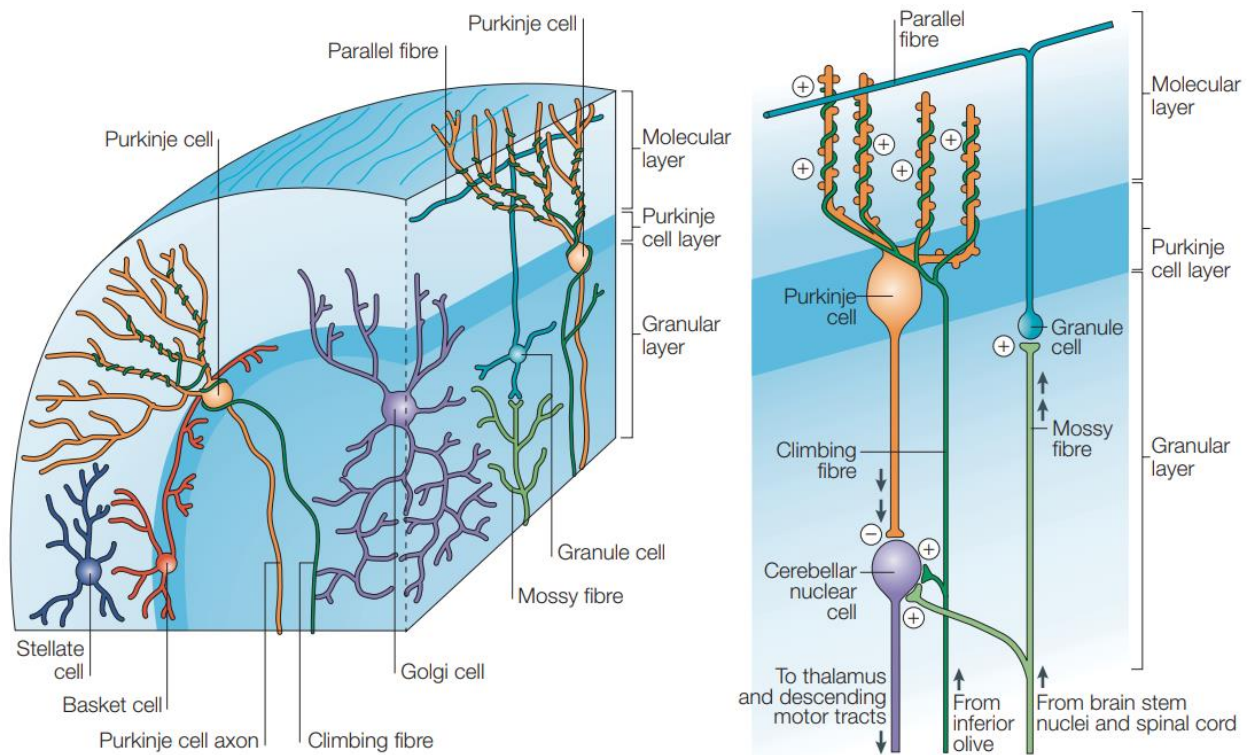
Figure 11. Basic structure and cell types of the cerebellar cortex. Excitatory (+) and inhibitory (-) connections are indicated.[11]

The cerebellum is structurally connected to the pons through three pairs of cerebellar peduncles (inferior, middle and superior) (Perrini et al., 2013). All afferent and efferent cerebellar fibres travel through these three bundles and allow information flow between the cerebellum and the regions of the CNS including motor cortex, spinal cord, thalamus and brainstem (Perrini et al., 2013). Almost all efferent fibres that originate from the dentate nuclei travel through the superior peduncle and transport information mainly to the red nucleus, the thalamus and the cerebral cortex (Sakai, 2013). Most of the fibres traveling through the inferior and middle peduncle are afferent and mediate sensorimotor information to the cerebellum (Perrini et al., 2013). Specifically, afferent projections of pontine cells travel through the middle cerebellar peduncle, whereas afferent and some efferent fibres arising from the posterior medulla travel through the inferior cerebellar peduncle (Glickstein & Doron, 2008).

For sensory-motor feedback of limb functions, afferent cerebellar fibres decussate near the junction of the pons and the midbrain before entering the brainstem and travel via the thalamus in the superior direction towards the contralateral M1 and the premotor cortex (cerebello-thalamo-cortical pathway) (Purves et al., 2004; Figure 12). The output from the cerebellum can then influence the output of these motor and sensory areas (Purves et al., 2004). In turn, efferent fibres from sensory and motor cortical regions synapse to pontine nuclei on the ipsilateral side of origin, and cross midline before entering the cerebellum via the middle cerebellar peduncle (Purves et al., 2012). Therefore, both types of input converge in the same cerebellar hemisphere that is representing the ipsilateral side of the body (Steward, 2000). A third source of cerebellar input comes from cortical descending pathways via the red nucleus and the inferior olive that is located in the medulla (cortico-rubro-olivo-cerebellar pathway) (Allen & Tsukahara, 1974). The red nucleus provides additional input on voluntary movement coordination received from the rubrospinal tract (Kawato, Furukawa, & Suzuki, 1987). However, the role of the rubrospinal tract in human motor control is less pronounced than the role of the corticospinal tract.
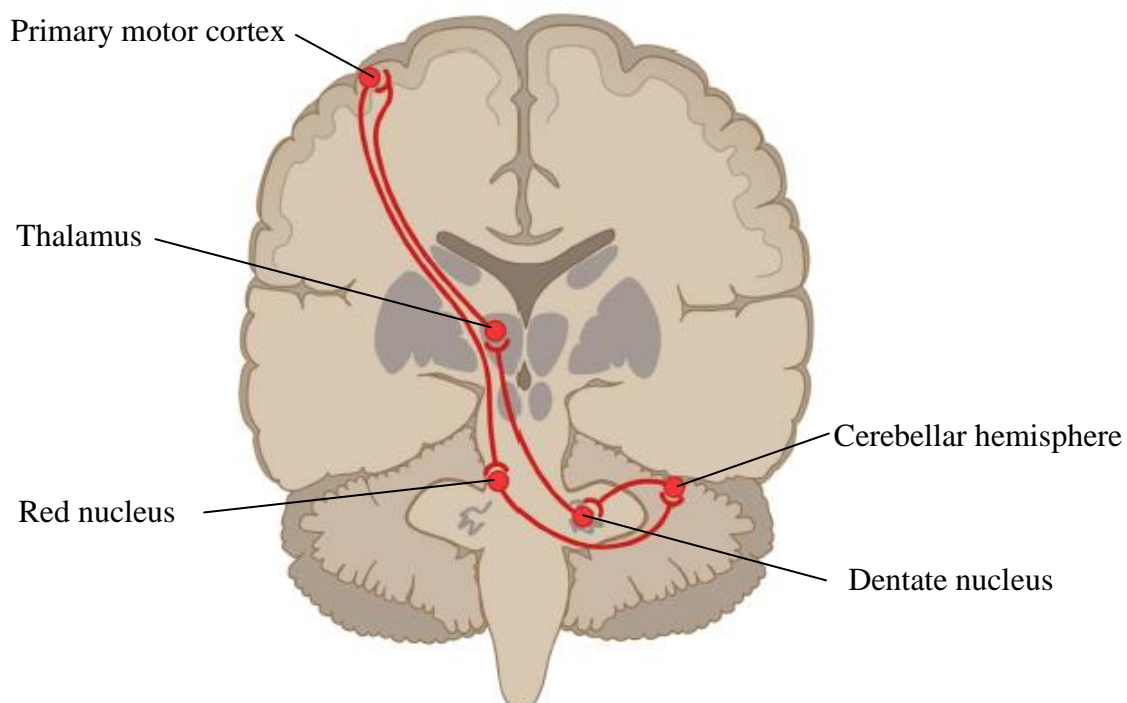
Figure 12. Cerebrocerebellar circuit.[12]

The majority of information on cerebellar-cortical connections has been derived from the corticospinal system in animals and humans. Only more recently, resting-state functional connective MRI (fcMRI) was used identify activations and functional connections within the cerebro-cerebellar network during different types of movements - including tongue movements as a motor task of the corticobulbar system, in addition to hand and foot movements (Buckner, Krienen, Castellanos, Diaz, & Yeo, 2011). Both the cerebral and cerebellar cortex had somatotopic organisations of motor representations for all three conditions that are functionally connected through contralateral cerebellar-cortical coupling in 26 young healthy adults (Buckner et al., 2011; Figure 13). In contrast to ipsilateral representation of unilateral hand and foot movements, the areas of motor representation for the tongue in the somatotopic cerebellar map was bilateral (Buckner et al., 2011). Furthermore, the motor representation of the tongue in the cerebellar cortex were the furthest posteriorly oriented and may therefore be particularly receptive to external noninvasive brain stimuli. If this bilateral and contralateral representation of volitionally controlled oral motor behaviour also translates to swallowing remains further investigation.
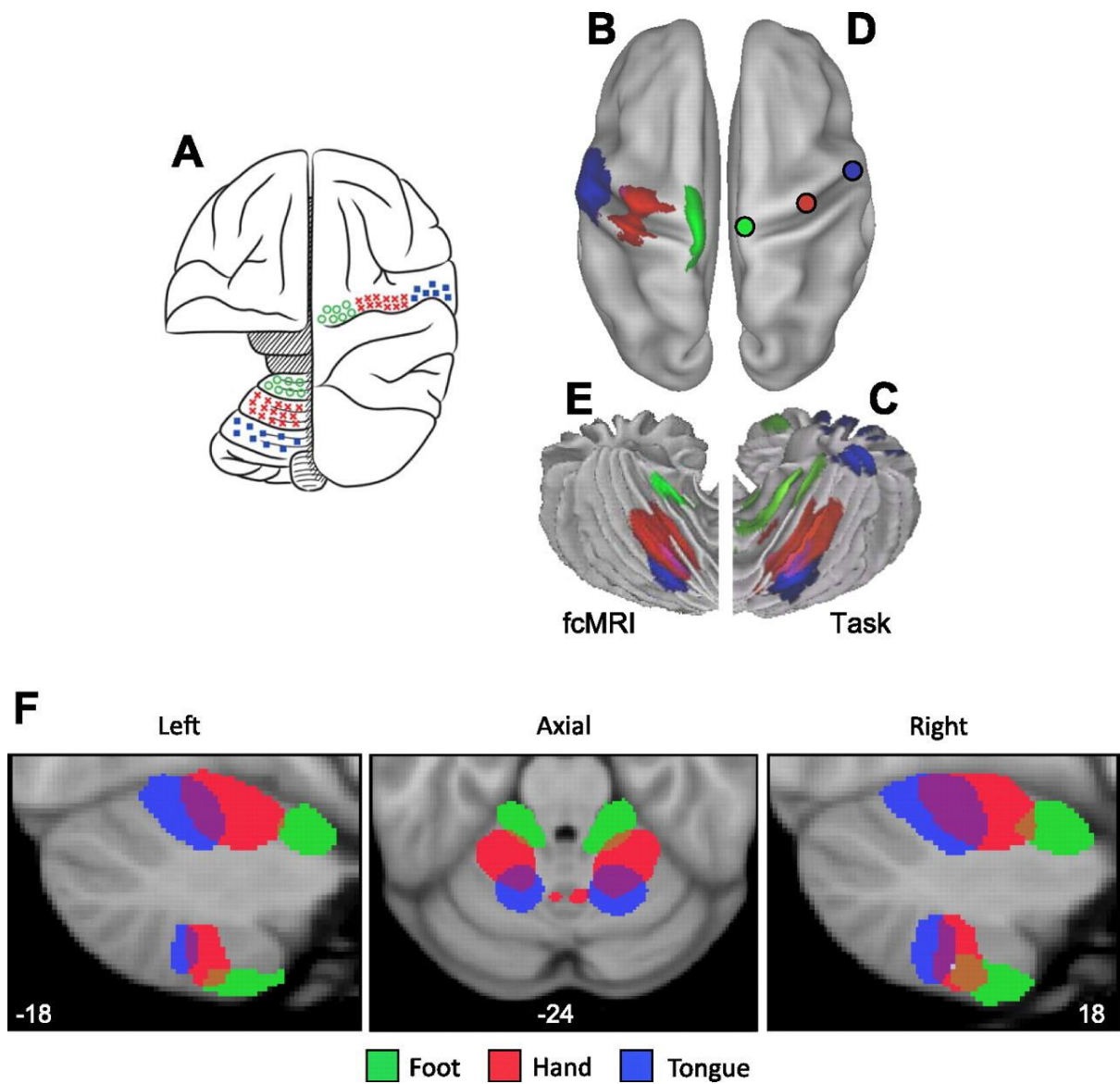
Figure 13. Functional connectivity (A) and activation patterns of the primary motor cortex (B, D) and the cerebellum (E,C,F) for three movement conditions: foot = green, hand = red, tongue = blue.[13]

[13] Reprinted with permission of The American Physiological Society: Journal of Neurophysiology 106(5), 2322-2345, Buckner, Krienen, Castellanos, Diaz, and Yeo, 2011, The organization of the human cerebellum estimated by intrinsic functional connectivity. Copyright © 2011.

6.2 Role of the cerebellum in sensorimotor learning

The cerebellum is primarily involved in motor learning and error correction of movements (De Zeeuw & Ten Brinke, 2015). Particularly, it modulates information about movement direction, timing, sequencing and force, and stays in permanent exchange with the motor cortex (Hikosaka et al., 1999; Keele & Ivry, 1990; Thach, 1992). It does not generate skilled movements, rather integrates sensory input from the spinal cord, the vestibular system and the sensory cortex with executive motor commands from M1 and the premotor cortex (Wolpert, Miall, & Kawato, 1998). In other words, it compares the plans for movement execution with the information received from the executed movement and makes adjustments accordingly (Blakemore, Frith, & Wolpert, 2001; Jörntell, 2016).

Several neuroplastic changes that are associated with sensorimotor learning take place within the cerebellum and influence this cerebellar-cortical loop system. For example, LTP-like mechanisms have been identified at mossy fibre and granule cell synapses (D'Angelo et al., 2005) as well as LTD-like mechanisms at the synapses between parallel fibres and Purkinje cells (Ito, 2002). Specifically for LTD-like mechanisms, it has been found that the release of glutamate from presynaptic terminals of parallel fibres interact with glutamate receptors in the dendritic spine of the Purkinje cells (Jörntell, Bengtsson, Schonewille, & De Zeeuw, 2010). A chain of chemical processes within the Purkinje cell dendrites results in the release of Ca^{2+} from intracellular stores (Jörntell et al., 2010). In addition to this, the depolarising effects of climbing fibre input leads to an activation of Ca^{2+}-channels within the membrane of the Purkinje cell dendrites (Barbour, Brunel, Hakim, & Nadal, 2007). This additional Ca^{2+} is pumped into the Purkinje cell postsynaptic spine. The increase in Ca^{2+} results in further internal chemical processes before evoking LTD at the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptors) (Soler-Llavina & Sabatini, 2006). This LTD results in a reduction in synaptic strength. The mechanisms of cerebellar plasticity are significantly different from neuroplasticity effects within the cerebral cortex, where high Ca^{2+} leads to an inversed effect of LTP (Bear & Malenka, 1994).

Neuroplastic changes in response to motor skill learning in the cerebellum have also been assessed via neuroimaging or NIBS techniques and provide further insights into cerebellar mechanisms. In an fMRI study on a small sample of eight healthy volunteers, cerebellar activity was absent during imagined motor processes (lack of sensory input), but present, when actual hand movements were executed (Nair, Purcott, Fuchs, Steinberg, & Kelso, 2003). The results of this study emphasise the importance of active execution of the task that generates sufficient sensory input to activate cerebellar-cortical feedback mechanisms. Using a paired-pulse TMS paradigm, Schlerf, Galea, Bastian, and Celnik (2012) found increased cerebellar activity associated with an increase in sensorimotor errors during the learning process of an unknown visuomotor perturbation task; i.e. the more complex the task, the more active the cerebellum. Furthermore, it was proposed that stronger output of the cerebellum, i.e. stronger inhibition, signifies increased motor learning (Schlerf et al., 2012). No increase was found during a gradual perturbation in the visuomotor condition, suggesting that only sudden unexpected errors require cerebellar involvement (Schlerf et al., 2012) . These results also support earlier findings that suggested that the cerebellum is highly involved in the early phases of motor adaptation, where larger errors are occurring but are quickly decreasing (Galea, Vazquez, Pasricha, de Xivry, & Celnik, 2011; Jayaram et al., 2012). However, in contrast to research findings from the lower limb perturbation tasks (Jayaram, Galea, Bastian, & Celnik, 2011), Schlerf and colleagues did not identify any relationship between excitability changes in the cerebellum and the amount of learning that occurred. A possible explanation for this may be the difference in the neural networks between upper and lower limb functioning, where errors in locomotor functioning result in more severe consequence (e.g. falls) (Schlerf et al., 2012). Hence, they get more attention within the CNS which may be reflected in the correlation between cerebellar excitability and behavioural outcomes.

The cerebellum is also involved when motor behaviours are relearned after stroke. Movements of a paretic limb, for example, results in substantial discrepancies between predicted and actual performance. Similar to motor learning processes of an unknown or perturbed task in healthy individuals, the cerebellum detects the movement error and optimises subsequent sensorimotor accuracy (Blakemore et al., 2001). The role of the cerebellum in post-stroke motor recovery has been confirmed by several longitudinal fMRI studies on cortical (Small, Hlustik, Noll, Genovese, & Solodkin, 2002) and

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subcortical stroke patients (Ward, Brown, Thompson, & Frackowiak, 2003). Both studies monitored changes in neural correlates in longitudinal fMRI studies over a timeframe of six-month post stroke and found a significant increase in cerebellar activity during this time. The cerebellum is particularly active two to three-month post-stroke and in patients that demonstrated a good behavioural motor recovery compared to poor recovery (Small et al., 2002). Unfortunately, the separation into good and poor motor recovery groups was not intended a priori which may have led to possible bias in the analysis of these results.

In conclusion, the cerebellum is critically involved in sensorimotor learning in the healthy and neurologically impaired system. Neuroplasticity within the cerebellum and the cerebellar-cortical networks has been identified, demonstrating LTD and LTP-like changes throughout the motor learning process. However, most of the research on cerebellar function in sensorimotor learning has been performed on voluntary distal limb movements. The demonstrated capacity of the cerebellum in coordination of limb movements and its involvement in motor learning processes may apply to corticobulbar-related motor function, such as swallowing. However, this has not been specifically investigated. The accessible position of the cerebellum and sensitivity to changes in the magnetic and electric field produced by NIBS, make it a site of particular interest for neurorehabilitation in motor recovery (Priori, Ciocca, Parazzini, Vergari, & Ferrucci, 2014).

7. Non-invasive brain stimulation

NIBS is the superordinate term for techniques that can induce and examine changes in neural excitability using external (non-invasive) magnetic or electrical stimuli (Liew, Santarnecchi, Buch, & Cohen, 2014). TMS and tDCS, are two NIBS techniques that have gained growing interest for neurorehabilitation over the past decade (Liew et al., 2014). While single or paired-pulse TMS is mainly used to test physiological changes in cortical excitability, repetitive TMS (rTMS) can be used as neuromodulatory technique in association with neurorehabilitative treatments, similar to tDCS (Liew et al., 2014). Both rTMS and tDCS modulate neural activity below the stimulation area and can secondarily influence interconnected neural networks (Liew et al., 2014). Emerging research in stroke neurorehabilitation uses NIBS to target brain regions, such as the M1 and the cerebellum that are involved in motor planning, motor execution and motor learning, to facilitate neuroplastic changes and hence motor recovery (Hummel & Cohen, 2006; Liew et al., 2014; Sandrini & Cohen, 2013). It is essential for researchers and clinicians to understand the underlying neurophysiological principles that support the development of these techniques.

7.1 Neurophysiological basis

Neurons are important components of the nervous tissue in the CNS and are responsible for cognitive processes, motor movements and sensory perception (Tresilian, 2012). These different functions are served by different types of neurons. Motor neurons, for example, are responsible for the efferent transport of motor information from the CNS, whereas sensory neurons transport afferent sensory information to the CNS (Tresilian, 2012). Lastly, interneurons are responsible for transmitting information between neurons (Tresilian, 2012). In addition, neurons have different polarities. For example, the majority neurons in the cerebral cortex are excitatory glutamatergic neurons, and others are inhibitory neurons, which are GABA-ergic (gamma-aminobutyric acid) (Sakakibara & Hatanaka, 2015). Typically, neurons have several dendrites to receive signals from other neurons and a single axon to transmit information to other neurons (Sakakibara & Hatanaka, 2015). The number of dendrites and axons can also be used to

classify neurons. Figure 14 illustrates three different types of neurons: unipolar, bipolar or multipolar. Motor neurons arising from the motor cortex and Purkinje cells in the cerebellum are considered as multipolar neurons (Tresilian, 2012). The vast amount of dendrites make the Purkinje cells, in particular, one of the most complex neurons in the human brain (Tresilian, 2012). Different stimulation parameters need to be considered to target different types of cell morphology with NIBS, e.g. protocols targeting neurons in the motor cortex may use different parameters than protocols targeting neurons within the cerebellum or vice versa (Rahman, Toshev, & Bikson, 2014).
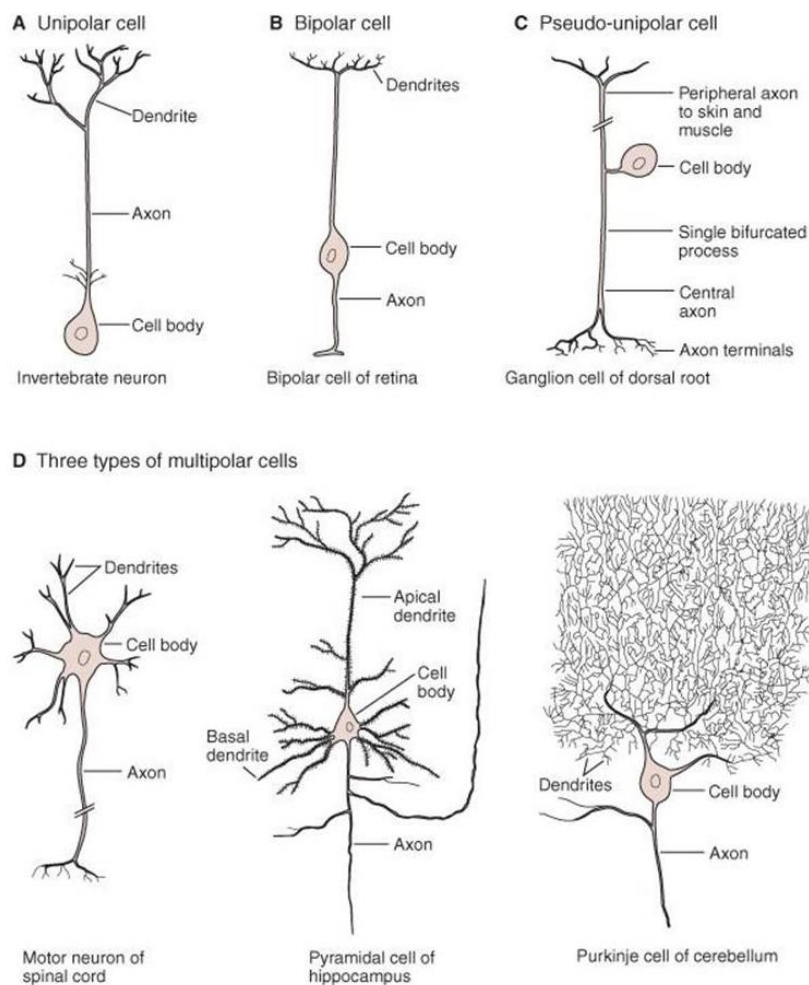
Figure 14. Illustration of the different neuron types in the human brain; including unipolar, bipolar, pseudo-unipolar, multipolar neurons.[14]

Neurons communicate with each other through electric impulses (Platkiewicz & Brette, 2010). Even when a neuron is not receiving or transmitting, intracellular measures reveal an electrical potential difference across the plasma membrane, so-called a resting membrane potential (Lodish et al., 2000). Neural membrane potentials are typically negative, usually around -65 mV (Purves et al., 2004). This polarisation results from an ionic imbalance between the inside and outside of a neuron (Purves et al., 2012). There are more positive charged potassium ions (K^+) on the inside, whereas the outside fluid contains an excess of sodium ions (Na^+) (Purves et al., 2004). The concentration of these two types of ions would create a more or less balanced transmembrane potential. However, the additional existence of negatively charged proteins and nucleic acid molecules contribute to the negative membrane potential (Purves et al., 2004). In contrast to the positively charged ions Na^+ and K^+, negatively charged ions cannot permeate the membrane (Purves et al., 2004). Therefore, unlike the positive ions, they do not follow electrical or chemical gradient differences (Purves et al., 2004). An inactive neuron is therefore polarized and remains in this state until an incoming stimulus alters ionic concentrations (Purves et al., 2004).

With arrival of an incoming stimulus, ion channels within the membrane open up and allow Na^+ to diffuse to the inside of the cell, which changes the membrane potential from polarised to depolarised (Purves et al., 2004). A high-intensity stimulus will cause more Na^+ channels to open up until the transmembrane depolarisation reaches a threshold level of around -50 mV (Purves et al., 2004). At this point, the neuron responds in an all-or-nothing manner: if the stimulus is strong enough, complete depolarization occurs and elicits an action potential (Purves et al., 2004). Different neurons have different firing thresholds that are determined by a variety of factors, such as changes in temperature, acidity or glucose levels (Mitry, McCarthy, Kopell, & Wechselberger, 2013). The depolarisation phase of the neuron is enhanced by the opening of K^+ channels until it reaches its maximum at around +50 mV (Purves et al., 2004). At its peak, Na^+ channels start to close again, launching the repolarisation phase (Purves et al., 2004). The repolarisation continues until it exceeds the resting membrane potential which initiates the K^+ channels to close as well (Purves et al., 2004). Figure 15 illustrates changes in the membrane potential over time. Interestingly, the magnitude of the action potential always stays the same despite an increased intensity (Purves et al., 2004). The phase following an action potential is characterised by hyperpolarisation and

is also called refractory period (Platkiewicz & Brette, 2010). It describes the time during which the membrane potential returns back to its polarised resting state. The neuron is not receptive to incoming stimuli during this period of time (Purves et al., 2004).
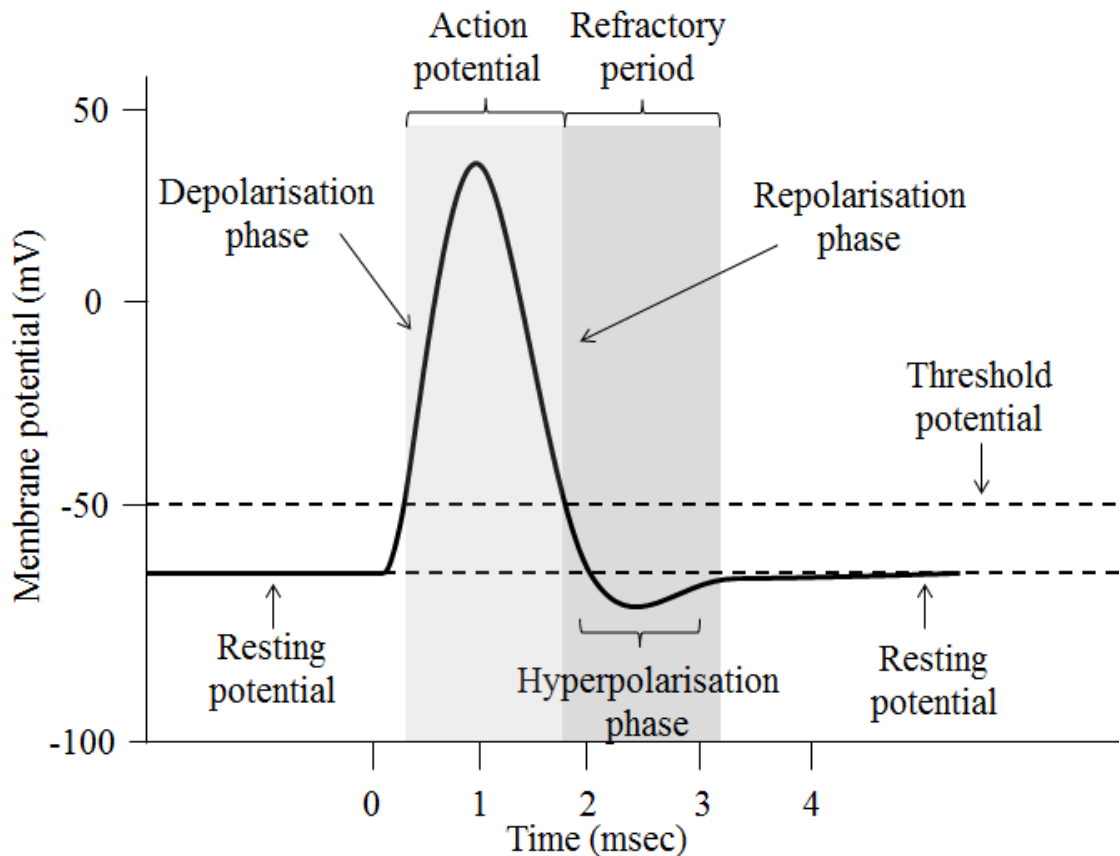
Figure 15. Illustration of temporal changes in the membrane potential, including neural membrane resting states before and after an action potential.

NIBS can be used to alter the neural membrane potential and consequently neural or cortical excitability (Woods et al., 2016). The stimulus intensity, the direction of the current flow and the focality of the stimulus determine if an action potential will be elicited or not (Woods et al., 2016). Different stimulation techniques have different effects on cortical excitability. They can be used to test and/or manipulate neural activation which provides opportunities to promote neuroplasticity and to develop and evaluate treatment approaches for motor recovery after stroke. Basic mechanisms, application and safety criteria of NIBS including TMS, rTMS and tDCS need to be understood to make the best use of their capacities for objective evaluation or modulation of the motor system for rehabilitation.

7.2 Transcranial magnetic stimulation (TMS)

TMS was first demonstrated in 1985 by Barker and colleagues (Barker, Jalinous, & Freeston, 1985) and has since been used to stimulate both the CNS and peripheral nerves (Rossini et al., 2015). TMS delivered over the primary motor cortex is most commonly used for neurophysiological assessments, such as functional brain mapping, to investigate interhemispheric connectivity or document changes in neural excitability before and after treatment or neuromodulatory interventions (Griskova, Höppner, Ruksenas, & Dapsys, 2006; Kobayashi & Pascual-Leone, 2003). It uses a focal high-intensity stimuli to produce a discharge of action potentials (Woods et al., 2016). However, TMS can also be used therapeutically when low-intensity repetitive stimuli pulses are applied to the motor cortex to modulate transmembrane neural potentials, so-called rTMS (Rossini & Rossi, 2007). In contrast to TMS, rTMS has the potential to evoke longer-lasting changes in the brain and has, therefore, become an emerging treatment approach (Rossini & Rossi, 2007). Although these two approaches are used differently and are targeting different outcomes, they share the basic mechanism of electromagnetic induction.

TMS uses the bidirectional transformation between electricity and magnetism, following two physical laws. Ampere's law, which describes that induction of an electric field subsequently induces a magnetic field (Griskova et al., 2006). Faraday's law states that a time-varying magnetic field can induce an electric field and current flow in a nearby conducting material (Daskalakis, Christensen, Fitzgerald, & Chen, 2002). Electrical pulses are generated inside the stimulator device and sent through the cable into a coil. The electric current that flows inside the coil produces a magnetic field (Figure 16). Magnetic impulses of TMS first penetrate largely non-conductive material, such as the scalp and skull, and subsequently induce an electric current in conductive material – the neurons beneath the coil (Griskova et al., 2006). When a magnetic impulse is discharged over the brain region of interest, it induces an electrical potential which then leads to neuronal depolarization and subsequently to the generation of action potentials (Di Lazzaro et al., 2004). The efferent volley, evoked by the delivered TMS impulse over the M1, travels along the corticospinal or corticobulbar pathway and can be measured as a MEP with EMG at the muscle or muscle group of interest (Macrae, Jones, et al., 2014; Rossini & Rossi, 2007). A variety of measures can be

taken from this recording, such as latency between the cortical stimulus and MEP response, the size of the MEP amplitude itself (peak-to-peak) or the cortical silent period following the MEP response (Ridding & Rothwell, 2007; Rossini & Rossi, 2007; Figure 17).
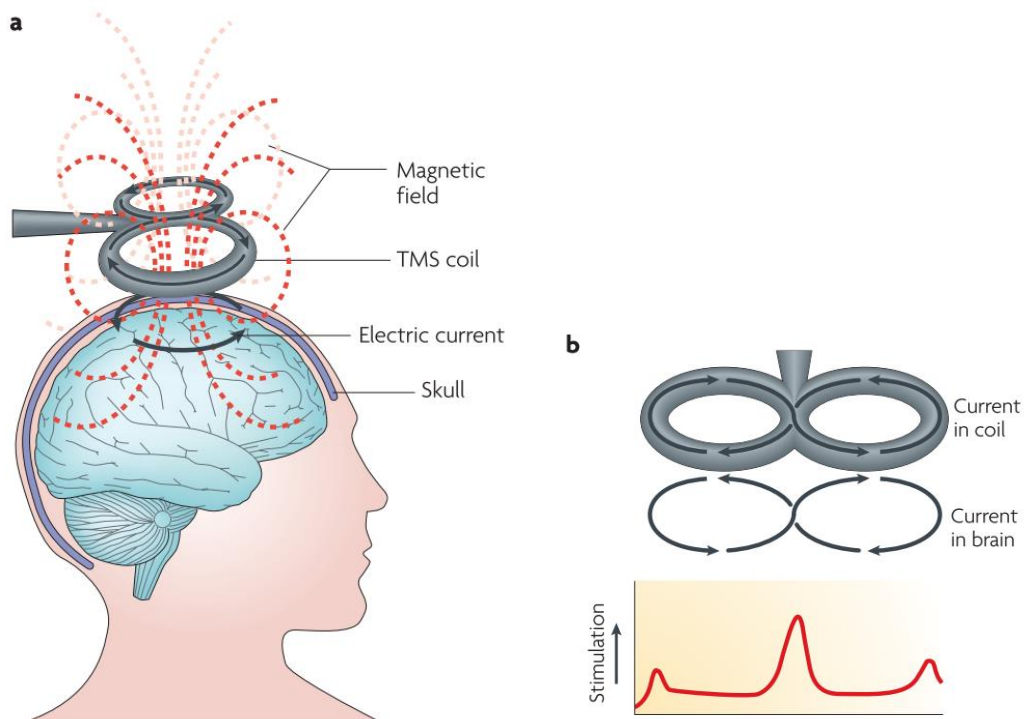
Figure 16. Picture a: Transcranial magnetic stimulation of the cortex with a figure-of-eight-coil. Picture b: Direction of current flow within the figure-of-eight coil in relation to the current flow in the opposite direction within the brain. The graph below displays the distribution of the stimulation intensity.[15]

The most commonly used measure of motor cortical excitability is the MEP amplitude recorded from the targeted muscle or muscles group (Ebmeier & Lappin, 2001). Therefore, this measure reflects the excitability along the entire pathway from cortex to muscle of interest. MEPs are influenced by a variety of internal and external factors. Internal factors include age, genetics, brain state, state of muscle activity, or anatomical features such as cranial or brain anatomy (Ebmeier & Lappin, 2001; Ridding & Ziemann, 2010). One method to account for some of these differences is to determine

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[15] Reprinted by permission from Springer Nature: Nature Reviews Neuroscience, 8(7), 559, Is there a future for therapeutic use of transcranial magnetic stimulation? Ridding, M. C., & Rothwell, J. C., Copyright (2007).

the individual motor threshold, which has been defined as the lowest stimulation intensity needed to evoke (five or more) stable MEP responses with an amplitude of 50 µV or more (McConnell et al., 2001; Pascual-Leone et al., 1995; Temesi, Gruet, Rupp, Verges, & Millet, 2014). This method was proven to provide reliable measures for the hand muscle (Pridmore, Fernandes Filho, Nahas, Liberatos, & George, 1998; Stokes et al., 2007).

Two different types of motor threshold exist: the resting motor threshold and the active motor threshold (Groppa et al., 2012). The determination of the resting motor threshold takes place with the targeted muscles relaxed (Groppa et al., 2012). The active motor threshold describes a measure where the muscle or muscle group is voluntary contracted and therefore preactivated during the stimulation (Groppa et al., 2012). Less stimulation intensity is needed when the muscle is preactivated (Groppa et al., 2012). The assessment of the motor threshold in the corticospinal system can be performed at a safe and tolerable stimulus intensity for both the resting and active motor threshold (Temesi et al., 2014). In contrast, resting motor thresholds from the orofacial musculature cannot always be found with a comfortable stimulation intensity, therefore requiring active motor thresholds in the assessment of these muscles (Doeltgen, 2010). Nevertheless, the motor threshold is commonly used as an intrinsic calibration value for the stimulus strength, which is essential in experiments comparing the effects of stimulation between subjects and for the safety of the participant (Groppa et al., 2012; Temesi et al., 2014).

In addition to participant related internal factors, MEP responses can be influenced by external factors such as coil shape, coil position, current orientation and stimulus intensity (Klomjai, Katz, & Lackmy-Vallee, 2015; Rossini & Rossi, 2007). These parameters determine the strength of the stimulation and therefore the type of tissue that is being stimulated (Daskalakis et al., 2002). The strength of the electrical current in the coil is usually $5-10$ kA and the induced magnetic field strength of $1-2.5$ Tesla (Ebmeier & Lappin, 2001; Groppa et al., 2012). Most commonly, figure-of-eight coils are used for the stimulation of cortical motor representations (Daskalakis et al., 2002). In this type of coil, currents flow in the opposite direction within each wing and converge at the centre point where the two coil circuits meet to produce a focal magnetic field (Daskalakis et al., 2002; Figure 16). Despite the fact that circular coils

are more powerful, the focality of figure-of-eight coils results in better spatial specificity of activation (Deng, Lisanby, & Peterchev, 2013). An increase in field strength would theoretically allow the stimulation of deeper brain tissue layers of the targeted area but come with the risk of seizure induction through overstimulation of the superficial cell layers (Ebmeier & Lappin, 2001). Alterations in coil orientation change the direction in which the impulse is sent into the neural structures. Fibres that lie parallel to the stimulating coil are more easily activated than perpendicularly oriented fibres (Ebmeier & Lappin, 2001). The coil is therefore held at an rotated angle of 45° degrees clockwise to the parasagittal plane for the stimulation of the motor cortex (Mills, Boniface, & Schubert, 1992).

TMS can be used for other externally accessible brain regions, such as the cerebellum (Grimaldi, Argyropoulos, Boehringer, et al., 2014). The coil orientation and stimulus intensity must be adjusted accordingly. It is important to keep the coil orientation constant as small changes can influence the size of the MEP amplitude (Mills et al., 1992). This has only been tested for cortical stimulation but is likely also relevant for the stimulation of other brain regions.
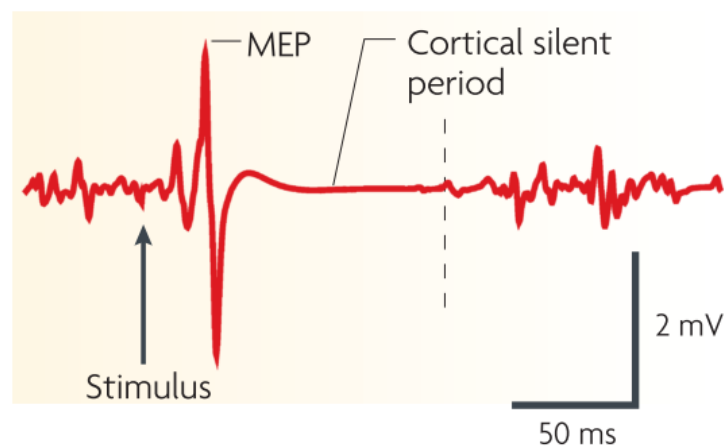
Figure 17. Representation of a motor evoked potential (MEP) as a response to single-pulse transcranial magnetic stimulation (TMS). Marked are the time point of the delivered TMS stimulus, the MEP response itself and the cortical silent period following the MEP. The end of the cortical silent period is marked by a dashed line.[16]

TMS can be applied using different frequencies of magnetic impulses: single-pulsed, paired-pulsed (also known as double-pulsed), repetitively pulsed TMS (repetitive TMS; rTMS) or theta burst stimulation (TBS) (Rossini & Rossi, 2007). Conventional single pulse TMS involves the delivery of one magnetic impulse to the cortical area of interest (Goss, Hoffman, & Clark, 2012). Recording of five to six consecutive MEPs are recommended for the assessment of excitability of the neuromuscular system (Groppa et al., 2012). In the corticospinal system, MEPs from the muscles are measured contralateral to the applied stimulus over the cortex (Barker, Jalinous, & Freeston, 1985). In contrast, MEPs assessed for swallowing are primarily measured from midline or bilaterally organised structures, such as the submental (Hamdy et al., 1996; Plowman-Prine, Triggs, Malcolm, & Rosenbek, 2008), pharyngeal (Ertekin et al., 2001; Hamdy et al., 1996, 1997; Michou et al., 2012; Michou, Mistry, Jefferson, Tyrrell, & Hamdy, 2014; Michou, Mistry, Rothwell, & Hamdy, 2013; Plowman-Prine et al., 2008) or osophageal muscles (Ertekin et al., 2001; Fraser et al., 2003; Hamdy et al., 1996; Khedr, Abo-Elfetoh, & Rothwell, 2009). In contrast to MEPs from the corticospinal system, the smaller size and overlapping positioning of the muscles for swallowing makes this measure less muscle specific. Furthermore, due to the assumed hemispheric lateralisation in swallowing neural control, single-pulse TMS is initially used to identify the dominant hemisphere, which subsequently serves as the side for further assessments (Hamdy et al., 1996; Mistry et al., 2007). In addition, subcortical structures relevant for swallowing neural control can be assessed with single-pulse TMS, such as the contributions of the cerebellum in swallowing (Jayasekeran et al., 2011).

Paired-pulse TMS is a variation of single-pulse TMS and uses two single TMS pulses in quick succession. It is mainly used to study intracortical excitability changes where the two stimuli in close sequence (typically < 50 ms) are applied through the same stimulation coil over the same cortical region (Maeda & Pascual-Leone, 2003). The priming pulse is applied at subthreshold, whereas the second pulse is applied at suprathreshold and will subsequently result in a MEP response in the target muscles (Ridding, Inzelberg, & Rothwell, 1995). Paired-pulse TMS with an interstimulus interval (ISI) of less than 5 ms can produce short-interval intracortical inhibition (SICI), mediated by the gamma-aminobutyric acid A receptor ($GABA_AR$) (Kujirai et al., 1993). In contrast, increasing the gap between the two pulses to greater than 8 ms and less than 30 ms can produce intracortical facilitation (ICF) which is likely mediated by N-

methyl-D-aspartate (NMDA) receptors (Ziemann, Chen, Cohen, & Hallett, 1998). However, paired-pulse TMS using two separate coils can also be used to assess excitability changes between two different brain regions, such as the cerebellar cortical connection (Grimaldi, Argyropoulos, Boehringer, et al., 2014; Jayasekeran et al., 2011). Measurements from the FDI muscle recorded the biggest response with an ISI of 5 ms (Daskalakis et al., 2004; Pinto & Chen, 2001; Ugawa, Uesaka, Terao, Hanajima, & Kanazawa, 1995; Werhahn, Taylor, Ridding, Meyer, & Rothwell, 1996), whereas an ISI of 50 ms evoked the largest MEP amplitudes measured from the muscles of the corticobulbar system (Jayasekeran et al., 2011). These findings indicate that the connection between the cerebellum and the motor cortex is inhibitory, whereas the connection may be facilitatory in the corticobulbar system.

In contrast to TMS being a diagnostic tool, rTMS is a preferred method for treatment since it induces lasting changes in the neuromuscular system, e.g. LTP in the target cells (Esser et al., 2006; Goss et al., 2012). rTMS uses the same set-up as single-pulse TMS but applies stimuli in a more frequent manner. Protocols using rTMS at a frequency of < 1 Hz inhibit cortical excitability post stimulation, whereas rTMS at > 5 Hz increases excitability (Klomjai et al., 2015). Applying rTMS in trains of 50 Hz stimulation in bursts of pulses every 200 ms is known as theta-burst stimulation (TBS) (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). Different rTMS protocols, including TBS protocols, have been used to increase excitability of the unaffected hemisphere or decrease excitability of the affected hemisphere. These protocols are based on the findings by Hamdy, Aziz, Rothwell, Hobson, and Thompson (1998), which demonstrated that the recovery of swallowing is associated with an increased motor cortical representation of swallowing in the unaffected hemisphere. Hence, rTMS has been used to facilitate the recovery of swallowing related cortical functioning with promising potential for dysphagia rehabilitation (Khedr et al., 2009; Mistry, Michou, Rothwell, & Hamdy, 2012). However, these techniques have the disadvantage of high cost, accessibility and potential adverse effects (discussed below) when compared to other NIBS techniques, such as tDCS.

For TMS applications, minor adverse effects have been reported in juvenile and adolescent participants, most commonly headache (37/322) or scalp discomfort (8/322) (Krishnan, Santos, Peterson, & Ehinger, 2015). Other TMS-related adverse events

included neck stiffness, twitching or fatigue (Krishnan et al., 2015). However, these minor side effects were mostly transient (disappeared within 24 h) and resolved spontaneously without medical intervention (Krishnan et al., 2015). Major side effects are rare but possible, with two reports of seizure induction (Chiramberro, Lindberg, Isometsä, Kähkönen, & Appelberg, 2013; Hu et al., 2011) and two reports of a syncope development (Kirton et al., 2008; Kirton, deVeber, Gunraj, & Chen, 2010). To minimize the risk of side effects, safety guidelines recommend avoiding TMS applications in individuals with a history of epilepsy or metal implants, including brain stimulators and cochlear implants (Krishnan et al., 2015; Rossi et al., 2009; Wassermann, 1998). The greatest concern is the potential risk of seizure induction and epileptogenic complications, in particular when using higher frequency rTMS (> 5 Hz) (Ebmeier & Lappin, 2001). Therefore, alternatives for rTMS interventions are in demand.

7.3 Transcranial direct current stimulation (tDCS)

Historically, electrical stimulation was used to cure psychiatric disorders of the early 18th century (Sarmiento et al., 2016). A reappraisal of the 'modern' tDCS as a form of NIBS only came about at the turn of this century (Brunoni et al., 2012), when Priori and colleagues (1998) and Nitsche and Paulus (2000) demonstrated that weak, direct electric currents delivered transcranially, induced bi-directional polarity-dependent changes in cortical function (Brunoni et al., 2012). Since then, tDCS has rapidly accelerated as tool in clinical and cognitive-neuroscience research over the past two decades. It has been used in a variety of disorders, such as depression (Shiozawa et al., 2014), pain (Fenton, Palmieri, Boggio, Fanning, & Fregni, 2009; Fregni et al., 2006; Fregni, Freedman, & Pascual-Leone, 2007), epilepsy (San-Juan et al., 2015), schizophrenia (Brunelin et al., 2012; Brunoni et al., 2014), tinnitus (Song, Vanneste, Van de Heyning, & De Ridder, 2012), neglect (Sunwoo et al., 2013), traumatic brain injury (TBI) (Demirtas-Tatlided, Vahabzadeh-Hagh, Bernabeu, Tormos, & Pascual-Leone, 2013) and stroke rehabilitation (Marquez, van Vliet, Mcelduff, Lagopoulos, & Parsons, 2015; Schlaug et al., 2008). Furthermore, it has been investigated as an adjuvant therapeutic tool within the healthy system to promote learning (reading, motor

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skills, math, threat detection) (Jacobson, Koslowsky, & Lavidor, 2012; Kang, Summers, & Cauraugh, 2016; Madhavan & Shah, 2012), memory (Berryhill & Jones, 2012; Coffman, Clark, & Parasuraman, 2014), and creativity (Zmigrod, Colzato, & Hommel, 2015).

In contrast to TMS using magnetic stimulation, tDCS uses electrical direct current to stimulate brain structures (Stagg & Nitsche, 2011). Direct current refers to a constant unidirectional current flow of electrons within an electric circuit, compared to an alternating current flow which changes direction periodically (Hahn et al., 2013). This ensures that sensitive components to current within the circuit, such as the body or brain structures, are not exposed to potentially damaging, abrupt increases or decreases in voltage (Paulus & Opitz, 2013). TDCS functions on Ohm's law, which describes the relationship between the components of the electrical DC circuit, consisting of voltage (volts) across a conductor, current flow through the conductor [(milli)amperes] and resistance of the conductor [(kilo)ohms] (Paulus & Opitz, 2013). Changes in one of the units affects the other two. Therefore, the voltage (typically under 20 volts) varies during the application of tDCS to maintain a constant current flow (Bikson et al., 2016; Hahn et al., 2013). An increase in resistance, e.g. through changes in skin conductivity, would result in a weaker relationship between the required voltage and the current intensity, which will consequently be upregulated by the tDCS device (Hahn et al., 2003). To ensure the safety of the participant, modern tDCS devices have a pre-defined upper voltage limit, that automatically terminates the stimulation process if the resistance is too high (Paulus & Opitz, 2013).

Conventional tDCS setups utilise conductive rubber electrodes (typically 5 x 5 cm or 5 x 7 cm) that are wrapped in saline-soaked sponge pockets and held in place on the head by a soft head strap (Kronberg & Bikson, 2012). The placement of the electrodes determines the direction of the current flow through the brain tissue, which may be adapted depending on which brain function or behaviour is targeted (Woods et al., 2016). Most commonly, both electrodes are placed on the head (Nasseri, Nitsche, & Ekhtiari, 2015). Alternatively, one electrode can also be placed on an extra-cephalic structure, such as the arm or shoulder (Woods et al., 2016). As illustrated in Figure 18, the current partially penetrates the skull and flows from the positively charged electrode (anode) to the negative (cathode). The current flows inward towards the brain

underneath the anodal electrode, where it hyperpolarises the apical dendrites and depolarises the soma of the neuron (Radman, Ramos, Brumberg, & Bikson, 2009; Figure 19). The opposite mechanisms occur underneath the cathodal electrode (Radman et al., 2009). The hyperpolarisation or depolarisation effects of neurons are highly dependent on the direction of the electric field in relation to the orientation of neurons within the brain tissue (Bikson et al., 2004). Bikson and colleagues (2004) found that the depolarisation maximum occurs when the electric field is parallel to the cortical surface while significant somatic depolarisation occurs when the electric field is orthogonal to the somatic-dendritic axis.
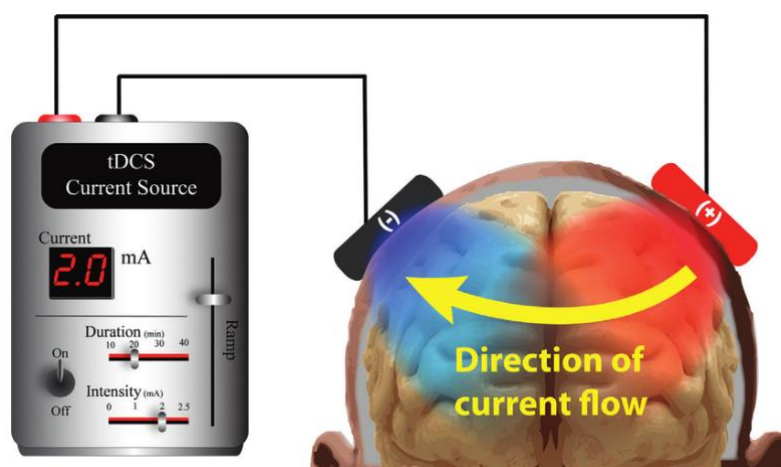
Figure 18. The electric current delivered by the tDCS device with electrodes overlying cortical structures. The current enters the brain through the anodal electrode (+), passes through cortical and subcortical regions and leaves through the cathodal electrode (-). On the tDCS device: Current intensity displayed in milliamperes (mA), duration minutes (min).[17]

The effects of tDCS on neural tissue can further be influenced by the type of neuron stimulated (e.g. motor, sensory or interneuron neuron, excitatory or inhibitory), neural state (active or passive), neural structure (single neurons or neural networks) and sensitivity of neurons (firing threshold) (Bikson et al., 2004; Moreno-Duarte et al., 2014). Furthermore, neurons are not purely hyperpolarised or depolarised, more

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[17] Reprinted from The Stimulated Brain (pp. 35-59), Transcranial electrical stimulation: transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS), transcranial pulsed current stimulation (tPCS), and transcranial random noise stimulation (tRNS), Moreno-Duarte, I., Gebodh, N., Schestatsky, P., Guleyupoglu, B., Reato, D., Bikson, M., & Fregni, F., Copyright © (2014) with permission from Elsevier.

frequently it is a combination of both, with one predominating (Bikson et al., 2004). Although these numerous variables make the neural response to tDCS difficult to predict, repeatable polarity dependent changes in cortical excitability have been identified (Lefaucheur et al., 2017). Cortical excitability is increased when anodal tDCS is applied over the M1 (Boros, Poreisz, Münchau, Paulus, & Nitsche, 2008; Nitsche, Fricke, et al., 2003). Cathodal tDCS in the same position decreases excitability (Ardolino, Bossi, Barbieri, & Priori, 2005). These polarity dependent effects of tDCS have also been confirmed for the cerebellum (Galea et al., 2009).
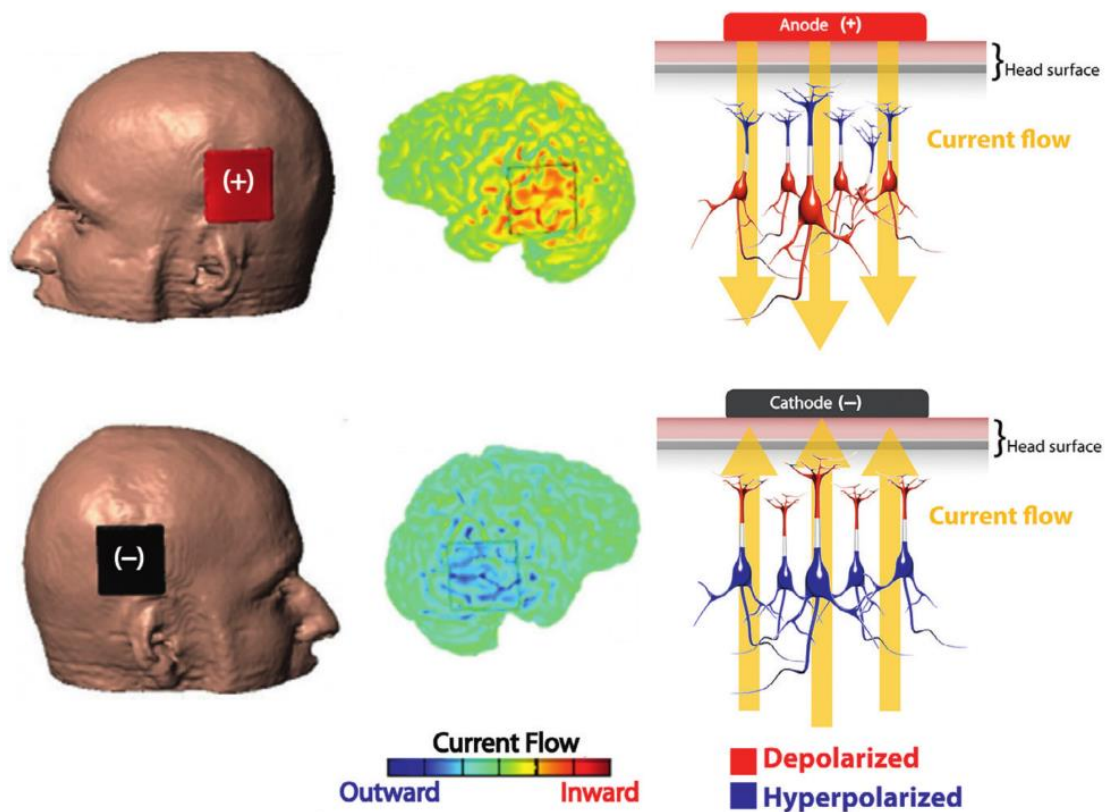
Figure 19. Brain regions underneath the tDCS electrodes are activated by the current flow. The electrode polarity of tDCS determines direction of current flow (outward or inward) in the brain. The Upper right: Direction of the tDCS current through the anodal electrode (+). Apical dendritic regions of the pyramidal cortical neurons become hyperpolarized (blue) whereas the somatic regions become depolarized (red). Lower right: Direction of the tDCS current through the cathodal electrode (-); apical dendritic regions of the neuron become depolarized (red) whereas the somatic regions become hyperpolarized (blue).[18]

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[18] Reprinted from The Stimulated Brain (pp. 35-59), Transcranial electrical stimulation: transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS), transcranial pulsed

The application of tDCS at subthreshold level (typically $1 - 2$ mA, for $10 - 25$ min) not only modifies the transmembrane neural potential and excitability level of neurons, it also changes their responsiveness to synaptic input (Rahman et al., 2013) and modulates their individual firing rate (Miranda, Lomarev, & Hallett, 2006; Wagner, Valero-Cabre, & Pascual-Leone, 2007). In addition to these short-term changes in the electric neural membrane potential, longer interventions of tDCS, with a duration of several minutes, induces lasting after effects of up to one hour or more (Nitsche, Fricke, et al., 2003; Nitsche & Paulus, 2001). The modulation of neuronal ionic channels, in particular, the N-methyl-D-aspartate (NMDA) receptors and L-type voltage-gated calcium channels (L-VGCC), are the main drivers of neuroplastic changes induced with tDCS (Paulus, 2011). Furthermore, tDCS has been demonstrated to reduce GABA neurotransmission for both stimulation polarities (Stagg et al., 2009). These neurotransmitter responses are thought to regulate LTP or LTD-like mechanisms which are important to evoke lasting therapeutic changes (Paulus, 2011).

To use tDCS therapeutically, it is essential to be aware of side effects and safety criteria concerning its application. Two reviews stated that most commonly reported side effects of tDCS are itching, tingling, burning sensation and observed skin redness (Brunoni et al., 2011; Krishnan et al., 2015). These side effects were reported to be rare, mild and transient, with no lasting effects over a period of more than two hours were reported (Krishnan et al., 2015). Interestingly, reports of participants' perception between the active and sham condition did not significantly differ from each other. Mild discomfort was mostly reported during the onset phase of stimulation and vanished within minutes following stimulation onset (Krishnan et al., 2015). This suggests that commonly used sham procedures are efficient in blinding participants to their stimulation group (Brunoni et al., 2011). The results of these two reviews are mainly based on short-term tDCS interventions (one to two sessions) in healthy volunteers.

Bikson et al. (2016) provided an update of this information, including adverse events following multiple sessions of tDCS, neuroanatomical changes (such as tissue damage), risk of seizure induction as well as special safety considerations in the ageing or

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impaired brain. This meta-analysis was performed across more than 33,200 sessions of tDCS, with over 1000 subjects receiving multiple sessions across days. It did not reveal any record of serious adverse effects, such as brain injury or tissue damage (Bikson et al., 2016). Although high-intensity electrical stimulation in animal research has been shown to produce epilepsy (Liebetanz et al., 2006), conventional tDCS protocols are far below the seizure induction threshold. There have been no reports for seizure generation in current animal and clinical studies using conventional tDCS protocols (Bikson et al., 2016). It is important to mention that reviews and reports on adverse effects are based on controlled studies that are not testing the extreme use of tDCS for its ability to produce injury or for an extensive amount of time. Furthermore, as research is primarily based on tDCS over cortical areas, side effects of other stimulation areas such as the cerebellum warrant more attention.

Based on the current evidence, no special considerations regarding the usage of tDCS in the ageing or stroke populations need to be taken (Bikson et al., 2016). However, it is difficult to assess side effects that are exclusively a result of tDCS and not part of the neurological condition, as approximately 30% of stroke survivors report mood-related changes (e.g. depression) and up to 20% experience post-stroke seizures without tDCS applications (Silverman, Restrepo, & Mathews, 2002). In these cases, frequent monitoring, including baseline assessments of behavioural changes, is advised. The evidence base for the tolerability and safety of tDCS of a more vulnerable patient population, such as acute and subacute stroke patients, is low (few studies with small sample sizes) and may therefore only be accepted provisionally (Bikson et al., 2016).

In summary, tDCS has presented with no serious adverse effects in the healthy and impaired participants. Only a minimal risk of mild and transient side effects to humans have been reported when it is used following the current safety recommendations:

> *"(1) the current is less than 2.5 mA, (2) it is applied through electrodes that are known to minimize skin burns at the specific current level, (3) the current application duration is less than 20-60 min per session, and (4) that sessions are not more frequent than twice per day."* (Fregni et al., 2015, page 5).

In addition to conventional tDCS, newer technology known as high-definition tDCS (HD-tDCS) has been developed. HD-tDCS increases the focality of the stimulation by using many small electrodes (Truong et al., 2015; Figure 20, middle). The 4x1 HD-tDCS approach also uses smaller electrodes but only five. Similar to conventional tDCS, 4x1 HD-tDCS uses a 2-channel system, in which the one electrode in the centre receives one type of output and the four surrounding electrodes receive the other type of output from the stimulator device (Truong et al., 2016; Figure 20, right). Using smaller electrodes in the two latter approaches results in an increased current density (calculated as current intensity divided by the electrode size) at each electrode. Experiments or modelling studies suggest peak current densities from 0.0828 to 0.211 A/m² should not pose risk for brain injury by tDCS applications (Bikson et al., 2016). Therefore, special conductivity gel is used to generate full contact to targeted skin area and is considered necessary to ensure patient safety (Bikson et al., 2016). HD-tDCS approaches are still in their early developmental stages and many questions regarding their technical, neurophysiological work and safety mechanisms require further investigation before they can be implemented into clinical work (Villamar et al., 2013). HD-tDCS approaches may enable better focality of the stimulation area in the future, nevertheless, their potential applications are similar to those of conventional tDCS (Villamar et al., 2013). Conventional tDCS has a greater empirical research provides established level B evidence ("probably effective") for therapeutic use in some areas of clinical practice (Lefaucheur et al., 2017).
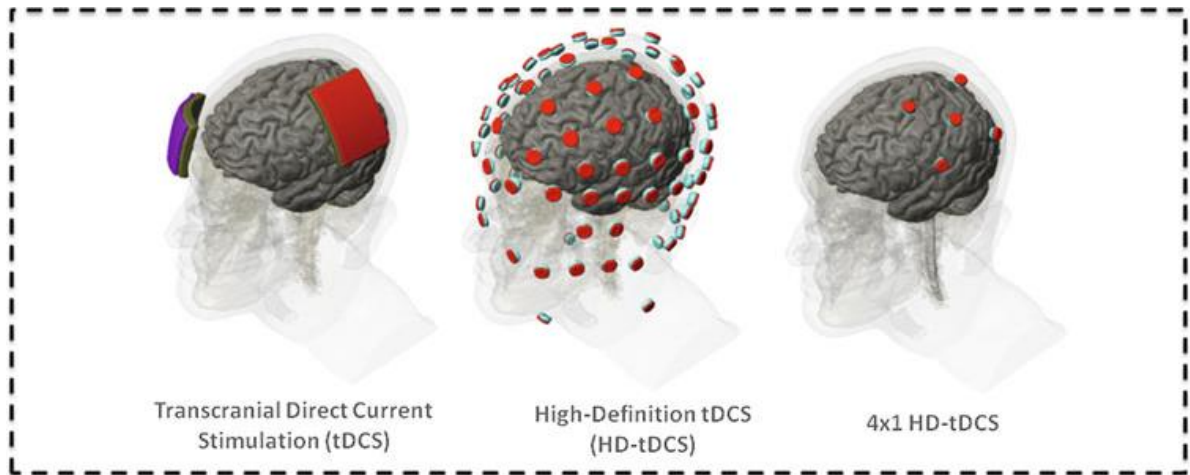
Figure 20. Transcranial direct current stimulation (tDCS) approaches using different electrode sizes and set-ups in comparison.[19]

Given the tolerability, flexibility, cost efficiency and transportability of conventional tDCS, it combines many advantages over rTMS as an emerging neuromodulatory adjunct to neurorehabilitation following stroke. Although the majority of studies have been performed utilising tDCS over the motor cortex in combination with behavioural skill training (Hummel & Cohen, 2006; Liew et al., 2014; Sandrini & Cohen, 2013), the cerebellum has recently become another popular target for tDCS interventions to facilitate motor (re)learning processes (Grimaldi, Argyropoulos, Bastian, et al., 2014; van Dun, Bodranghien, Mariën, & Manto, 2016).

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[19] Reprinted by permission from Springer Nature: In Textbook of Neuromodulation (pp. 113-126), Springer New York, A Role of Computational Modeling in Customization of Transcranial Direct Current Stimulation for Susceptible Populations, Truong, D., Minhas, P., Mokrejs, A., & Bikson, M., Copyright © (2015).

8. Cerebellar tDCS and motor learning

The potential for neuroplastic change and its role in motor learning make the cerebellum an intriguing target for neurorehabilitation using tDCS interventions. Following research on tDCS over the motor cortex to enhance motor learning, research protocols have now been adapted to target the cerebellar-cortical circuit network by stimulating the cerebellum directly (Grimaldi, Argyropoulos, Boehringer, et al., 2014). Differences in cell morphology and the complex folding of the cerebellar hemispheres can influence the effects of stimulation intensity or polarity when compared to cortical mechanisms (Rahman et al., 2014). Therefore, a critical review of the cerebellar tDCS literature on changes in neurophysiology and motor behaviour with respect to motor cortical stimulation protocols is essential. There is currently a lack of studies investigating the effects of cerebellar tDCS on corticobulbar excitability; the following review is therefore mainly based on findings from the corticospinal system.

8.1 Cerebellar tDCS

Cerebellar tDCS protocols vary regarding electrode placement, current intensity, duration and timing of stimulation, which can influence the neurophysiological and behavioural outcomes. Computational studies have been used to predict the electric field generated across the brain for different tDCS configurations over the cerebellum (Truong et al., 2015). Parazzini et al. (2014) were the first to analyse current flow across subcortical structures and modelled cerebellar tDCS on three virtual human head models of different ages and genders, constructed of 77 different tissue types, segmented into a (hexahedral) voxel-based format (1 mm voxels) (Parazzini et al., 2014). A midline setup (Figure 21) was used with one electrode centred on the median line over the cerebellum, 2 cm below the inion, and the electrode with the other polarity over the right arm (5×7 cm). The authors found that a current intensity of 2 mA produces the highest electric field directly below the stimulating electrode and bilateral in the posterior cerebellum (Parazzini et al., 2014). Their findings support previous research using neurophysiological measures (Galea et al., 2009), demonstrating a minimal current spread to surrounding structures (e.g., occipital cortex, brainstem). The

researchers state that this spread is unlikely sufficient to produce relevant functional effects (Parazzini et al., 2014). Furthermore, current spread to the heart was also found to be low, which also diminishes safety concerns of this electrode placement (Parazzini et al., 2014). Unfortunately, this research group did not specify the polarity of current applied nor did they investigate the direction of the electric field.

midline   unilateral
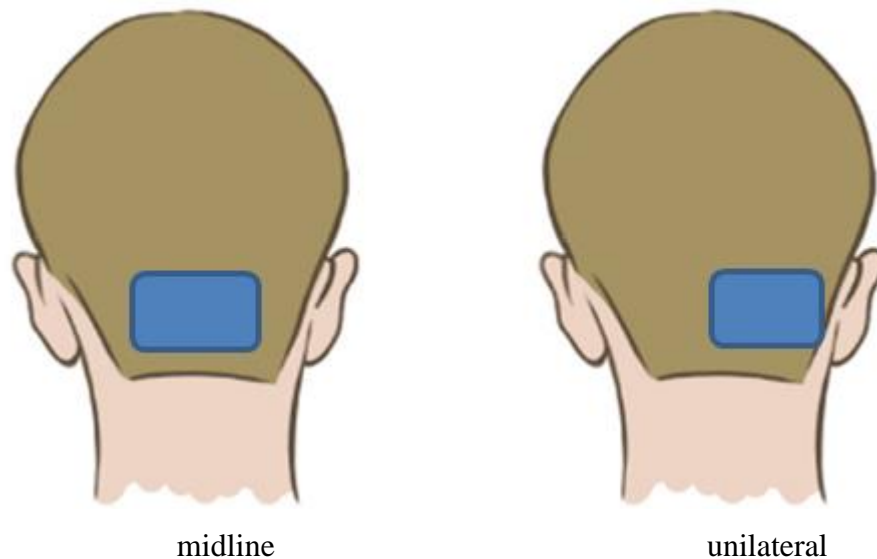
Figure 21. Common electrode placements for cerebellar transcranial direct current stimulation (tDCS).[20]

The effects of cerebellar tDCS have primarily been investigated on unilateral corticospinal tasks, targeting the dominant hand. This led to the development of a unilateral electrode placement over the cerebellum; commonly 1 – 2 cm below and 3 – 4 cm lateral to the inion (Ferrucci et al., 2015) with a second electrode of the other polarity positioned ipsilateral over the buccinator muscle (Galea et al., 2009; Hamada et al., 2012; Hardwick & Celnik, 2014; Herzfeld et al., 2014; Jayaram et al., 2012; Shah, Nguyen, & Madhavan, 2013). This electrode placement has been found to produce a predominantly lateral current flow through the anterior parts of the cerebellum and was classified as the most efficient setup when targeting unilateral motor behaviours (Rampersad et al., 2014; Yavari et al., 2016).

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[20] Head models adapted and downloaded from https://jeffsearle.blogspot.de/2015/09/drawing-head-from-different-angles.html (20.03.2018).

Studies that investigated changes in cognitive functions or the corticobulbar system (only tested on eye movements) typically used a centred position for stimulation (i.e. the anode over the inion), as the task was not specific to one hemisphere (Ferrucci et al., 2013). The right arm was used for the placement of the second electrode during central cerebellar stimulation (Ferrucci et al., 2013). However, the bigger the distance between the electrode over the cerebellum and the second electrode, the smaller the magnitude of the neuromodulatory effect (Dmochowski, Datta, Bikson, Su, & Parra, 2011). A placement with less distance between the electrodes should be considered in future studies to increase the magnitude of the stimulation effect in this study domain.

Another important variable in the application of cerebellar tDCS is the timing of intervention. Most clinical studies employed cerebellar tDCS prior to treatment (Bation, Poulet, Haesebaert, Saoud, & Brunelin, 2016; Ferrucci et al., 2016; Gironell et al., 2014; Grimaldi & Manto, 2013; Grimaldi, Oulad Ben Taib, Manto, & Bodranghien, 2014; Ho et al., 2014; Minichino et al., 2015). Studies on healthy volunteers investigating the effects on motor learning employed a stimulation protocol, where tDCS was mainly applied during the motor task (Block & Celnik, 2013; Cantarero et al., 2015; Foerster et al., 2013; Galea et al., 2009; Hardwick & Celnik, 2014; Herzfeld et al., 2014; Jayaram et al., 2012; Madhavan & Shah, 2012; Panouillères, Joundi, Brittain, & Jenkinson, 2015; Panouillères, Miall, & Jenkinson, 2015; Yavari et al., 2016). The results of studies in post-stroke recovery using tDCS of M1 indicate facilitation of long-term motor learning when tDCS was employed before or during motor practice but not after (Kang et al., 2016). Research using tDCS of M1 on healthy volunteers suggests that stimulation prior to motor training has the greatest effects (Cabral et al., 2015; Pirulli, Fertonani, & Miniussi, 2013). However, there is no research on the timing of intervention-related changes for cerebellar tDCS.

Common cerebellar tDCS investigations have used a single session design on healthy individuals with a stimulation duration between $15 - 25$ min (Ferrucci et al., 2015). Neurophysiological changes of cerebellar tDCS in a single sessions lasted up to 30 min, e.g. when 2 mA for 25 min were applied (Galea et al., 2011). Behavioural changes were measured up to 90 min post cerebellar tDCS (15 min of 1 mA) in combination with motor training (Shah et al., 2013). In contrast, most clinical studies employed cerebellar tDCS over multiple days (Bradnam, Graetz, McDonnell, & Ridding, 2015; Ferrucci et

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al., 2016; Gironell et al., 2014; Ho et al., 2014; Minichino et al., 2015) or two stimulation sessions a day (Bation et al., 2016). These clinical studies have been performed on patients with ataxia or psychological disorders, e.g. depression. There is no published research on the use of cerebellar tDCS for stroke recovery.

8.2 Effects of cerebellar tDCS on corticospinal excitability

The effects of cerebellar tDCS on corticospinal excitability have been tested by measuring changes in the cerebello-thalamo-cortical pathway. Electrical (Ugawa et al., 1991) and magnetic (Pinto & Chen, 2001; Ugawa et al., 1995) stimulation have been used to examine the disynaptic cerebello-thalamo-cortical pathway in healthy human volunteers. Using a paired-pulse TMS paradigm, several studies demonstrated that delivering a conditioning TMS pulse over the cerebellum approximately 5 ms prior to a second TMS pulse over M1, resulted in a reduction of the MEP amplitudes measured from the FDI muscle (Daskalakis et al., 2004; Pinto & Chen, 2001; Ugawa et al., 1995; Figure 22). This inhibitory phenomenon was described as cerebellar brain inhibition (CBI) (Daskalakis et al., 2004). It was postulated that CBI in humans reflects the activation of the inhibitory Purkinje cells resulting in an increased inhibition of the dentate nucleus within the disynaptic facilitatory dentate-thalamo-cortical pathway (Daskalakis et al., 2004; Iwata & Ugawa, 2005; Pinto & Chen, 2001). Pinto and Chen (2001) further reported a markedly reduced cerebellar inhibition when the target muscle was active, which may be the result of reduced excitability of the cerebello-thalamo-cortical pathway (Pinto & Chen, 2001). Therefore, suppression of the cerebellum could only be assessed when the targeted muscle was at rest and not when the muscle was active (Pinto & Chen, 2001). This is an important finding when translating this assessment to the corticobulbar system. As previously reported, it may not be possible to collect MEP recordings from submental muscles at rest (Doeltgen, 2010).
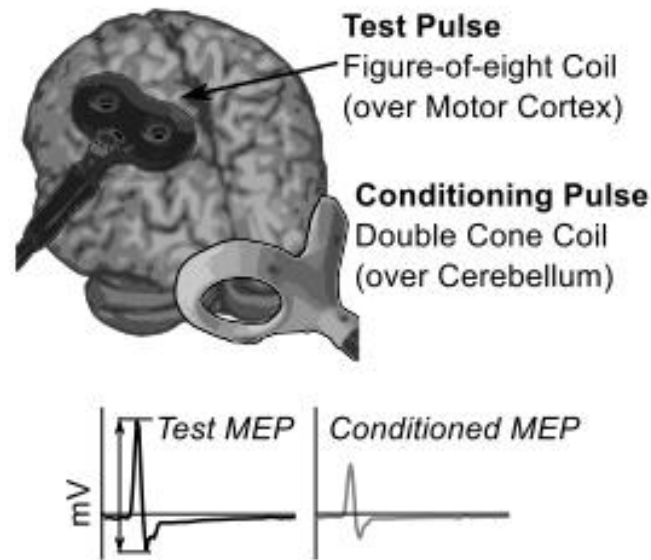
Figure 22. Paired-pulse transcranial magnetic stimulation (TMS) setup for the assessment of the cerebellar brain inhibition (CBI). The difference between the test motor evoked potential (MEP) responses in millivolts (mV) in the two conditions (test and conditioned) is used to explore the influence of the cerebellum over the motor cortex.[21]

Changes in CBI can be used to assess the effects of cerebellar tDCS on corticospinal excitability. Galea and colleagues (2009) were the first to reveal polarity dependent effects of cerebellar tDCS on CBI using the previously determined paired-pulse TMS technique (Galea et al., 2009). They applied 25 min of either cathodal tDCS or anodal tDCS over the right cerebellar hemisphere, which decreased or increased CBI respectively (Galea et al., 2009). These outcomes were thought to be the result of strengthening or weakening the inhibitory output of the Purkinje cells respectively (Galea et al., 2009; Figure 23). A recently published study by Doeltgen, Young, and Bradnam (2015) confirmed that anodal cerebellar tDCS affects the inhibitory tone the cerebellum exerts over the M1. However, anodal cerebellar tDCS led to a reduction in CBI in this study, which is contradictory to the findings of Galea et al. (2009).

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[21] Reprinted by permission from the Society for Neuroscience: Journal of Neuroscience, 32(34), 11610-11617, Dynamic modulation of cerebellar excitability for abrupt, but not gradual, visuomotor adaptation, Schlerf, J. E., Galea, J. M., Bastian, A. J., & Celnik, P. A., Copyright © (2012).
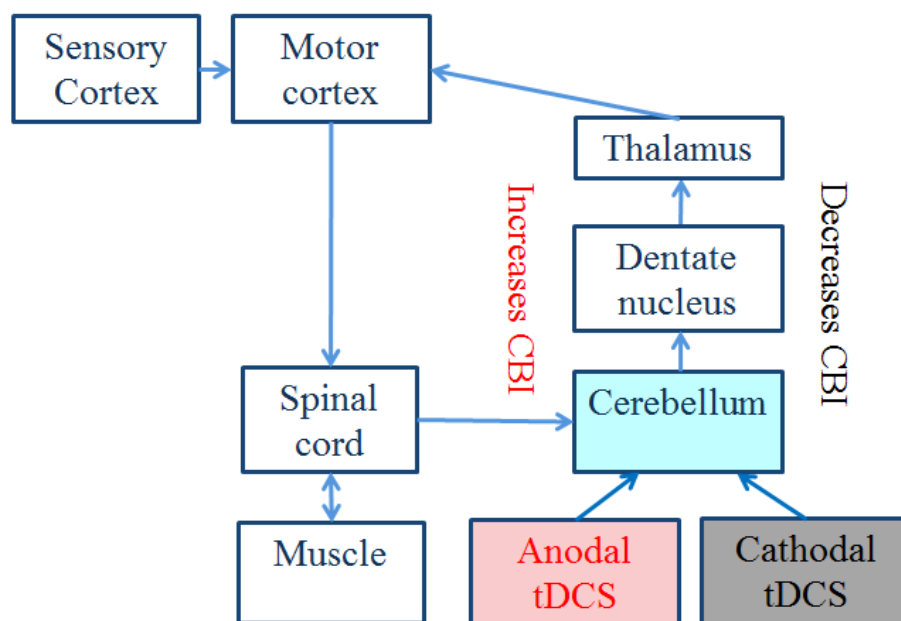
Figure 23. Framework of the influence of the polarity-dependent effects of transcranial direct current stimulation (tDCS) on the cerebellar brain inhibition (CBI). Anodal cerebellar tDCS increases cerebellar excitability and CBI and hence inhibits the motor cortex. On the other hand, cathodal cerebellar tDCS decreases cerebellar excitability and CBI and hence facilitates the motor cortex (Galea et al., 2009).[22]

Both studies used similar parameters for tDCS: the same electrode placement (right cerebellar hemisphere and ipsilateral buccinator muscle), stimulation intensity of 2 mA and similar stimulation durations 25 min (Galea et al., 2009) and 20 min (Doeltgen et al., 2015). Consequently, it is more likely that the heterogenic results arise from the different paired-pulse TMS protocols used to assess changes in CBI. Hardwick et al. (2014) demonstrated that stimulation intensity and coil geometry have a noticeable effect on the efficacy of TMS stimulation to assess CBI (Hardwick, Lesage, & Miall, 2014). Galea et al. (2009) used the stimulus intensity that evokes an MEP response of 1 mV as the test stimulus intensity, whereas Doeltgen et al. (2015) used 50% of the intensity that is needed to evoke a maximal MEP response. Galea et al. (2009) found that a lower stimulation intensity (~ 25% of the brainstem threshold) of the conditioning stimulus revealed effects of anodal tDCS on CBI, whereas the originally used higher

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[22] Adapted from Grimaldi, G., Argyropoulos, G. P., Boehringer, A., Celnik, P., Edwards, M. J., Ferrucci, R., ... & Lesage, E. (2014). Non-invasive cerebellar stimulation—a consensus paper. The Cerebellum, 13(1), 121-138.

intensity of 5% below brainstem threshold produced a ceiling effect. The intensity of the conditioning stimulus used by Doeltgen et al. (2015) was set to 100% of the resting motor threshold of M1 and must have been similarly low compared to Galea's setting of 25% below brainstem threshold, as they were able to detect effects of anodal tDCS on CBI without plateauing. Stimulation intensity plays an important role as it dictates the depth of stimulus brain tissue penetration (Hardwick et al., 2014). Another factor that influences the depth of the stimulus is the coil geometry. Both studies used a figure-of-eight coil to deliver the test stimulus over M1, however, their choice of the coil for the conditioning cerebellar stimulus differed. Galea and colleagues (2009) used a double-cone coil, whereas Doeltgen et al. (2015) used a figure-of-eight coil. A double-cone coil has been found to be more reliable and achieve greater depth of stimulation compared to a figure-of-eight coil (Hardwick et al., 2014). However, it also causes more discomfort to the participant resulting from the depth of stimulation. Hence, the participant is more aware of the stimulus, anticipates a possible painful experience and consequently increases tension which could influence the outcome. The use of the different TMS and tDCS protocols makes it difficult to compare the results of these two studies but suggest that stimulation intensity and the coil type highly influence the polarity-dependent effects on CBI and should be carefully selected in future research.

The effects of cerebellar tDCS have also been tested on intracortical interactions using subthreshold and suprathreshold paired-pulse TMS of M1. No effects were observed on short interval intracortical inhibition (SICI), intracortical facilitation (ICF) nor short-interval intracortical facilitation (SICF) reflecting excitability of cortical interneurons (Doeltgen, Young, & Bradnam, 2015; Galea et al., 2009; Hamada et al., 2012). This may indicate that cerebellar tDCS evokes changes at the cerebellum directly and not within the intracortical network excitability.

In summary, cerebellar tDCS is demonstrated to be an effective method to modulate cerebellar excitability as measured by changes in CBI (Grimaldi et al., 2016; Grimaldi, Argyropoulos, Bastian, et al., 2014). Nevertheless, current research is limited by inconsistent TMS and tDCS protocols to assess CBI tested on small sample sizes. Although these studies provide initial insights into the polarity-dependent effects of unilateral cerebellar tDCS on corticospinal excitability, there is a lack of evidence using more direct measures of plasticity, such as brain imaging studies or direct recording of

cellular activity from animal models. Furthermore, there are currently no studies investigating the effects of cerebellar tDCS on corticobulbar excitability.

8.3 Effects of cerebellar tDCS on motor learning

Based on measurable changes evoked in cerebellar excitability, cerebellar tDCS has been used as adjunct to motor training with the goal of enhancing motor learning (Buch et al., 2016; Hardwick & Celnik, 2014). The exploration of this approach is of particular interest to support rehabilitation of individuals who have lost control over limb movements. The studies have been summarised in regards to the different types of motor learning paradigms: skill acquisition, skill learning and adaptation. Skill acquisition and (motor) skill learning are in line with the definitions in section 5 of this thesis. Motor adaptation is similar to skill acquisition, also achieved over a shorter period of time (e.g. within a single training session) (Buch et al., 2016). However, in adaptation paradigms, motor performance has to be restored in response to environmental perturbation (Shmuelof, Krakauer, & Mazzoni, 2012).

Motor adaptation: Most research investigating the influence of cerebellar tDCS has been performed using motor adaptation paradigms in healthy individuals. Galea and colleagues (2011) investigated the specific role of the cerebellum and the primary motor cortex on motor learning using a screen cursor rotation task (Galea et al., 2011). They tested the effects of 15 min tDCS at 2 mA during visuomotor adaptation in twenty-seven healthy right-handed volunteers. Using different electrode placements, the active electrode over the right cerebellum or left M1 in comparison to sham, they found faster error reduction following anodal cerebellar tDCS compared to M1 or the sham condition. This superiority of cerebellar tDCS was true for both stimulation protocols, applying stimulation before and during the performance of the adaptation task. In a second experiment, they assessed the effects of tDCS over the cerebellum or M1 on retention. They concluded that stimulation over M1 during motor adaptation leads to increased retention compared to cerebellar stimulation or sham. However, the use of the term 'retention' can be misleading, as it is not expressing the retention of a motor skill following a break period, more so the rate of forgetting immediately following the

adaptation task with concurrent stimulation. Nevertheless, these investigations indicate that anodal cerebellar tDCS may be a valuable tool to enhance motor learning in an adaptation task, whereas tDCS over the motor cortex might an option to facilitate the retention of skills.

In a later study, this research group found that anodal tDCS can eliminate age-related differences in performance of a motor adaptation task (Hardwick & Celnik, 2014). Even though younger individuals are typically faster in reducing movement errors in a motor adaptation task (Bock, 2005; Seidler, 2006), older individuals receiving anodal cerebellar tDCS during motor adaptation were able to improve their motor performance to the level of younger adults (Hardwick & Celnik, 2014). This study did not have a control group of young participants receiving anodal tDCS; the researchers are referring to the findings of the previous study for comparison. Furthermore, unlike their other studies, they only used single-blinding in this study. More recently, Panoullières, Joundi, et al. (2015) also compared the effects of anodal tDCS on young and older adults using a similar tDCS protocol and visuomotor rotation task within a single study. In contrast to the results from Hardwick and Celnik (2014), there was no effect of cerebellar stimulation on motor adaptation, but improvements of motor adaptation for both groups with anodal tDCS of M1. Modest changes of stimulation protocols, e.g. electrode placement, may have influenced the overall outcome of cerebellar tDCS related changes compared to previous reports. The distance between the two electrodes was increased by placing the second electrode over the shoulder and not the buccinator muscle, which might have weakened the magnitude of the neuromodulatory effect (Dmochowski et al., 2011). These contrasting results of anodal cerebellar tDCS on motor adaptation in young adults demonstrate that electrode placement may be an important variable to consider in future study designs.

Block and Celnik (2013) investigated the capability of cerebellar tDCS to influence intermanual transfer, which is the ability to transfer a learned skill from one hand to the other hand, utilising the same visuomotor adaptation paradigm. Neither 15 min of 2 mA anodal cerebellar tDCS over the trained or untrained hemisphere nor the same dose of tDCS over M1 affected intermanual transfer. Although intermanual transfer was not affected, anodal tDCS over the trained cerebellar hemisphere facilitated visuomotor adaptation. Unfortunately, these results are based on the data that has been collected

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during the first half of the adaptation task only. When taking the whole dataset into account, no difference between the stimulation groups was found. Specifying data analysis methods a priori, e.g. analysing different stages of learning within the session, would have been desirable, as the approach to subdivide the data post hoc for analysis might have biased results.

Most recently and in contrast to the previously discussed studies, Yavari et al. (2016) investigated the effects of both anodal and cathodal cerebellar tDCS (15 min of 2 mA) in a similar visual adaptation task. In line with the results of previous studies (Izawa, Criscimagna-Hemminger, & Shadmehr, 2012; Miall & Jackson, 2006), cerebellar involvement in the prediction of movement errors, using a localisation task where position estimation of the hand was required without visual feedback, was confirmed. Anodal cerebellar tDCS led to a higher reduction rate of errors compared to cathodal tDCS. Compared to previous studies, the effects of anodal tDCS were not superior to sham, but there were significant differences between the two stimulation condition, anodal and cathodal tDCS. These results suggest weaker effects of each stimulation condition when compared to sham and contrasting effects of the two different types of stimulation leading to a significant difference between the two conditions. A subsequent study by this research group demonstrated the same polarity dependent effects of cerebellar tDCS in a lower limb locomotor adaptation paradigm using the same tDCS protocol in forty healthy individuals (Jayaram et al., 2012). Cerebellar stimulation over the ipsilateral hemisphere of the perturbed leg enhanced (anodal tDCS) and diminished (cathodal tDCS) cerebellum-dependent locomotor learning in the early phase of the motor adaptation process. It is positive to note that a control condition was used, demonstrating that cerebellar stimulation on its own has no influence on walking behaviour, nor did it affect the vestibular system as measured by changes in walking trajectory.

Using a different type of adaptation paradigm, a force field task in the upper limb, Herzfeld and colleagues (2014) also demonstrated polarity dependent effects of cerebellar tDCS. Anodal cerebellar tDCS increased the rate of adaptive learning in the dominant arm, whereas cathodal stimulation decreased it and, beyond this, exhibited impaired retention (Herzfeld et al., 2014). A stimulation intensity of 2 mA with an extended duration of the stimulation of 25 min has been used compared to other studies.

Using the same experimental task, no effect on error-dependent learning was seen anodal tDCS over the left motor cortex (Herzfeld et al., 2014). This suggests a cerebellar specific role in error-dependent learning that has not been found for the motor cortex, which is line with the findings from Galea et al. (2011). However, another study using force field perturbation of the dominant arm revealed opposite effects of anodal cerebellar tDCS in that it impaired error-based learning compared to sham (Taubert et al., 2016). Taubert and colleagues (2016) used the same tDCS protocol with exception of the stimulation duration with only 20 min compared to 25 min in Herzfeld et al.'s study (2014). Either shorter stimulation duration or the different behavioural tasked used in this study may explain the differences in the results between these two studies. This suggests that stimulation and task-specific characteristics highly influence polarity dependent effects of tDCS.

In summary, the majority of research on cerebellar tDCS in motor adaptation paradigms suggests that anodal cerebellar tDCS increases motor adaptation, whereas cathodal tDCS did not produce any effects or generates effects in the opposite direction to anodal tDCS. There are at least two possible explanations for the differences in these results. First, the results vary depending on the different types of stimulation parameters or the behavioural interventions used between the different studies. Or second, behavioural results may not always reflect the polarisation effect that has been identified on cerebellar excitability following cerebellar tDCS (Galea et al., 2009).

Skill acquisition: Two studies have investigated the influence of cerebellar tDCS on motor skill acquisition in the corticospinal system. Foerster and colleagues (2013) evaluated the effects of anodal tDCS (2 mA for 13 min) on motor imagery of handwriting, testing different electrodes placements, including a cerebellar placement (Foerster et al., 2013). Surprisingly, only tDCS over M1 and the dorsolateral prefrontal cortex enhanced motor function following mental practice of the handwriting task, whereas anodal cerebellar tDCS impaired motor performance. The authors discuss that based on Galea's results (2009) who demonstrated an increased inhibitory tone of the cerebellum over the M1 following anodal tDCS that this inhibition of M1 may lead to a decreased ability in handwriting. However, it might also be due to the lack of sensory input during mental practice compared with motor execution of the task that cerebellar-cortical loops are not involved enough to evoke neuroplastic changes.

Shah and colleagues (2013) tested the effects of cerebellar tDCS (1 mA for 15 min) during a visuomotor task on skill acquisition in the lower limb. Although polarity dependent effects were found for M1 tDCS, both excitatory and inhibitory cerebellar tDCS led to a significant improvement in accuracy of the learned task compared to a sham group. With the greatest improvements achieved following cathodal tDCS. The effects stayed unchanged for up to 60 min post-termination of training and stimulation. This study used a lower current intensity with 1 mA compared to all the other studies on cerebellar tDCS in motor learning (1.5 – 2 mA) suggesting that the effect of tDCS may be different for different stimulation intensities. This hypothesis is supported by findings from motor cortical tDCS studies which indicated that increasing the current intensity does not reflect a linear change in excitability (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013). The results of these two studies on the effects of cerebellar tDCS in motor skill acquisition suggest that the effects of tDCS may be task specific, e.g. depend on amount and type of sensory input, and/or change with different stimulation intensities.

Motor skill learning: Lastly, only one study has investigated the effects of cerebellar tDCS on motor skill learning in 33 healthy volunteers (Cantarero et al., 2015). Participants performed a sequential visual isometric pinch task (Reis et al., 2009) during 20 min of right cerebellar stimulation at 2 mA over three consecutive days and completed a follow-up assessment after one week. The results indicate that anodal cerebellar tDCS improved skill learning compared to the sham and cathodal tDCS. Furthermore, greater gains were identified during online rather than offline learning. These results are in line with findings from studies using the same task during concurrent stimulation of M1, where anodal tDCS also enhanced motor skill learning (Reis et al., 2009; Schambra et al., 2011). The analysis of the single components of skill learning, error rate and movement time, lead to the conclusion that improvements in motor skill mediated by anodal stimulation were mainly a result of a reduction in error rate rather than a reduction in movement time (Cantarero et al., 2015). Even though the cerebellum plays a primary role in error-dependent learning in motor adaptation tasks, the results of this study indicate that the cerebellum might instead be relevant for both, adaptation and skill learning paradigms.

Taken together, the divergence of experimental protocols makes it difficult to compare the results between studies. More precise conclusions about tDCS mechanisms of anodal and cathodal cerebellar stimulation on neurophysiological changes and motor learning require further investigation. Current findings demonstrate that changes in neurophysiologic excitability do not necessarily coincide with changes in motor behaviour. Although the effects of cathodal tDCS on motor learning in the corticospinal system are inconsistent, the majority of studies demonstrated enhanced motor learning with anodal tDCS.

9. Developing effective neuromodulation protocols for dysphagia rehabilitation

Primarily anodal tDCS, but also cathodal tDCS, of the motor cortex, have been found to enhance motor learning when used in combination with motor tasks of the corticospinal system in healthy participants and patients following stroke. TDCS, being a non-invasive, safe and low-cost tool, is also appealing to explore as a possible adjunct to motor skill training in dysphagia therapy. It has been reported for use in dysphagia therapy where the centre of attention for the application was the manipulation of the motor cortex in combination with unspecific or strength-based swallowing training (Pisegna et al., 2015). However, the recent shift to more skill-based swallowing rehabilitation methods and the role of the cerebellum in motor learning, make cerebellar stimulation an appealing approach that might enhance therapeutic swallowing outcomes.

9.1 tDCS in swallowing and dysphagia rehabilitation

Neurophysiological protocols of tDCS from the corticospinal system have been replicated for swallowing. Jefferson and colleagues (2009) compared low-intensity (1 mA for 10 min) and high-intensity (1.5 mA for 10 min or 1 mA for 20 min) of anodal and cathodal tDCS over the pharyngeal representation of M1 in healthy participants to a sham condition (Jefferson, Mistry, Singh, et al., 2009). The double-blind application of the different conditions took place on separate days in a randomised order. In contrast to findings from the corticospinal literature (Nitsche & Paulus, 2001), low-intensity anodal or cathodal tDCS did not evoke polarity dependent effects on pharyngeal motor evoked potentials. However, increasing the time and intensity of the applied current, increased or decreased corticobulbar excitability following anodal and cathodal tDCS respectively (Jefferson, Mistry, Singh, et al., 2009). Nevertheless, suppression of pharyngeal cortical excitability with cathodal stimulation was weaker and rather short-lived compared to the changes with anodal stimulation. High-intensity anodal stimulation resulted in immediate and lasting effects of up to 60 min post-termination of tDCS.

These excitatory effects of anodal tDCS over the pharyngeal motor cortex were subsequently confirmed by later investigations in healthy participants (Suntrup et al., 2013; Vasant et al., 2014). In line with previous research, cathodal tDCS only evoked differences in pharyngeal motor cortical excitability at the higher intensity of 1.5 mA at 15 min and 30 min post. The authors discuss that suppression of the pharyngeal motor cortical regions might be harder compared to the suppression of other motor cortical regions, e.g. for limb motor functions, because of the bilateral cortical innervation and strong cross-cortical connections (Jefferson, Mistry, Singh, et al., 2009). Therefore the results of these studies suggest that higher stimulation intensities and extended durations of tDCS may be required for the swallowing system to evoke similar changes to what has been demonstrated for the corticospinal system.

Zhao et al. (2015) used Jefferson and colleagues' (2009) stimulation parameters as guidance and applied tDCS for 20 min at 1.5 mA with the same electrode placement over M1 in 31 healthy volunteers. In addition, they combined the application of tDCS with effortful swallowing (40 swallows) (Zhao et al., 2015). Anodal tDCS applied over the hemisphere with weaker suprahyoid projections led to a bilateral increase in MEPs, for up to 90 min following the stimulation. On the other hand, anodal tDCS over the hemisphere with stronger suprahyoid projections resulted only in an increase of MEPs for the same side. Unfortunately, in this well-designed study, cathodal tDCS was excluded from the investigation. Furthermore, although the researchers used a strengthening task in combination with the stimulation protocol, they acknowledge that a lack of coordination in the pharyngeal musculature could also be the cause of swallowing difficulties which should be explored for future clinical application protocols.

Suntrup et al. (2013) used magneto-encephalography to determine the effects of tDCS on cortical network activity in swallowing. They applied tDCS overlying the pharyngeal areas of M1 for each hemisphere separately in a crossover design (Suntrup et al., 2013). Anodal tDCS or sham was applied to the left or the right swallowing motor area during the performance of swallowing reaction tasks in three separate sessions with 21 healthy volunteers. Increased bilateral activation was found with unilateral stimulation of either side. These findings support earlier research showing that tDCS is able to alter the inter-hemispheric connectivity (Meinzer et al., 2012; Polanía, Nitsche, & Paulus, 2011; Sparing et al., 2009). In contrast to interactions

between the hemispheres for limb movement control that are mainly inhibitory (Vines, Nair, & Schlaug, 2006) this study suggests interhemispheric connections for swallowing might be more synergistic (Suntrup et al., 2013). These differences between the corticospinal and corticobulbar system regarding inter-hemispheric interaction have also been confirmed by evidence from Vasant and colleagues (2014). They demonstrated excitatory effects of anodal tDCS on a pre-conditioned hemisphere with inhibitory rTMS, which in turn increased the excitability of pharyngeal projections in M1 from both hemispheres to the pharynx (Vasant et al., 2014). Unfortunately, no additional assessment in the unconditioned system was performed to confirmed the inhibitory effect of rTMS or the effects of anodal tDCS.

Cosentino et al. (2014) were the first to investigate the effects of tDCS of both polarities in a double-blind randomised trial with healthy volunteers. They confirmed the facilitating effect of anodal cortical tDCS within the swallowing system. Only anodal tDCS over the right M1 (20 min at 1.5 mA), not cathodal tDCS, enhanced oral sucking and swallowing-related EMG measures compared to the sham group. Unfortunately, tDCS was applied over the right hemisphere exclusively. The likelihood of a dominant hemisphere for swallowing was not considered (Hamdy et al., 1996), and hence if the dominant or non-dominant hemisphere was stimulated. It is yet to be investigated how these changes noted in healthy participants would translate to patients with dysphagia.

The results of studies using tDCS on the healthy brain networks provide the foundation for the development of post-stroke rehabilitation protocols using tDCS. In general, tDCS protocols that have been used following stroke were based on the theoretical model of an imbalance in interhemispheric activation of motor commands caused by the stroke (Feng, Bowden, & Kautz, 2013; Figure 24, A). TDCS can be used in three different ways to modulate this imbalance. First, anodal stimulation is used to upregulate excitability of the lesioned hemisphere (B). Second, cathodal stimulation over the contra-lesional hemisphere is used to down-regulate excitability (C). Third, bi-hemispheric stimulation combining A and B simultaneously (D) (Edwardson, Lucas, Carey, & Fetz, 2013; Feng et al., 2013; Figure 24) may encourage a decrease in activity in the lesioned hemisphere and increase activity in the contra-lesional hemisphere (Feng et al., 2013). Cortical modulation and corrections of interhemispheric imbalance using tDCS in combination with functional motor therapy of the paretic limb have led to

motor improvements in limb function in patients after stroke (Bastani & Jaberzadeh, 2012; Butler et al., 2013).
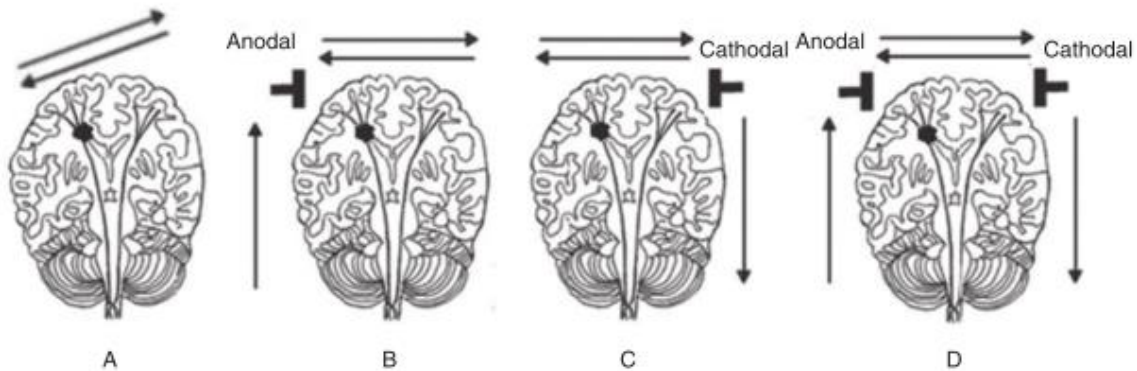
Figure 24. Schematic model of interhemispheric-imbalance post stroke (A) and possible interventions to re-establish balance with different polarities of transcranial direct current stimulation (tDCS).[23]

Four studies investigated the effects of anodal tDCS applied over the motor cortex combined with dysphagia rehabilitation in patients post stroke (Ahn et al., 2017; Kumar et al., 2011; Shigematsu, Fujishima, & Ohno, 2013; Yang et al., 2012). The majority of these studies altered the tDCS protocols from the corticospinal system since in contrast to the cortical recovery process of limb function, spontaneous recovery of dysphagia has been associated with increased activity of the undamaged motor cortex (Hamdy et al., 1998). Kumar and colleagues (2011) targeted the unaffected sensorimotor cortical representation of swallowing using anodal tDCS (2mA) (Kumar et al., 2011). Using this placement, the authors wanted to diminish dysfunctional effects on swallowing function from the lesioned hemisphere. They randomly assigned 14 acute stroke patients (24 to 168 hours after their first ischemic stroke) with mild to severe dysphagia (≤ 5 on the Dysphagia Outcome and Severity Scale (DOSS)) either to the anodal or sham condition. All patients received the same swallowing treatment consisting of one session of 60 effortful swallows (one swallow every 30 s) while sucking on a flavoured lollipop in combination with tDCS on five consecutive days. They revealed a significant improvement for patients in the anodal tDCS group [2.6 (CI 95 %: 1.91 – 3.29)] compared with the sham group [1.26 (CI 95 %: 0.57 – 1.95)] using a multivariate analysis of the DOSS scores pre to post-treatment. However, therapist and researchers

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in this trial were not blinded to the group allocation which is a major limitation when using a subjective outcome measure, such as the DOSS. There is no information regarding the validity of the assessment tool and its reliability is highly dependent on the experience of the assessor. Another limitation of this study is the overall treatment approach of effortful swallowing, in that it is used for every patient without considering their underlying pathophysiological mechanisms, even though a VFSS has been performed. The study confirmed the location of the stimulating electrode using the patients' MRI scan. In 2015, this research group proposed an extension of this study performing a phase I/II randomised double-blind trial (Marchina et al., 2015). It was planned to investigate the effects of tDCS in the acute-subacute stroke population, including 99 unilateral cortical and subcortical stroke patients. The patients would be assigned into one of the three stimulation groups: high dose tDCS (ten sessions active tDCS, twice daily) low dose tDCS (five sessions active tDCS, five sessions sham), or sham. Although the researchers included more objective swallowing measures into the analysis, they still proposed similar swallowing treatment to their pilot study, consisting of 40 swallows per session alternating between regular and effortful swallows. Furthermore, the inclusion of subcortical stroke patients might lead to complications as their electrode placement is based on the rationale of unilateral cortical strokes.

Two other studies chose a placement of the anodal electrode over the lesioned hemisphere to modulate the cortical imbalance (Shigematsu et al., 2013; Yang et al., 2012). This placement follows the same rationale as is used in limb motor recovery treatment. Furthermore, both studies followed a similar experimental protocol consisting of 10 sessions of treatment consisting of 20 min combined tDCS (1 mA) (or sham) and swallowing training (plus an additional 10 min of training only in the Yang et al. study). The dysphagia treatment was based on patient's swallowing function and was a mix of direct (compensatory strategies) and indirect (physical manoeuvres such as oral motor exercises or sensory stimulation). The primary outcome measure was the Functional Dysphagia Scale (FDS). Improvements on the FDS have been revealed in both groups without significant differences directly after the treatment period. However, a posthoc test three month after the intervention revealed significant improvements of the anodal group compared to the sham group (Yang et al., 2012). Secondary outcome measures based on the VFSS, such as oral or pharyngeal transit time, did not significantly differ between the two groups at any of the test time points (Yang et al.,

2012). Most recently, Ahn and colleagues (2017) completed a double-blind randomised controlled trial using bihemispheric anodal tDCS paired with conventional dysphagia therapy on 26 chronic stroke patients (6 months or more) (Ahn et al., 2017). The anodal electrodes were placed on either side over M1 and the cathodal electrodes bilateral over the supraorbital regions of the contralateral hemisphere. Patients were assigned to either the anodal or sham condition and received 10 sessions of 20 min at 1 mA current intensity. In line with Kumar et al., the DOSS was performed as the only outcome measure immediately before and after the intervention. There was no difference between the anodal tDCS and sham group post-treatment (U = 70.50, Z = –0.83, p = 0.48). However, they reported a mean significant improvement of 0.62 points (SD 0.77) in the DOSS scores of the anodal tDCS group, from 3.46 (SD 1.27) to 4.08 (SD 1.50) (Z = -2.27, p = 0.02). Although this study used a double-blind design, it is questionable if the DOSS is sensitive enough to capture changes in swallowing function.

In summary, the use of different neurostimulation parameters (e.g. electrode montage or duration of stimulation) does not allow direct conclusions to be made regarding the effects of tDCS. Overall, the application of anodal tDCS in combination with dysphagia treatment provided promising results. However, these results need to be interpreted with caution as most of the studies have major limitations in their study designs. Single evaluation tools might not be sufficient to find adequate changes in swallowing function. Only one of the four studies made use of objective assessments of swallowing to derive their outcome measures (Yang et al., 2012) which should the preferred method in future study designs. In addition, blinding of the researcher, therapist and patient is essential to ensure an unbiased analysis and minimise a possible placebo effect. Furthermore, long-term post hoc assessments of treatment studies (weeks or month) should be used as the effects of stimulation might be delayed in some cases. Most importantly, however, is the use of specific treatment approaches based on the patient's underlying pathophysiology.

9.2 Cerebellar tDCS for motor learning in swallowing

TDCS demonstrated to be a well-tolerated, safe and non-invasive brain stimulation technique in healthy and patients subjects with various neurological disorders including stroke patients (Bikson et al., 2016). The motor cortex has become a popular target for tDCS interventions in conjunction with motor rehabilitation of limb function following stroke (Buch et al., 2016). For dysphagia rehabilitation, tDCS over M1 provides additional benefit to stroke patients in their recovery process (Kumar et al., 2011; Shigematsu et al., 2013). Nevertheless, the application of tDCS over M1 has primarily been used in addition to non-specific treatment approaches. New avenues to target specific problems in the underlying pathophysiology of patients with dysphagia following stroke have been developed (Huckabee & Macrae, 2014). Skill training in swallowing therapy using surface sEMG have demonstrated potential for dysphagia rehabilitation in patients with PD and following stroke (Athukorala et al., 2014; Stepp et al., 2011).

Skill training leads to neuroplastic changes within the corticospinal and corticobulbar system (Dayan & Cohen, 2011; Svensson et al., 2003). These changes can be facilitated with the use of NIBS. TDCS modulates neuronal excitability and plasticity of different neuroanatomical regions, including M1 and the cerebellum, in a way that it enhances neuroplastic changes for limb function (Galea et al., 2009; Nitsche & Paulus, 2001). The majority of studies targeted M1 to facilitate motor learning processes using tDCS. However, cortical lateralisation for swallowing neural control remains unclear. Most likely, a hemispheric dominance of motor cortical representations of swallowing exists (Hamdy et al., 1998). Identification of the hemisphere that is to be targeted with the stimulating electrode for tDCS is a tedious and expensive procedure. Furthermore, current tDCS protocols used for dysphagia rehabilitation following stroke were adapted from the more established knowledge in the corticospinal system, which led to many heterogeneous approaches of M1 stimulation. Stimulating the cerebellum may offer an alternative to motor cortex tDCS for enhancing the effects of motor skill learning in swallowing and swallowing treatments. Cerebellar tDCS alleviates many problems caused by hemispheric interactions for a bilaterally-controlled neuromuscular event. No identification of the cortical motor hot spot for swallowing would be necessary, which reduces time and costs and also simplifies the implementation into clinical practice.

Cerebellar tDCS has been shown to facilitate neuroplasticity and motor learning in limb movement tasks (Buch et al., 2016). These findings provide a justification to evaluate similar effects in corticobulbar-related motor functions, like swallowing. The process of skill-learning in swallowing in both healthy subjects and patients with dysphagia could be facilitated with cerebellar tDCS. Elucidating the optimal methods of rehabilitation for patients with dysphagia requires investigation of the role of the cerebellum in skill-learning of swallowing events.

10. Hypotheses

10.1 Behavioural Study I

The effects of cerebellar tDCS on motor skill learning in swallowing.

Motor skill learning induces neuroplastic changes in cortical and subcortical brain regions that remain in the absence of practice (Dayan & Cohen, 2011). The cerebellum is known to correct movement errors and plays an important role in motor learning (Ito, 2000; Sokolov et al., 2017). Cerebellar tDCS has been demonstrated to change cerebellar excitability in a polarity-dependent manner (Galea et al., 2009) and to enhance motor skill learning in the corticospinal system (Cantarero et al., 2015). However, it is unknown if possible similar effects can also be found in corticobulbar-related motor functions, like swallowing.

Research questions: Does cerebellar tDCS improve motor skill learning for swallowing? Which stimulation polarity would be more effective? Do these effects maintain over a follow-up period of up to one week post training?

Objective: To evaluate immediate and long-term polarity dependent effects of cerebellar tDCS on motor learning in swallowing.

Hypotheses: Anodal cerebellar tDCS (20 min, 2 mA) when applied prior to swallowing skill training will enhance motor skill learning and maintenance of these effects for up to one week post training as assessed by the precision of submental muscle contraction relative to baseline and to the sham condition. The opposite effects are proposed for cathodal cerebellar tDCS using the same experimental protocol, i.e. cathodal tDCS will inhibit motor skill learning in swallowing.

Rationale: The majority of research indicates that anodal cerebellar tDCS in combination with motor learning paradigms for the corticospinal system enhances motor learning in healthy volunteers (Buch et al., 2016). However, results regarding the effects of cathodal cerebellar tDCS are inconclusive, although with some indication of

inhibitory effects on motor learning (Buch et al., 2016). Nevertheless, polarity dependent effects were found for neurophysiological investigations on cerebellar excitability following tDCS (Galea et al., 2009). It is, therefore, important to investigate the effects of both stimulation types of tDCS to identify polarity dependent effects on motor learning within the corticobulbar system. And if the effects of repeated application of tDCS in combination with visuomotor skill training that resulted in lasting effects of up to one week post training (Cantarero et al., 2015), can also occur for more reflexive motor behaviours.

Significance: The promise of cerebellar tDCS as an adjuvant technique to motor skill training offers new directions to enhance rehabilitative skill training approaches for motor recovery following stroke. With existing evidence for the effectiveness of skill training for dysphagia rehabilitation (Athukorala et al., 2014), cerebellar tDCS may provide additional benefit in the rehabilitation process by improving functional and physiological outcomes of swallowing in patients with dysphagia.

Proposed Study: In a double-blind randomised controlled trial, 39 healthy volunteers (> 50 years of age) will be assigned to one of three conditions (anodal tDCS, cathodal tDCS or sham), with approximate age and gender matching (Behavioural Study I in section 11). Two training sessions will be completed on consecutive days. Sessions will consist of a 2 mA current applied over midline cerebellum for 20 min, followed by a training task using sEMG biofeedback to target volitional control of magnitude and timing of submental muscle activation during swallowing. Effects on motor acquisition will be evaluated immediately after each training session; retention effects will be evaluated on days three and ten post training. Linear mixed effects models will be used to identify the effects of stimulation on accuracy measures indicative of skill learning.

10.2 Behavioural Study II

The effects of cerebellar tDCS on motor skill learning swallowing rehabilitation post stroke.

There is increasing evidence that motor cortical tDCS enhances motor recovery of stroke patients (Kang et al., 2016). However, there is no research which investigates the effects of *cerebellar* tDCS in this population. This study investigates the effects of cerebellar tDCS on motor skill learning and functional changes in swallowing physiology in patients with dysphagia following stroke.

Research questions: Does cerebellar tDCS enhance motor skill learning in post-stroke dysphagia rehabilitation? Furthermore, would tDCS related changes also be reflected in physiological and functional outcome measures of swallowing?

Objective: To evaluate immediate and long-term polarity dependent effects of cerebellar tDCS on motor skill learning in stroke patients with dysphagia.

Hypotheses: Anodal cerebellar tDCS (20 min, 2 mA) when applied prior to swallowing skill treatment is hypothesised to enhance motor skill learning and swallowing performance as assessed by the precision of submental muscle contraction relative to baseline and to the sham condition. The effects of this intervention will be maintained for up to one week post-treatment. Opposite effects were proposed for cathodal cerebellar tDCS; i.e. cathodal tDCS would inhibit motor skill learning in patients with dysphagia. Physiological and functional changes of swallowing will be assessed in a behavioural swallowing examination, including a cranial nerve examination, the TOMASS, the TWST and the EAT-10.

Rationale: Cerebellar tDCS has polarity dependent neurophysiological and behavioural effects that can enhance motor skill learning in healthy individuals. It is important to determine if the effects of this intervention can also be of merit for neurorehabilitation to facilitate recovery of dysphagia after stroke.

Significance: Effective treatment protocols are necessary for the recovery of patients with dysphagia. Adjuvant cerebellar tDCS could facilitate greater treatment effects from motor skill treatment for swallowing than the treatment in isolation.

Proposed Study: In this randomised double-blind proof-of-concept study, six stroke patients will be randomly allocated into one of three conditions: anodal cerebellar tDCS, cathodal cerebellar tDCS or sham (Behavioural Study II in section 12). This study uses the same experimental protocol as used with healthy volunteers. Data will be analysed using descriptive statistics.

10.3 Methodological study

The effects of midline cerebellar tDCS on motor evoked potentials from corticospinal and corticobulbar projections.

Unilateral cerebellar tDCS has previously been used to modify CBI (Galea et al., 2009); such modification is behaviourally measurable and significantly alters motor behaviour in the corticospinal system (Galea et al., 2011; Jayaram et al., 2011; Cantarero et al., 2015). Unlike limb motor control, motor tasks involving swallowing, speech, or proximal musculature of the torso, rely primarily on bilateral innervation. Midline electrode placement is hypothesised to be more efficient for these bilaterally-symmetrical motor tasks at midline.

Research question: Does *midline* cerebellar tDCS evoke neurophysiological changes in the corticobulbar or corticospinal system?

Objective: To investigate changes in CBI following cathodal tDCS applied over the cerebellum at midline, in order to evaluate potential use of this modality for bilaterally innervated motor tasks.

Hypotheses: Cathodal midline cerebellar tDCS would decrease CBI in the corticobulbar system as assessed by larger MEPs in the submental muscles at midline

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and/or in the corticospinal system as assessed by larger MEPs in the FDI muscle of the dominant hand relative to baseline and relative to sham condition.

Rationale: The investigation of neurophysiological changes for this electrode placement over the cerebellum at midline is hypothesised to provide an alternative and more effective stimulation for motor skill learning of bilaterally innervated body functions. Cathodal tDCS will be used to identify the effects of midline cerebellar tDCS, as anodal tDCS may result in a ceiling of the effect when measured using the CBI (Galea et al., 2009). Measurements of the FDI muscle will be included as it has previously been shown to be a reliable outcome measure of CBI (Galea et al., 2009; Doeltgen et al., 2015).

Significance: Significant changes of cerebellar tDCS at midline would imply effective stimulation of the cerebellum and would, therefore, broaden the scope of the application to more proximal bilateral motor functions including swallowing.

Proposed Study: In this randomised double-blind crossover study, fifteen healthy individuals will be recruited to assess changes in CBI using a paired transcranial magnetic stimulation paradigm before and up to 20 min following midline cathodal cerebellar tDCS (20 min, 2 mA) (Methodological study in section 13). Linear mixed effects modelling will be used to analyse changes in MEP amplitude following tDCS intervention compared to baseline and the sham condition.

PART II: Behavioural and methodological studies

11. Behavioural study I: The influence of cerebellar tDCS on motor learning in swallowing

11.1 Introduction

Cerebellar tDCS has been demonstrated to change cerebellar excitability in a polarity-dependent manner; anodal tDCS increases and cathodal tDCS decreases cerebellar excitability (Galea et al., 2009). These findings indicate a potential role of this technique when utilised as an adjunct to treatment approaches for neurological motor rehabilitation. However, the effects of cerebellar tDCS on motor skill learning for corticobulbar related motor functions, such as swallowing, are currently unknown. Investigating the effects of cerebellar tDCS for swallowing will identify the possible therapeutic role of this technique as an adjunctive tool for dysphagia rehabilitation. This study investigated polarity dependent effects of cerebellar tDCS on motor skill acquisition and retention for a swallowing task in healthy volunteers. It was hypothesised that anodal cerebellar tDCS would reduce movement errors of submental muscle activity (timing and magnitude) during swallowing; whereas cathodal tDCS would lead to the opposite effect (increase in movement errors) relative to baseline and the control condition.

11.2 Methods

Ethical approval was obtained from the New Zealand Health and Disability Ethics Committee (15/STH/46) and registered in the WHO-approved Australia New Zealand Clinical Trial Registry (ACTRN: 12615000451505). Informed consent was obtained from all participants prior to commencement of data collection (Appendix I).

11.2.1 Participants

Healthy volunteers aged 50 years and older were recruited. Recruitment continued until at least 36 complete sets of data (12 for each condition) were collected. Approximate age and gender matching was achieved by assigning the participants into three age-based subgroups within one condition; with age groups ranging from 50 – 64 years,

65 – 79 years and 80 + years. For the purpose of matching by group, at least two complete sets of data were collected from two participants of each gender within the two younger age groups and at least one of each gender within the oldest age group.

All participants were screened prior to enrolment using a questionnaire (Appendix II) to ensure they meet the inclusion criteria and were safe to undergo NIBS procedures based on recommendations by Pope (2015). No participant reported a medical history of existing swallowing, neurological or muscular impairments. In line with guidelines for participant enrolment into NIBS studies (Nitsche, Liebetanz, et al., 2003; Rossi et al., 2009), participants with recent brain or eye surgery (within the last 6 month), metallic implants in the skull or a history of epilepsy were also excluded from participation. Furthermore, participants with limitations in their visual function that would interfere with the use of visual feedback for task completion were also excluded. This was ensured by excluding patients with reported colour blindness and obtaining verbal confirmation from the patient that they could see and read everything on an example computer screen of the task.

11.2.2 Equipment

tDCS: The tDCS device (TCT Research Limited) was powered by two 9 V alkaline batteries. The device allowed a current output ranging from 0.5 mA to 2.0 mA with a precision of ± 0.004 mA and a current correction time of 45 ms. The maximal output voltage was 28 V. TDCS was delivered using the research version of the Transcranial Stimulation Kit including sham mode and a password protected stimulation setting for operator blinding. The direct current was applied via three rectangular rubber electrodes in saline-soaked (0.9% sodium chloride solution) sponge covers (5 cm $\times$ 5 cm). The sponge electrodes were held in place using a soft neoprene montage set.

sEMG: Submental muscle activity for the swallowing skill assessment and training was recorded using triode patch electrodes (EMG Triode[TM] Electrode from Thought Technology Ltd.). The spacing between the electrodes measured 2 cm. The submental muscle activity was recorded with a portable EMG biofeedback device (NeuroTrac® Simplex device, Verity LTD, UK). From there, data were sent via a fibre optic cable into a USB serial port which was plugged into a computer. The EMG device was a

single channel device, that displayed the linear envelope of the raw sEMG signal, with a sensitivity of 0.2 µV. The sampling frequency oscillated between 15 Hz − 20 Hz. The Biofeedback in Strength and Skill Training software (BiSSkiT^{CE}) version 1.0.0.1 displayed the acquired information as a time by amplitude waveform, in real-time, on a computer screen. The software offers assessment and training options for both swallowing strength and skill. Only the swallowing skill features were used for this study.

11.2.3 Study Protocol

The study was performed as a double-blind RCT. Participants were randomly assigned to one of three conditions: anodal tDCS, cathodal tDCS and sham. The participant and the researcher present during the procedure were blinded to the participant's allocated group. A second researcher was responsible for the randomised group allocation of the participants after receiving information about age and gender from the researcher who scheduled and performed the study. The second researcher also programmed the tDCS device into the active or sham stimulation setting depending on the group allocation of the participant prior to commencement of each training session. The researcher present during the study was blinded to the setting of the tDCS device (active or sham) to ensure unbiased handling of the participant throughout the study. However, the researcher performing the study was aware of the stimulation polarity (anodal or cathodal) of the electrodes, as this is evident for viewing the participant during stimulation.

All participants underwent the same study protocol and participated in two training sessions on consecutive days consisting of cerebellar tDCS (anodal, cathodal or sham) preceding sEMG biofeedback swallowing skill training. Retention and transfer tests (see section 5) of swallowing skill were performed before and after these sessions, and in two follow-up sessions, on day three and day 10 (one week post-stimulation) (Figure 25). Immediate retention (IR) of swallowing skill performance was assessed directly after the swallowing skill training sessions and preceding tDCS. Delayed retention (DR) tests were performed to assess the level of swallowing skill performance one day after the first and second day of training, and after one week following the first training session. Similarly, transfer tests were performed immediately (IT) and delayed

(DT) to assess swallowing skill learning, i.e. how the learned skills were transferred to an untrained condition. The results of the transfer tests performed during the first two days inform about the cumulative effects of *practice*. The assessments on day three and day 10 are more reflective measures of skill *learning* since they capture longer-term changes following two days of skill practice and consolidation periods, in which no additional training was completed.

Distinct differences of behavioural and neurophysiological processes have been described as contributing to changes during the training and after training ends (see section 5.1). Therefore, data from the retention and transfer tests were used to assess online and offline effects on swallowing skill performance and learning, as well as the effects during the consolidation phase.

At the end of day one and two, a comfort rating questionnaire regarding tolerance and side effects of the tDCS intervention (adapted from Brunoni et al., 2011; Appendix II) was completed by the participant at the end of each training session.
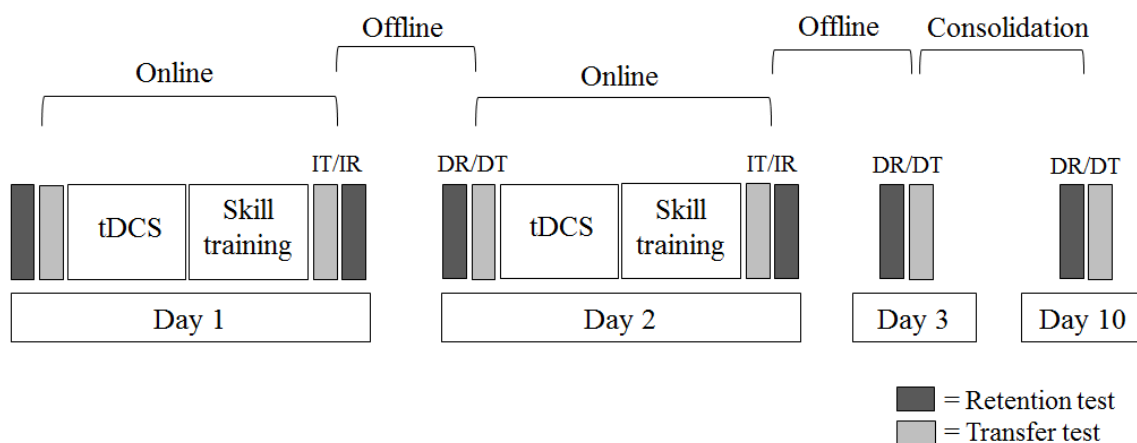
Figure 25: Experimental design of Behavioural study I. tDCS = transcranial direct current stimulation, IT = immediate transfer test (without feedback), IR = immediate retention test (with feedback), DR = delayed retention test (with feedback), DT = delayed transfer test (without feedback).

11.2.4 Experimental procedures

All equipment was prepared before the participant entered the laboratory. The participant was asked to sit on a chair in front of the computer screen. In preparation for attachment of sEMG electrodes, the participant's skin surface overlying the submental muscles was cleaned with an alcohol wipe. A triode patch electrode was placed with the two recording electrodes in anterior-posterior direction on the midsagittal plane to the skin overlying the submental muscles (Athukorala et al., 2014; Sella, 2012). After the participant was set up with the sEMG electrodes, a printed screenshot of the swallowing skill training task was used to explain (in the first session) or recapitulate (subsequent sessions) the swallowing task. The researcher verbally guided the participant through the characteristics of the time by amplitude waveform. It was explained that the electrodes under the chin were measuring muscle activity in real-time. The x-axis represented time in seconds, with a pre-set duration of each screen to 30 s. The y-axis on the computer screen represents the magnitude of submental muscle activity measured in µV. The amplitude of the waveform depended on the activity of the muscles underlying the electrodes. The waveform moved along the bottom of the screen when the participant's submental muscles were relaxed. However, the amplitude changed during each swallow when the muscles were active, i.e. more electrical activation of these muscles resulted in a greater waveform. The aim of the task was to swallow, such that the peak of the waveform was placed inside the green target box and to get as close as possible to the centre of the box, which was marked by a red cross. The participant was given time to ask questions following these explanations.

sEMG calibration: After responding to all questions, the skill assessment function of the BiSSkiT[CE] software was started. This initiates a two-step sEMG calibration process, which was performed at the beginning of every session. The first part of the calibration was the assessment of the participant's resting muscle activity. The participant was asked to relax and sit quietly without any movement of the tongue or mouth during this first part of the calibration process. The resting muscle activity was measured for a minimum of 10 s before the DC offset was removed, which set a mean amplitude displacement of the waveform for this participant back to zero. This calibration accounted for inter- and intra-individual variability, e.g. anatomical differences between the participants or slightly different electrode placement.

The second part of the calibration assessed the maximal muscle activity during five effortful swallowing trials. The participant was asked to swallow with maximal effort, "contracting all the muscles in mouth and throat as hard as possible during swallowing". The five swallows were performed with a frequency of approximately one every 30 s (one swallow per screen). The average peak amplitude during swallowing of those five swallows was derived by the software and set as the maximal value of the y-axis. This calibration value was also used to determine the size and the placement of the target for the skill assessment and training task. The participants were then encouraged to explore their individual swallowing behaviour, e.g. swallowing with more or less effort or at different points in time, in regards to the changes of the real-time sEMG feedback on a computer screen without a target for one minute. This provided them an opportunity to understand better how the waveform was influenced by their intrinsic motor behaviour, and how it related to the upper limit of the y-axis.

Cerebellar tDCS: The sponge covers for the tDCS electrodes were soaked in saline solution and excess was removed before placement on the participant's head. Any excess solution that led to irritation of the participant, e.g. solution running down the neck or cheeks, was removed using a paper towel. The sponge electrodes were held in place using the soft neoprene montage set which is adjustable in size using Velcro®. The cerebellar electrode was centred at midpoint 2 cm below the inion (Ferrucci et al., 2013; Parazzini et al., 2014) and the current of the other polarity was divided across two electrodes, allowing for bilateral placement over the buccinator muscles (Figure 26). This is in contrast to the commonly used unilateral placement of the active and reference electrode in the corticospinal literature (Ferrucci, Cortese, & Priori, 2014). However, this placement was considered appropriate for bilaterally controlled, midline tasks as it would allow for an equal current distribution to both sides of the head (see section 8).

Figure 26. Electrode montage and device used for cerebellar transcranial direct current stimulation (tDCS). Electrode placement of the second electrode on the left cheek mirrors the placement on the right cheek but is not visible in this picture.

The stimulating current was pre-set at 2 mA intensity and delivered for 20 min over the cerebellum. Stimulation using these parameters induces a current at a density of 0.08 mA/cm^2. This level of stimulation has previously been shown to change cerebellar excitability (Ferrucci et al., 2013; Galea et al., 2009, 2011) and is well below the threshold for tissue damage (Liebetanz et al., 2009). The onset of the tDCS current was ramped up over 30 s, which is an established part of tDCS protocols to reduce irritation (van Dun et al., 2016). If the sham operation setting of the tDCS device was turned on, no current flowed through the electrodes during the application. The researcher explained to the participant that the stimulation would be applied for 20 min. It was also explained that the participant might feel a slight tingling sensation during this time or might not feel anything. It was said that the sensation of the stimulation depends on everyone's individual sensibility threshold. The participant was instructed to sit comfortably in the chair throughout the stimulation and to only speak if necessary. However, the participant was specifically asked to provide an indication to the researcher in any event of pain or discomfort, so the researcher was able terminate the stimulation immediately. The researcher was present throughout the stimulation procedure and ensured the participant's well-being half-way through, by verbal questioning. At this time, the researcher also applied 10 ml of saline solution to the three sponge pads on the side that was in contact with the head using a syringe. This

stopped the sponge material from drying out throughout the stimulation process. The tDCS device automatically monitored the electrode resistance to ensure sufficient skin contact. Additional saline was applied to the sponges to prevent skin irritation or injury if indicated by the device. All equipment used for the tDCS intervention was removed on completion of the stimulation protocol. The sEMG electrodes remained in position during the stimulation; however, the cables were disconnected from the portable sEMG device to ensure that there was no interference of electrical circuits.

Swallowing skill training: Cerebellar tDCS was followed by 20 min of biofeedback-assisted swallowing skill training. The participants used their own saliva to swallow once per screen (i.e. every 30 s), resulting in 40 swallowing trials in total. This swallowing frequency has successfully been used in studies on healthy participants (Sella, 2012) and patients with Parkinsons Disease (Athukorala et al., 2014). The duration of the swallowing skill training was held equal to the duration of tDCS period. The aim of this training was to increase the precision of volitional control of submental muscle activity during swallowing. The participants were instructed to place the peak of the sEMG waveform inside the target and to get as close as possible to the centre of the target by controlling the timing and strength of activation of these muscles during swallowing. The target was randomly placed in a different positions on each screen sweep; therefore, the required muscle activity during swallowing was not predictable. Additional feedback on performance was provided for motivation in four different ways and explained to the participant. First, the waveform and its movements in relation to the target were visible throughout each trial. Hence, the participants received online feedback during the execution of each swallowing trial. Secondly, the target changed size depending on the participant's performance. It decreased in size if the participant hit the target three time in a row, which consequently made it more difficult to hit the target in the subsequent trial. Conversely, the target increased in size following three consecutive misses. Thirdly, the words 'Hit' or 'Miss' appeared next to the target on completion of each swallowing trial (Figure 27). These words indicated if the peak fell inside ("Hit") or outside the target ("Miss"). Lastly, a number appeared simultaneously and underneath the written feedback. This number indicated the distance (in cm) from the centre of the target to the peak to the swallowing waveform. The smaller the number, the closer the peak was to the target centre. Zero would indicate that the peak of the sEMG waveform was exactly placed on top of the centre of the target.

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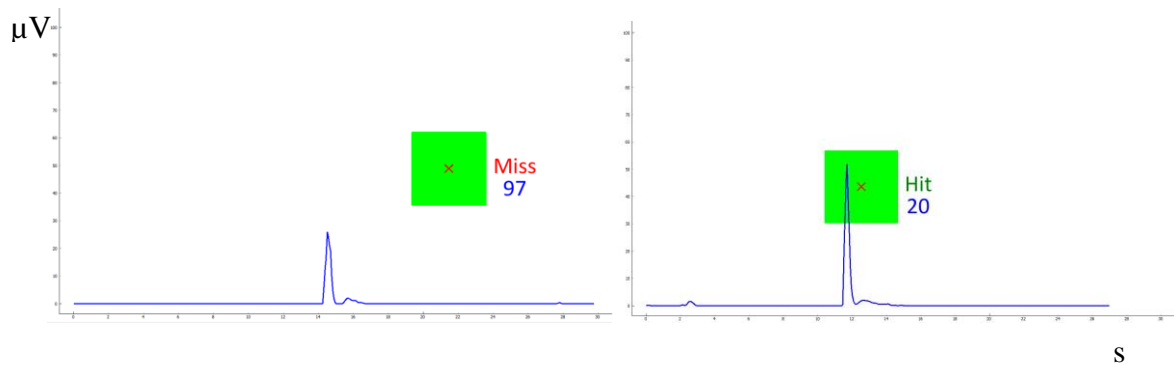
Figure 27. An unsuccessful and successful trial with visual and additional written feedback in the swallowing skill training task using the BiSSkiT^CE software. The y-axis represents amplitude in microvolts (µV) and x-axis represents time in seconds (s).

The BiSSkiT^CE software randomly positioned the target, in both the time and amplitude domain, on the computer screen. The x-axis denoted time in seconds and used a four second margin at the beginning and the end of the screen where the target could not be placed, i.e. the target could only be placed between 4 s – 26 s. This setting was implemented to give the participant enough time to visually recognise and prepare for the next swallow (a minimum of 8 s). The y-axis ranged from 0 – 100% of the calibration value (average peak amplitude of the five effortful swallows). Placement of the target varied between 20% - 70% of the calibration value (target movement range). The upper limit ensured that swallowing with maximal effort, as would be used for strength training, was not required for this skill training paradigm. The initial size of the target square was set to 50% of the allowed target movement range. The target size decreased or increased by 10% following three successful or unsuccessful trials respectively. These settings were adapted from the skill training protocol used by Athukorala et al., 2014. The provided feedback incorporated both KP, by monitoring the movements of the sEMG waveform in real-time, as well as KR, by viewing the peak of the sEMG waveform in relation to the centre of the target, with the additional quantitative feedback after the attempt. The important role of feedback, including KP and KR, in achieving novel and volitional swallowing tasks has previously been demonstrated for participants using VFS as biofeedback (Macrae, Anderson, Taylor-Kamara, & Humbert, 2014). The protocol for this skill training was designed based on the three vital components of a skill-based therapy described by Huckabee and Macrae

(2014): specificity of practice, task challenge and feedback. In particular, specificity of practice was achieved by using swallowing as the task itself, task challenge was given through the changes in target size, and lastly, additional quantitative visual feedback was provided to the participant supplementary to the real-time feedback.

Outcome measurements

Swallowing skill performance: Retention tests were performed to assess swallowing skill performance using the same conditions under which swallowing skill was practised during the training, i.e. with online feedback. However, this task was adapted in that the target was always positioned in the centre of the screen and did not vary its position. This was necessary to ensure a stable point of comparison across participants (Figure 28). In accordance with the skill training protocol, the size of the target measured 50% of the allowed target movement range for amplitude.
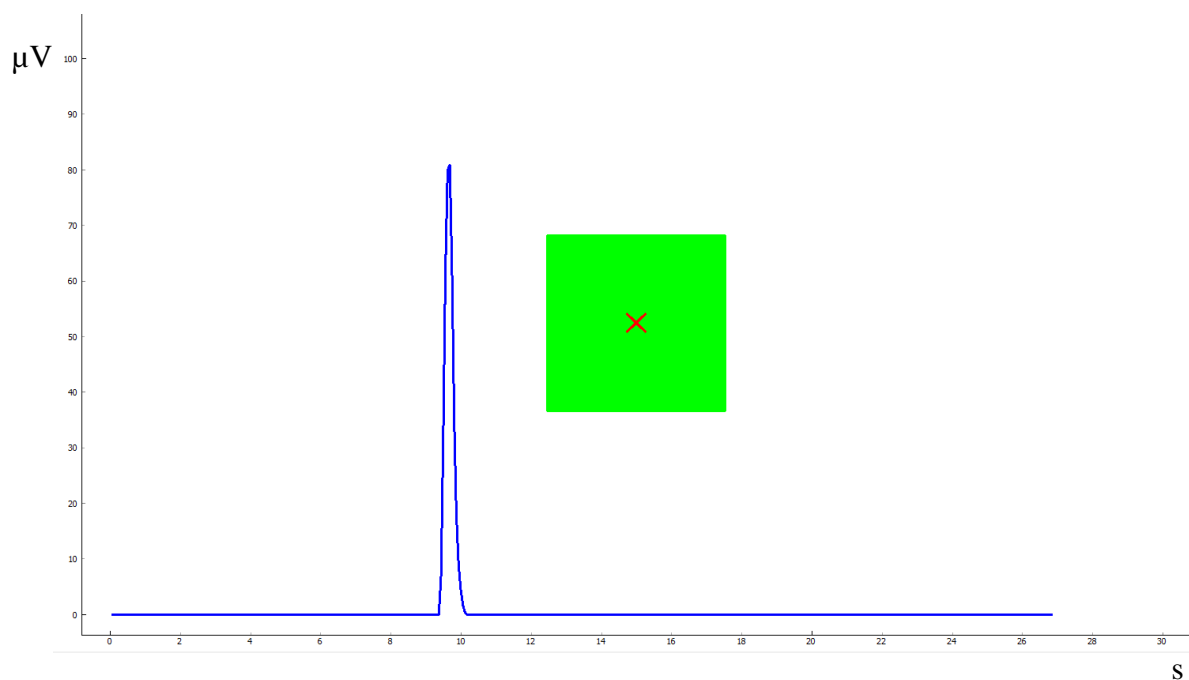
Figure 28. Screenshot of one swallowing trial during the retention test using the BiSSKiT[CE] software. Amplitude in microvolts (μV) and time in seconds (s).

Swallowing skill learning: Since transfer tests require a variation of the practiced skill (Kantak & Winstein, 2012) and motor skill learning can only be assessed in the absence of the feedback by which the skill was obtained (Salmoni et al., 1984), no visual feedback was provided during these transfer test trials. The waveform disappeared randomly at a time between two and five seconds prior to the target, requiring the participants to perform the task based on their intrinsic representation of the screen and motor control model (Figure 29). Furthermore, a smaller target size was chosen (30% of the allowed target movement range) to provide continual challenge throughout the task, even for individuals with no reported or perceived swallowing impairment. In line with the skill training protocol, the target varied in position within the same time and amplitude constraints. The researcher monitored the patient during this assessment closely and marked each swallow by pressing a key on the keyboard. This procedure ensured that the EMG peak representing the swallow could be identified in later analysis.
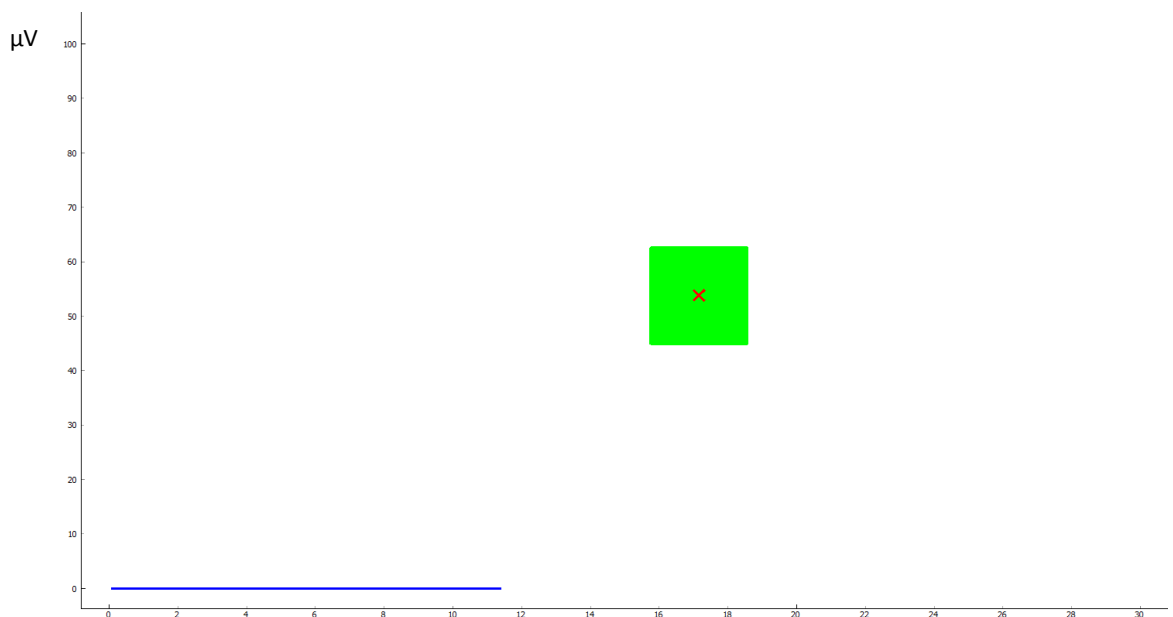
Figure 29. Screenshot of one swallowing trial during the transfer test using the BiSSKiT^CE software. Amplitude in microvolts (μV) and time in seconds (s).

Both retention and transfer tests consisted of 10 saliva swallowing trials each. In accordance with skill training, the goal of the task remained the same - to place the peak of the waveform inside the target as close as possible to the centre. The participants were reminded of the task goal and familiarised with the specifics of the upcoming assessment before each of the two assessments.

Transfer tests were performed immediately before and after the skill training task to avoid additional practice effects of the retention test with visual feedback. On request, the participants were allowed to drink sips of water between the swallowing assessments, or between the tDCS and skill training, but not during the swallowing training or assessments. Less than half the participants (n = 19) underwent the calibration and retention test as part of a concurrent skill and strength study in the swallowing laboratory with another researcher. However, the exact same protocol and instructions have been used, and the assessment took place immediately before participating in the rest of the first session of this study.

tDCS tolerance questionnaire: Following the training sessions, a questionnaire was completed to probe participant comfort and perceived side effects (Appendix II). The researcher asked if the participant experienced any of the listed side effects. If side effects were experienced, a second question was asked to identify if they perceived that the stimulation was the cause. The side effect "skin redness" was rated by the researcher.

Data collection and post-processing

The BiSSkiT^{CE} software automatically selects the highest peak of the sEMG signal as the swallowing event. If the maximum value did not correspond with the swallowing attempt (e.g. artefact due to movement) as visually obeserved by the investigator on site, the researcher manually selected the sEMG peak associated with swallowing, as indicated by the marker. During the transfer tests, where the sEMG waveform was not visible to the participant or the researcher for the swallowing trial, the researcher pressed a key on the keyboard immediately after the participant swallowed to mark this attempt. This allowed selecting the sEMG peak associated with the swallowing attempt in the post-analysis process using the BiSSkiT^{CE} software review mode.

A trial was discarded from the overall analysis in the event of peak-clipping. These events may be explained by as disproportionate submental muscle activation used for the execution of a particular swallow, unstable electrode to skin contact, extraneous and simultaneous body movements of the participant during the swallowing attempt or accidental contact between the connection cable and the sEMG device. The researcher immediately responded following invalid trials, ensuring sufficient electrode to skin contact and by reminding the participant to relax while swallowing. Invalid swallowing trials were discarded and not included in the analysis.

11.2.5 Data analysis

The differences between the peak of the sEMG waveform and the centre of the target for temporal (s) and amplitude (μV) domains were calculated (Figure 29). These absolute error measurements were then normalized to the maximum value of each axis. For the temporal error, the maximum duration of each trial was fixed at 30 s, whereas the maximal amplitude was set to the individual's calibration value (see section 11.2.4). The relative error was chosen to account for the differences in amplitude between participants and within individual participants across sessions, as the amplitude scale was recalibrated for each session. The relative temporal and amplitude error measurements were exported and averaged across the 10 swallowing trials of each assessment block, using a spreadsheet program (Microsoft Excel 2010).

Linear mixed effects model analysis was performed in RStudio (version 3.2.5) using the packages lme4 and lmerTest (Bates, Maechler, & Bolker, 2012). First, the model assumptions (linearity, homoscedasticity, normality of residuals) were checked. Transformations of the data were performed if the assumptions were violated. Changes in motor performance and learning between and within stimulation groups across time points were evaluated in separate models for each of the two response variables, the temporal error and the amplitude error. The mixed model consisted of the fixed-effects stimulation condition (anodal, cathodal, sham) and timepoint (Baseline, Post day 1, Pre day 2, Post day 2, Follow-up 1 day, Follow-up 1 week) as well as the random effect "subject", assuming a different intercept for each subject. The mixed models with interaction and without interaction were compared to identify interaction effects and the best-fit model. Observed power calculations of the interaction effects were performed

using the function "powerSim" from the library "simr" for n = 1000 simulations and will be reported with their 95% confidence intervals.
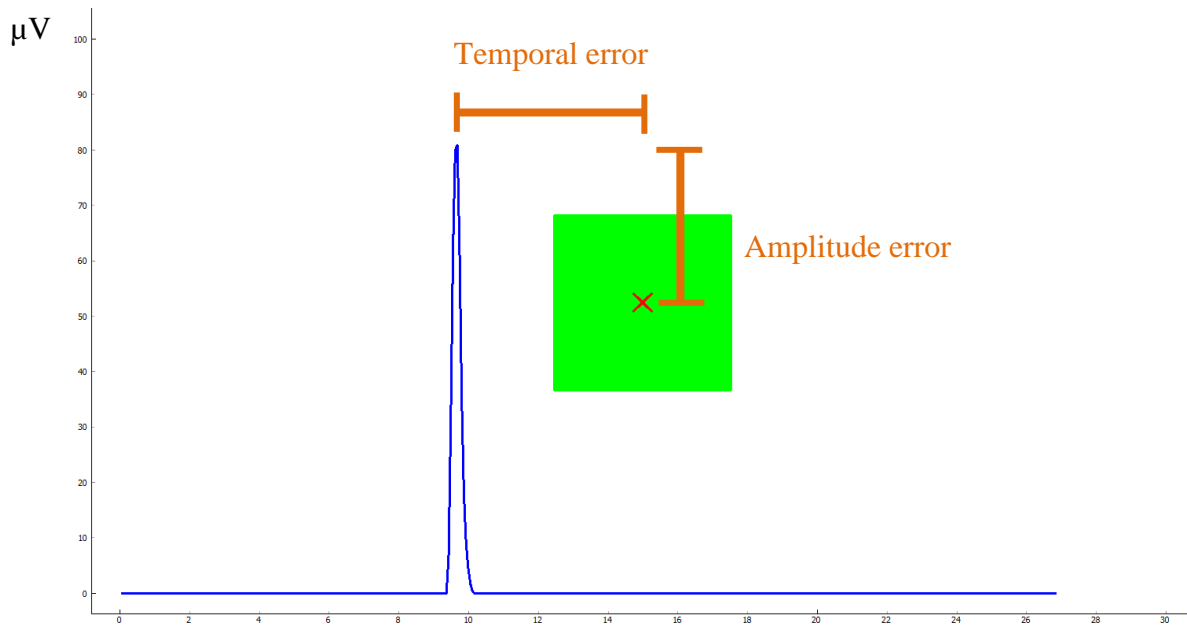
Figure 30. Computation of the temporal and amplitude error. Amplitude in microvolts (µV) and time in seconds (s).

Further analysis was undertaken with the best-fit model as a result of these analyses. The stimulation condition "Sham" and the timepoint "Baseline" were set as reference categories. This allowed evaluation of differences in motor skill learning and performance of the stimulation conditions compared to the sham condition over time.

The impact of cerebellar tDCS on different stages of motor performance and learning were tested in a separate mixed effects analysis for the two response variables (RV; temporal error and amplitude error). The phase of learning (online, offline, consolidation) and stimulation condition (anodal, cathodal, sham) were identified as fixed effects and subjects as the random effect of this analysis. Online effects were defined as the changes from before to after the training sessions, offline effects as the changes between consecutive sessions and consolidation effects as changes over the

follow-up period where no stimulation or training took place (Figure 25, page 114). Calculation of the effects were performed as follows:

- Online effects = ($RV_{\text{Post day 1}} - RV_{\text{Baseline}}$) + ($RV_{\text{Post day 2}} - RV_{\text{Pre day 2}}$)
- Offline effects = ($RV_{\text{Pre day 2}} - RV_{\text{Post day 1}}$) + ($RV_{\text{Follow-up 1 day}} - RV_{\text{Post day 2}}$)
- Consolidation effects = ($RV_{\text{Follow-up 1 day}} - RV_{\text{Follow-up 1 week}}$)

11.3 Results

11.3.1 Participant and group composition

Forty-four healthy volunteers consented to participation in this study. Thirty-nine participants successfully completed the study. The results of four participants were discarded for different methodological reasons. Insufficient electrode contact resulted in an inaccurate display of the sEMG signal during data collection for one. Another performed non-swallow movement to achieve peaks in the sEMG signal. Two other participants were not able to produce maximal muscle activity during the calibration swallows, which led to invalid trials (peak-clipping) throughout the skill assessment and training. Lastly, one participant withdrew from participation because of illness unrelated to the research on day two of the training. Table 2 displays participant details for the three stimulation groups for all remaining participants.

Table 2. Participant details for the three groups of Behavioural Study I.

	Anodal	**Cathodal**	**Sham**
Quantity	13	13	13
Average age in years (range)	71 (56 - 84)	71 (56 – 84)	71 (57 – 86)
Gender	6 male/ 7 female	6 male/ 7 female	6 male/ 7 female

11.3.2 The effects of cerebellar tDCS on skill learning in swallowing

Approximately 5% of the swallowing trials were discarded prior to the analyses because of the previously defined reasons (see section 11.2.4). Log-transformation (natural logarithm with base e) was used for all valid data to account for a violation of the non-constant residual variance assumption for both error measures (amplitude and temporal).

Amplitude error: For the amplitude error, there was no significant interaction effect for stimulation condition and timepoint $\chi^2(10) = 9.40$, $p = 0.50$ [observed power 48.90% (45.76, 52.05)] nor a significant main effect of stimulation group $\chi^2(2) = 1.56$, $p = 0.46$. There was a significant main effect of session $\chi^2(5) = 21.705$, $p < 0.001$. All groups were significantly different to baseline at all measurements post training including the follow-up measurements (Table 3), but there was no difference between the post-training and follow-up measurements (e.g. post-training session 1 was not significantly different to one week post training).

Table 3. Skill learning: Percentage of change in the amplitude error from baseline for each timepoint regardless of stimulation conditions.

Timepoint	% change from baseline	Lower 95% CI	Upper 95% CI	*p*-value
Post 1	-22.87	-33.44	-10.48	<0.001*
Pre 2	-19.31	-30.48	-6.34	<0.005*
Post 2	-23.61	-34.19	-11.34	<0.001*
Follow-up 1 day	-28.88	-37.86	-16.29	<0.001*
Follow-up 1 week	-16.45	-28.02	-3.03	=0.019*

*Note: Post 1 = Post training session 1 assessment; Pre 2 = Pre session 2 assessment; Post 2 = Post training session 2 assessment; 95% CI = 95% confidence interval; * = statistically significant effect. Raw data was used for the statistical analysis, change from baseline is used for demonstration purposes only.*

Temporal error: There was an overall significant interaction effect of stimulation condition and timepoint for the temporal error $\chi^2(10) = 20.85$, $p = 0.02$ [observed power 91.20% (89.27, 92.88)]. The three stimulation conditions were not significantly different from each other at baseline ($p > 0.30$). The change in the anodal group was significantly different to the sham condition at all timepoints, using the sham baseline measures (statistical estimates) as reference point (Figure 31). The change in the cathodal group was only significantly different from sham at the follow-up assessments. Only the sham group significantly decreased in the temporal target error from baseline to all other timepoints (Table 4). For the changes of the anodal and cathodal group see Appendix III.
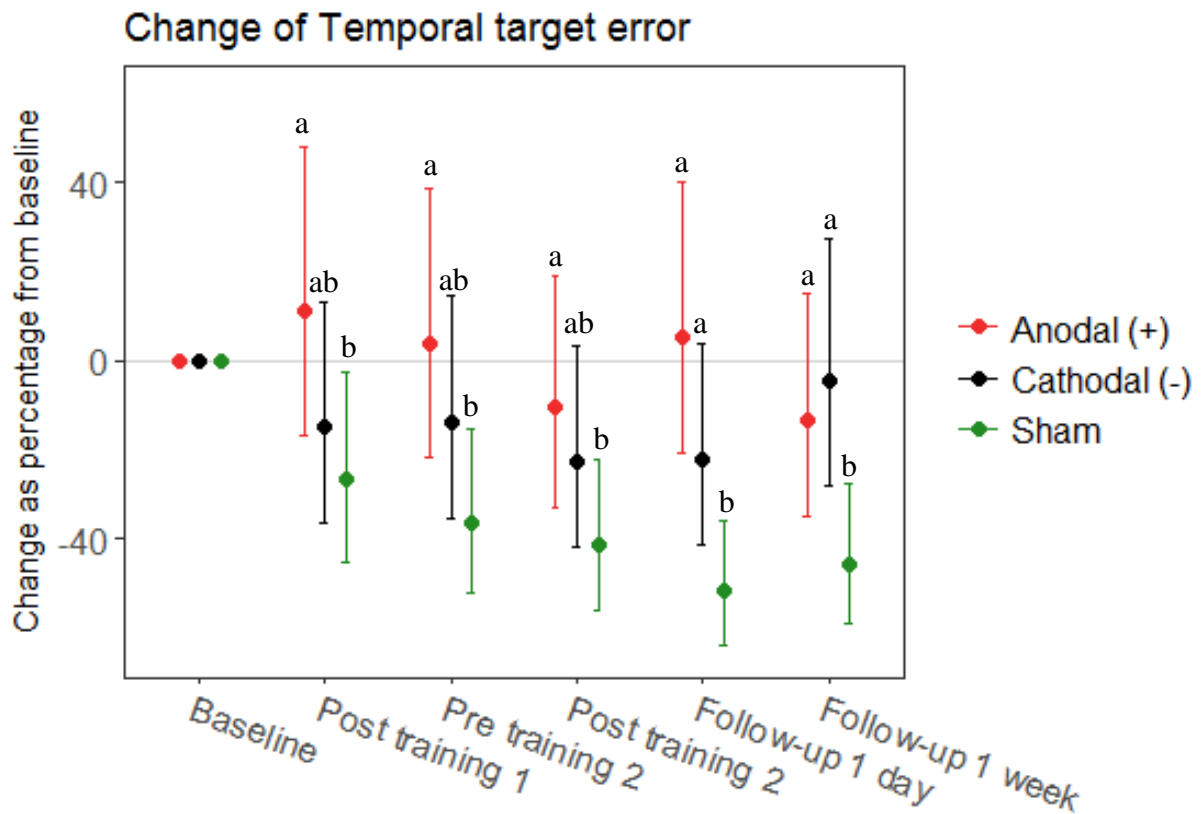
Figure 31. Percentage change in the temporal error (95% confidence interval) from baseline for skill learning in all three conditions (Post 1 = Post training session 1 assessment; Pre 2 = Pre training session 2 assessment; Post 2 = Post training session 2 assessment). Negative values represent a decrease in error, i.e. higher accuracy. Raw data was used for the statistical analysis, change from baseline is used for illustration purposes only. The letters a and b indicate statistically significant differences ($p < 0.05$) between stimulation within timepoint; the same letters indicate no statistical significance, different letters indicate statistical significance in the change from baseline for this timepoint.

Table 4. Skill learning: Percentage changes from baseline in the temporal error for the sham group.

Timepoint	% change from baseline	Lower 95% CI	Upper 95% CI	*p*-value
Post 1	-26.93	-45.15	-2.64	=0.034*
Pre 2	-36.50	-52.34	-15.39	=0.002*
Post 2	-41.58	-56.15	-22.16	<0.001*
Follow-up 1 day	-51.88	-63.88	-35.89	<0.001*
Follow-up week	-45.81	-59.32	-27.79	<0.001*

*Note: Post 1 = Post training session 1 assessment; Pre 2 = Pre training session 2 assessment; Post 2 = Post training session 2 assessment; 95% CI = 95% confidence interval; * = statistically significant effect. Raw data was used for the statistical analysis, change from baseline is used for demonstration purposes only.*

Testing the effects of cerebellar tDCS on different phases of learning (online, offline and consolidation) for both error measures revealed no significant interaction for the temporal error $\chi^2(4) = 8.49$, $p = 0.08$ [observed power 64.20% (61.14, 67.18)] nor the amplitude error $\chi^2(4) = 4.07$, $p = 0.40$ [observed power 32.60% (29.70, 35.60)]. There were no significant main effects for stimulation $\chi^2(2) = 3.08$, $p = 0.22$ nor learning phase $\chi^2(2) = 5.05$, $p = 0.08$ for the temporal error. There was a significant main effect for learning phase $\chi^2(2) = 15.83$, $p < 0.001$ but not for stimulation $\chi^2(2) = 0.37$, $p = 0.83$ for the amplitude error.

11.3.3 The effects of cerebellar tDCS on skill performance in swallowing

Prior to the analysis of skill performance, approximately 5% of the swallowing trials were discarded because of the reasons defined in section 11.2.4. Log-transformation (natural logarithm with base e) was used to account for a violation of the non-constant residual variance assumption for both error measurements. The effects of stimulation on swallowing skill performance were assessed, as before, by comparing the changes in separate analyses for the two error measures across timepoints, and within and between groups. There was no significant interaction effect between stimulation and timepoint

for both error measures [amplitude error: $\chi^2(10) = 13.28$, $p = 0.21$; temporal error: $\chi^2(10) = 11.45$, $p = 0.32$], nor a significant main effect of stimulation group [amplitude error: $\chi^2(2) = 3.46$, $p = 0.18$; temporal error: $\chi^2(2) = 0.24$, $p = 0.88$]. However, the main effect of timepoint was significant [amplitude error: $\chi^2(5) = 21.90$, $p < 0.001$; temporal error: $\chi^2(5) = 14.92$, $p = 0.01$]. All groups were significantly different to baseline at all of the follow-up measurements (Table 5; Table 6).

Table 5. Skill performance: Percentage of change in the amplitude error from baseline for each timepoint regardless of stimulation condition.

Timepoint	% change from baseline	Lower 95% CI	Upper 95% CI	*p*-value
Post 1	-24.47	-34.58	-12.798	<0.001*
Pre 2	-13.47	-25.05	-0.1	=0.049*
Post 2	-21.38	-31.9	-9.23	<0.001*
Follow-up 1 day	-23.79	-33.99	-12.011	<0.001*
Follow-up 1 week	-23.35	-33.61	-11.51	<0.001*

*Note: Post 1 = Post training session 1 assessment; Pre 2 = Pre session 2 assessment; Post 2 = Post training session 2 assessment; 95% CI = 95% confidence interval; * = statistically significant effect.*

Table 6: Skill performance: Percentage of change in the temporal error from baseline for each timepoint regardless of stimulation condition.

Timepoint	% change from baseline	Lower 95% CI	Upper 95% CI	*p*-value
Post 1	-18.1	-31.11	-2.63	=0.025*
Pre 2	-27.08	-38.66	-13.31	<0.001*
Post 2	-21.95	-34.35	-7.21	=0.006*
Follow-up 1 day	-21.45	-33.93	-6.62	=0.007*
Follow-up 1 week	-21.55	-34.01	-6.73	=0.007*

*Note: Post 1 = Post training session 1 assessment; Pre 2 = Pre session 2 assessment; Post 2 = Post training session 2 assessment; 95% CI = 95% confidence interval; * = statistically significant effect.*

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11.3.4 Tolerance and side effects of tDCS intervention

Mild to moderate tingling was the most commonly reported side effect for both of the stimulation groups (Table 7). The reports of side effects were similar between the two active stimulation groups, and between the first and second session of the intervention. Even in the absence of stimulation, two of the thirteen participants reported a mild tingling sensation in the sham condition. None of the participants experienced headache, neck pain or acute mood change associated with the tDCS intervention. Other side effects, associated with the active tDCS conditions, included scalp pain, itching, burning sensation, stinging, slight warm pressure during the stimulation. Skin redness was rare, being reported for no more than two participants in each stimulation group. One participant reported a mild headache the next morning. However, he was unsure if it was a definite side effect of the stimulation. Since he did not experience a headache the morning following the second tDCS session, it is questionable whether it can be linked to the stimulation. Another participant reported sleepiness and two others had mild trouble concentrating during tDCS. However, both participants did not consider these events to be the result of tDCS. One participant reported enhanced concentration during the stimulation. Another reported a slight change in eyesight which returned to normal after the session and only occurred in one of the two sessions. Since the questionnaire was asked at the end of each session and not immediately after tDCS, the participant was unsure if this side effect was due to the stimulation or because of the high concentration on the biofeedback task.

Table 7. Intensity and result of stimulation ratings for tingling sensation below the electrodes (the most commonly reported side effect) for session and condition.

		Total # participants	No	Possibly	Probably	Definitely
Anodal						
Session 1	Absent	3				
	Mild	9		2	2	5
	Moderate	1				1
	Severe	0				
Session 2	Absent	4				
	Mild	6	2	1	2	1
	Moderate	3			1	2
	Severe	0				
Cathodal						
Session 1	Absent	2				
	Mild	9		2	1	6
	Moderate	2				2
	Severe	0				
Session 2	Absent	2				
	Mild	8		2		6
	Moderate	3	2			1
	Severe	0				
Sham						
Session 1	Absent	11				
	Mild	2	1			1
	Moderate	0				
	Severe	0				
Session 2	Absent	11				
	Mild	2			1	1
	Moderate	0				
	Severe	0				

11.4 Discussion

This is the first study to assess the effects of cerebellar tDCS on motor skill learning in swallowing. Both anodal and cathodal tDCS had a relative inhibitory effect on skill learning in swallowing when compared to the sham condition. Cerebellar tDCS applied prior to swallowing skill training affected temporal accuracy but not the magnitude accuracy of submental muscle activation during swallowing. In contrast to the hypotheses and the corticospinal literature, anodal tDCS inhibited the temporal aspects of motor skill learning in swallowing. Similar to the corticospinal literature, cathodal tDCS inhibited motor skill learning of temporal accuracy gains compared to the sham condition. Interestingly, cerebellar tDCS only affected skill learning in swallowing, but not the performance measures. All groups regardless showed immediate and lasting improvements in measures of swallowing skill performance compared to baseline.

Polarity independent result

Although the majority of neurophysiological and behavioural studies on cerebellar tDCS documented opposing effects for the two polarity protocols (Chen et al., 2014; Galea et al., 2009; Herzfeld et al., 2014; Jayaram et al., 2012; Yavari et al., 2016b), both anodal and cathodal tDCS inhibited the learning of temporal accuracy of submental activation during swallowing in the current study. Nevertheless, in line with the results of this study, Ferrucci and colleagues (2008) found an inhibitory effect of both anodal and cathodal cerebellar tDCS at midline on reaction time in a working memory task. In contrast, the study by Shah and colleagues (2013) demonstrated enhanced behavioural effects for both polarity types in motor performance of a lower limb tracking task. The contrary effects of the two polarities may be explained by the different tasks used to document outcomes or the different electrode placements over the cerebellum used for these investigations. For example, Ferrucci et al. (2008) and the current study used an electrode placement at midline that targeted both cerebellar hemispheres simultaneously, whereas the study by Shah and colleagues (2013) utilised a unilateral electrode placement. Since the electrode placement influences the direction of the current flow through neural tissue (Woods et al., 2016), this is could be one explanation for the opposing polarity dependent results.

Inhibitory effects of anodal tDCS

Anodal tDCS is most commonly reported to enhance motor function in the corticospinal system (Buch et al., 2016). Contrary to the predictions made for this study, anodal cerebellar tDCS resulted in an inhibition of temporal motor skill learning following tDCS.

One explanation might be found in the nature of neural control mechanisms for the different types of motor behaviours. It could be hypothesised that connections of the cerebellum to higher cortical centres might have different neurophysiological mechanisms for motor functions in the corticospinal system than for motor functions of the corticobulbar system. This idea is supported by reports of inhibited behavioural effects following anodal cerebellar tDCS in the corticobulbar system (Panouillères, Miall, et al., 2015), which are in contrast to enhanced effects of anodal tDCS reported for the corticospinal system (Galea et al., 2011). Furthermore, Jayasekeran and colleagues (2011) provided neurophysiological evidence for this hypothesis. They demonstrated facilitory neural connections of the cerebellum for motor cortical output from the pharyngeal area using paired-pulse TMS to test CBI. This is contrary to the corticospinal system where inhibitory connections have been demonstrated using a similar neurophysiological assessment (Daskalakis et al., 2004). These findings indicate that neurophysiological differences between the two motor systems might explain the inhibitory effects of anodal cerebellar tDCS in the corticobulbar system found in this study. Stimulation parameters that have successfully been used to enhance motor skill learning in the corticospinal system might therefore not be transferable to corticobulbar motor tasks. Future studies assessing these differences in neural control systems with neurostimulation and neuromodulation are required.

The inhibitory effects of anodal cerebellar tDCS could also be the consequence of different tDCS parameters used in this study, such as stimulation intensity or electrode placement. Computer modelling studies of cerebellar tDCS and animal studies suggest that an intensity of 2 mA is required to reach the neurons within the cerebellum (Priori et al., 2014; Rampersad et al., 2014). A midline placement over the cerebellum is strong enough to evoke changes in cerebellar excitability without spreading to brainstem structures (Parazzini et al., 2014) and to result in behavioural changes, e.g. slower reaction time in a working memory task (Ferrucci et al., 2008). Therefore, a stimulation

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intensity of 2 mA over the cerebellum at midline was used in this study. However, dividing the reference electrode to the buccinator muscles to suit a bilaterally innervated motor task, might have diminished the electric field in either of the cerebellar hemispheres to approximately 1 mA. In contrast to studies that used 2 mA over one cerebellar hemisphere, studies that used 1 mA or 1.5 mA of unilateral cerebellar tDCS reported significant but heterogeneous behavioural effects (Avila et al., 2015; Dutta, Boulenouar, Guiraud, & Nitsche, 2014; Shah et al., 2013). For example, Shah and colleagues (2013) utilised 1 mA unilateral cerebellar tDCS and demonstrated surprising findings of an excitatory effect for cathodal tDCS in a behavioural locomotor task. The inhibitory effect of anodal tDCS in the current study might, therefore, be explained by an overall smaller current intensity applied to each cerebellar hemisphere as a result of the modified electrode placement. In detail, 2 mA anodal direct current has been applied midline over the cerebellum; however, by splitting up the cathodal electrodes to either side of the head only 1 mA of the current may have reached each side of the cerebellar hemispheres. In fact, decreasing tDCS intensity to 1 mA per hemisphere might have shifted the direction of excitability changes. Similar effects were demonstrated for cathodal tDCS of M1, where inhibition changed into excitation by increasing the stimulation intensity from 1 mA to 2 mA (Batsikadze et al., 2013). There is no study that directly compares the effects of different stimulation intensities over the cerebellum using a midline electrode placement on motor behaviours in healthy volunteers. More research is required to identify effects of different tDCS intensities and electrode placements on the same behavioural or neurophysiological outcome measures for cerebellar tDCS.

Cerebellar tDCS affects temporal not magnitude accuracy

Cerebellar tDCS influenced the learning of the temporal control of submental muscle contraction but not the magnitude of muscle activation during swallowing. These findings are in line with findings from Cantarero et al. (2015), who found that cerebellar tDCS only affected one component of motor learning. In their study on motor learning in the upper limb, only accuracy but not speed in a speed-accuracy trade-off task was affected by cerebellar tDCS. Cantarero et al. (2015) discussed that "different components of motor skill learning (i.e. error and speed) may be predominantly controlled by different neural circuits with the cerebellum largely influencing error reduction" (Cantarero et al., 2015; page 3289). In contrast to volitional cortical control

of accuracy and speed in upper limb movements, temporal and magnitude control of submental muscle activity during swallowing are components of a primarily brainstem driven, semi-reflexive motor function. However, oral and pharyngeal motor sequences during swallowing can be modulated from higher cortical centres (Ertekin, 2011; Lamvik et al., 2015). Therefore, the results of the current study support Cantarero et al.'s findings, indicating that different neural control mechanisms may be used for different components of motor skill learning and that they may respond differently to tDCS. In addition, findings from the current study demonstrate that this may also apply for mainly brainstem driven motor functions. However, this hypothesis would have to be tested directly.

Cathodal tDCS only inhibited long-term retention of motor skill learning

While anodal cerebellar tDCS inhibited temporal motor learning across all timepoints, the effects of cathodal tDCS developed over time and only inhibited temporal motor learning following consolidation periods and two day of skill training. In other words, the effects of cathodal cerebellar tDCS needed longer to evoke changes but then lasted for up to one week. The retention of the learned motor skills has been recognised to be the result of more permanent alterations in cerebellar connections in the form of LTD of Purkinje cells (Vigot, 2003). The current study demonstrated that both polarities of cerebellar tDCS in combination with motor training might evoke LTD-like processes in the cerebellum for motor skill learning of corticobulbar motor functions.

Cerebellar tDCS did not differentially affect different phases of motor learning

There were no significant differences between the three groups for the online learning phases (pre to post session) of the two days of motor skill training, nor the offline learning phases between the training and follow-up sessions. The finding of an insignificant difference between groups for online learning is contrary to findings of a similar single case study performed by Cantarero and colleagues (2015) in the corticospinal system. This could be the result of differences in the study protocols. Cantarero and colleagues (2015) evaluated the effects of tDCS on motor learning over three days of training, whereas two days were assessed in the current study. The additional session of training or stimulation might have been responsible for the significant difference of anodal tDCS compared to cathodal tDCS or the sham group.

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Another explanation for these results might be the timing of the stimulation. In the current study, a study design where cerebellar tDCS prior to swallowing skill training was chosen for the following reasons: Several minutes of cortical tDCS have been demonstrated to result in long-term changes of neural excitability after the stimulation was terminated (Nitsche, Fricke, et al., 2003; Nitsche & Paulus, 2001; Sehm et al., 2012). In addition, concurrent application of tDCS and submental sEMG elevated the baseline of the EMG signal (~ 20 µV) and introduced oscillation due to interference. Lastly, studies exploring the effects of tDCS over M1 found that only stimulation applied prior to motor training, but not during or after the training, had an effect on corticospinal excitability (Cabral et al., 2015). However, when cerebellar tDCS was applied during the performance of a limb motor task, significant differences between online and offline learning have been demonstrated (Cantarero et al., 2015). These results are contrary to the non-significant findings in this study, suggesting that the timing of cerebellar tDCS, applied to an active or passive neural network, might be a crucial variable. These findings are in line with research using tDCS over the motor cortex during a cognitive and motor task which demonstrated that synaptic plasticity is influenced by the state of neural network activity (Antal, Terney, Poreisz, & Paulus, 2007). Technical solutions to reduce the interference of concurrent tDCS in the EMG signal would be required to test the effects of cerebellar tDCS during swallowing skill training using submental sEMG.

Swallowing skill training improves performance

All groups, independent of stimulation condition, improved in their performance measures of temporal and spatial accuracy of submental muscle contraction throughout the swallowing skill training and maintained this performance level up to one week following the training. The improvements in skill performance are in line with Caruana (2015), who found that one hour of swallowing skill training using the BiSSKiT[CE] software increased the accuracy of submental muscle contraction during swallowing in healthy volunteers. Caruana measured skill performance in ten swallowing trials at the beginning and the end of the training session, dividing the mean target hit rate of ten trials by the mean target area. In addition, an increased swallowing capacity (ml per second) following one skill training session has been demonstrated (Caruana, 2015). Similar changes, a significant increase in swallowing volume (ml per swallow) and decrease in time per swallow, following two weeks of swallowing skill training were

also demonstrated for patients with PD (Athukorala et al., 2014). However, increased swallowing capacity, swallowing volume or less time needed per swallow may not necessarily indicate more efficient or safe swallowing for patients with dysphagia. Future research is required to identify possible functional changes in swallowing as a result of skill training in different patient populations with swallowing impairment.

Changes in swallowing performance following skill training and in functional measures of swallowing physiology may indicate neurophysiological changes as the result of motor adaptation or motor learning. Caruana (2015) investigated excitability changes of corticobulbar projections from the motor cortex following one session of swallowing skill training but did not find significant changes. The author discusses that this negative result is likely caused by the high variability between subjects in the outcome measures. In contrast, other studies using a tongue-protrusion task for skill training over seven consecutive days found neurophysiological changes immediately and at one day post-training (Svensson et al., 2003, 2006). These findings suggest that more than one session of skill training might be necessary to evoke neurophysiological changes as a result of motor learning. A neurophysiological assessment before and after the two day skill training in this study would have strengthened this study design.

Conclusion

Both anodal and cathodal cerebellar tDCS inhibited temporal motor skill learning in swallowing, thus suggesting that the cerebellum is an important component of circuitry involved in skill learning for swallowing. Having adapted commonly used parameters of cerebellar tDCS to modulate motor skill learning in the corticospinal system, the results of this study demonstrated changes in the opposite direction. Therefore, recent neurophysiological findings about functional differences of the connection between the cerebellum and the cortex for corticobulbar and corticospinal tasks are supported (Jayasekeran et al., 2011). A direct comparison of the effects of cerebellar tDCS on neurophysiological and behavioural measures is necessary to receive more insights into the differences between these two systems. The swallowing skill training and assessment using the BiSSKiT[CE] software has been demonstrated to be sensitive in evoking and measuring changes in swallowing skill-learning, even in the healthy population. It can, therefore, be a useful tool to gain further insights into motor skill learning in swallowing and stimulation related changes.

12. Behavioural study II: The effects of cerebellar tDCS on motor skill learning swallowing rehabilitation post stroke.

12.1 Introduction

The use of motor skill training for cortical reorganisation of motor networks is a well-established concept of neurorehabilitation in physical therapy and is emerging into practices for dysphagia rehabilitation. Unilateral tDCS over the motor cortex, combined with skill training, enhances motor recovery of the contralateral limb in stroke patients (Kang et al., 2016). Swallowing, however, is a bilaterally innervated, brainstem driven motor behaviour, with lateralised hemispheric input during swallowing. Targeting lower brain regions, such as the cerebellum, bilaterally with tDCS might be an option to influence motor skill learning in swallowing more effectively. There is no research investigating the effects of cerebellar tDCS on swallowing in patients following stroke. This proof-of-concept study investigates the feasibility of cerebellar tDCS on motor skill learning and functional changes in swallowing physiology in patients with dysphagia following stroke.

12.2 Methods

Ethical approval was obtained from the New Zealand Health and Disability Ethics Committee (15/STH/46) and registered in the WHO-approved Australia New Zealand Clinical Trial Registry (ACTRN: 12615000436572). Informed consent was obtained from all patients prior to commencement of data collection.

12.2.1 Participants

For this proof-of-concept study, six stroke patients (18 years and older) with oropharyngeal dysphagia were recruited. Using a random number generator to assign two participants each to one of three conditions (anodal tDCS, cathodal tDCS or sham) without replacement. Stroke patients were recruited through a specialised stroke rehabilitation research clinic. Prior participation in swallowing rehabilitation did not exclude participation; current and ongoing rehabilitation did. All participants were screened prior to enrolment to ensure they meet the inclusion criteria and were safe to

undergo NIBS following the recommendations from Pope (2015) (Appendix II). Patients with recent brain surgery (within the last 6 months), metallic implants in the skull, and a history of drug use or epilepsy were excluded from participation. Furthermore, patients with difficulty following instructions because of cognitive impairment or significant limitations in visual function that inhibited research task performance were excluded.

12.2.2 Equipment

This study utilised the same equipment for the tDCS intervention and the swallowing skill training that was used for Behavioural Study I. In particular, tDCS was delivered using the research version of the Transcranial Stimulation Kit (TCT Research Limited). TDCS was applied via three rectangular rubber electrodes in sponge covers (5 cm × 5 cm) that were soaked in a 0.9 % sodium chloride solution and held in place using a soft neoprene montage set. Furthermore, sEMG from the submental muscles was recorded using triode patch electrodes (EMG Triode^{TM} Electrode from Thought Technology Ltd.). The same portable sEMG biofeedback device (NeuroTrac® Simplex device, Verity LTD, UK) and Biofeedback in Strength and Skill Training software (BiSSkiT^{CE}) version 2.0 were used to process and display the acquired information on the computer screen. In addition, a small digital video camera, stopwatch, Arnotts Salada^{TM} cracker, measuring cup, torch and water mist spray bottle were needed for the behavioural assessment and treatment.

12.2.3 Study protocol

The same study protocol as in Behavioural study I with healthy participants was used for this study, including swallowing skill assessments (retention and transfer tests), cerebellar tDCS (double-blinded) and skill training. In addition, pre-existing standard evaluation measures were used to assess swallowing function prior to the commencement of the study and in the follow-up assessments (Figure 32). This behavioural swallowing exam was performed by a second clinician to avoid bias from the clinician providing the training interfering with the subjective outcome measures.

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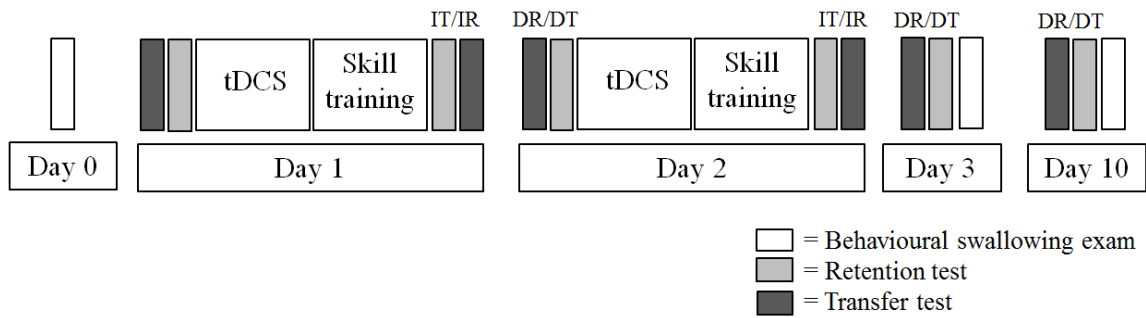
Figure 32: Experimental design of Behavioural study II. tDCS = transcranial direct current stimulation, IT = immediate transfer test (without feedback), IR = immediate retention test (with feedback), DR = delayed retention test (with feedback), DT = delayed transfer test (without feedback).

12.2.4 Experimental procedure

Behavioural swallowing examination: The behavioural swallowing examination was completed by a speech-and-language therapist (SLT) with 10 years of experience in dysphagia assessment in neurologically impaired patients. The clinician was blinded to the type of intervention that was received and was not present during the treatment sessions. The majority of instructions given throughout the assessment were standardised but extended if the patient required further explanation to complete the assessment.

Cranial nerve (CN) exam: The function of the CNs was assessed using a standard clinical CN exam protocol. The patient was told that the muscles of their face and throat will be assessed. Facial symmetry and functioning of the facial muscles (CN VII) was tested by observations of various movements of the upper and lower face, including movements of the eyebrows, eyelids and lips without and against resistance. CN V (motor) was assessed by asking the patient to open and close their mouth with resistance to the jaw. Furthermore, hyolaryngeal elevation and anterior movement was palpated. The sensory component of CN V was tested by touching the patients face in various areas and asking where the sensation was felt. The patients eyes were closed during this assessment to avoid visual localisation of the touched area. In addition, the patient was asked to volitionally cough (CN X) and to says "ah" to test palate elevation (CN IX and X) and vocal quality (CN X). The function of CN XII was assessed via

tongue movements, including protrusion, retraction and lateral movements against resistance. The sensory integrity of CN VII, IX and X were not tested. An additional assessment (e.g. testing the sensation of taste of the anterior tongue or cough testing for pharyngeal sensation) was considered too time-consuming for this already overall lengthy protocol, when balanced against the value of information it would provide. Lastly, the structural integrity of the oral cavity and teeth was assessed through inspection with a torch.

Timed water swallow test (TWST): The protocol outlined by Hughes and Wiles (1996) was followed for the TWST, including adaptation of the amount of water by using 150 ml for patients below 74 years and 100 ml for patients older than 75 years. A video camera recorded the patient's lip, jaw, and throat movements thought the test. The clinician instructed the patient: "Please drink all of this water as quickly and comfortably as possible, without stopping". During task execution, the number of swallows, time taken and total volume swallowed were noted. Timing commenced when the cup reached the participant's lower lip and stopped when the larynx dropped into baseline position after water consumption. The number of swallows during this time was counted by observation of the participant's thyroid elevation. If the patient was unable to drink the entire amount of water, e.g. due to the swallowing impairment, the remaining water was measured and subtracted from the total amount. This number was then used to calculate the volume of water per swallow in ml. Furthermore, the time used per swallow in s, and the swallowing capacity as volume of water swallowed per time (ml/s) were calculated for comparisons with normative data (Hughes & Wiles, 1996).

Test of Masticating and Swallowing Solids (TOMASS): The patient was given one quarter of an Arnotts Salada™ cracker and instructed: "I would like you to eat this cracker. I'd like you to finish it without any water. Please eat this as quickly as is comfortably possible. When you have finished, say your name". The number of swallows, number of masticatory cycles, number of bites and total time taken to ingest the full amount were recorded by the clinician. The timing was started when the cracker touched the lips or the teeth and stopped when the patient said their name. The number of swallows was assessed by observing thyroid elevation associated with swallowing.

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The number of masticatory cycles was counted with one masticatory cycle defined as an up and down movement of the jaw. Therefore, a bite of a segment of the whole cracker was also considered to be one masticatory cycle. The number of bites was assessed by counting the number bites used to taken the whole cracker or cracker segments into the mouth. If the whole cracker was taken in at once, it was counted as one bite. For uncertain results, the video recordings of the TWST and TOMASS were used by the same investigator to ensure accuracy of the data.

Swallowing skill assessment and intervention: The primary researcher of this study performed the swallowing skill assessment and intervention with the patient. Analogously to the study with healthy volunteers, all equipment was prepared prior to the session. Once seated in front of the computer screen, the skin surface overlying the submental muscles was cleaned with an alcohol wipe before the triode patch electrode was placed in anterior-posterior direction on the midsagittal plane to the skin overlying the submental muscles (Athukorala et al., 2014; Sella, 2012). The researcher verbally guided the patient through the characteristics of the task and visual feedback using the printed screenshot of the BiSSKiT^{CE} swallowing skill training task. The aim of the task was to swallow, such that the peak of the waveform was placed inside the green target box and to get as close as possible to the centre of the box, which was marked by a red cross. The patient was given time to ask questions following these explanations.

sEMG calibration: The two-step sEMG calibration process was performed at the beginning of every session. First, the patient's resting muscle activity was assessed for 10 s, before the DC offset was removed. Secondly, the maximal muscle activity during five effortful swallows was assessed. The patient was asked to swallow with effort in a frequency of approximately once per 30 s (one swallow per screen), contracting all the muscles in mouth and throat as hard as possible during swallowing. The number of calibration swallows was reduced to three swallows if the patient had severe difficulties executing this task, e.g. swallowing initiation. In line with the previous experiment, the average peak amplitude was set as the maximal value of the y-axis and determined the size and the placement of the target for the skill assessment and training task. Following calibration, the patients were encouraged to explore their individual swallowing behaviour for one minute using the real-time sEMG feedback on a computer screen without a target.

Cerebellar tDCS: Saline-soaked sponge electrodes were placed on the head using the soft neoprene montage set. For cerebellar tDCS, the stimulating electrode was centred at midpoint 2 cm below the inion (Ferrucci et al., 2013; Parazzini et al., 2014) and the reference electrodes were divided, allowing for bilateral placement over the buccinator muscles (Figure 26; Behavioural Study I). The stimulating current was pre-set at 2 mA intensity and delivered for 20 min over the cerebellum (30 s ramp-up time). In the sham condition, no current flowed through the electrodes during the application. As in the prior study, it was explained that a slight tingling or no sensation might be felt during the stimulation. The patient was instructed to sit comfortably in the chair throughout the stimulation and to only speak if necessary. Half-way through the stimulation procedure, the researcher ensured the patients well-being by verbal questioning. In addition, 10 ml of saline solution were applied to the three sponge pads on the side that was in contact with the head using a syringe. Additional saline was applied to the sponges if indicated by the device, which automatically monitored the electrode resistance to ensure sufficient skin contact. All equipment used for the tDCS intervention was removed on completion of the stimulation protocol. The sEMG electrode remained in position during the stimulation; however, the cables were disconnected from the portable sEMG device to ensure that there was no interference of electrical circuits.

Swallowing skill training: Following tDCS, 20 min of swallowing skill training using the BiSSkiT^{CE} software was completed. The patients were asked to swallow their saliva once per screen, i.e. approximately every 30 s, resulting in 40 swallowing trials in total. The patients were instructed to place the peak of the sEMG waveform inside the target and to get as close as possible to the centre of the target by controlling the timing and strength of swallowing. Additional feedback on performance was provided for motivational purposes. This included online feedback of the waveform in relation to the target, changes in target size following three consecutive successful or unsuccessful trials and written feedback. The written feedback consisted to the written words 'Hit' (successful trial) or 'Miss' (unsuccessful trial) and provided distance (in cm) from the centre of the target to the peak of to the swallowing waveform, where a small number indicated a close distance to the centre of the target. The target was randomly positioned on the computer screen, placed between 4 s – 26 s on the x-axis and between 20% - 70% of the calibration value on the y-axis. The initial size of the target square was set to 50% of the allowed target movement range and decreased or increased in size

by 10% following three successful or unsuccessful trials respectively (Athukorala et al., 2014).

Outcome measurements

In line with Behavioural study I, retention and transfer tests were performed immediately and delayed to assess the effects of cerebellar tDCS on motor *performance* and *learning* (see section 11.2.4). Retention tests were performed to assess swallowing skill performance with online feedback during the swallowing trials (Figure 28, Behavioural study I). Swallowing skill learning was assessed in the absence of visual feedback in the transfer test trials (Figure 29, Behavioural study I). Both assessment tasks used saliva swallows and consisted of 10 swallowing trials each. In accordance with skill training, the goal of the task was to place the peak of the waveform inside the target as close as possible to the centre. The participants were reminded of the task goal and familiarised with the specifics of the upcoming assessment before each of the two assessments.

Following the training sessions with tDCS, a questionnaire was completed to probe participant comfort and perceived side effects (Appendix II). The researcher asked if the participant experienced any of the listed side effects. If side effects were experienced, a second question was asked to identify if they perceived that the stimulation was the cause. The side effect "skin redness" was rated by the researcher.

In addition, the results of the behavioural swallowing examination including the CN exam, TWST and the TOMASS were used as indicators of change associated with the stimulation condition.

12.2.5 Data analysis

The results of the behavioural swallowing examination were reported based on the clinician's notes performing the assessment. The temporal and amplitude error were computed to analyse swallowing skill performance and learning. Analogous to Behavioural Study I, the differences between the peak of the sEMG waveform and the centre of the target for temporal (s) and amplitude (μV) domains were calculated (Figure 30, Behavioural Study I). These absolute error measurements were then

normalised to the maximum value of each axis. The relative temporal and amplitude error measurements were then exported and averaged across the 10 swallowing trials of each assessment block using a spreadsheet program (Microsoft Excel 2010). Given the small sample size, with only two individuals per stimulation condition, descriptive statistics were used to summarise and visualise the main features of the data. Figures were generated using RStudio (version 3.2.5).

12.3 Results

12.3.1 Patients

Eight stroke patients consented to participate in this feasibility study. One patient withdrew from participation on the first day due to an inability to meet the time commitment for this study. Another patient was excluded post-study, as she was not able to complete the swallowing skill training during the first session. Table 8 displays patient details and allocation into the three stimulation conditions.

Table 8. Patient demographics and stimulation condition for Behavioural Study II.

	Age	Gender	Medical diagnosis	Months post-onset	Diet	EAT-10
Anodal						
103	79	Male	Brainstem CVA; Previous stroke in 1996 (left hemiparesis)	59	Puree diet, thin liquids	19
104	69	Male	Cerebellopontine angle CVA	45	Normal diet, thin liquids	5
Cathodal						
107	69	Male	Right posterolateral medullary infarct with right occipital infarct	5	NPO	31
108	67	Male	Brainstem CVA (posterior fossa)	28	Normal diet, thin liquids	17
Sham						
102	70	Female	Brainstem CVA; Previous stroke in 2012	41	NPO	22
105	74	Female	Ischemic CVA (left hemiparesis); Previous stroke in 2002	135	Normal diet, thin liquids	4

Note: EAT-10 = Eating Assessment Tool 10; CVA = Cerebral vascular accident; NPO = Non per oral

12.3.2 Methodological adaptations

The following methodological adaptations of the swallowing skill assessment or training were undertaken if the patient required these to complete the task:

- Moisten the oral cavity: The frequent initiation of dry swallows was too challenging for three (patient 103, 105, 108) out of the six patients with oropharyngeal dysphagia. Therefore, half a squirt of water mist spray (< 1 ml) was provided to moisten the patient's oral cavity on request.

- Calibration value: For the calibration value – average value of five effortful swallows that is determined for the scaling of the y-axis and target placement – the differentiation between normal and effortful swallowing is required. However, this was difficult for four out of the six patients. If no difference between the effortful and normal swallowing condition was observed, even after training trials (as also explained under section 11.2.3), a manual change of the calibration value (e.g. 200%) was performed. The position of the target box adapted automatically in relation to the new calibration value. Without adaptation of the calibration value, the patients would have not been able to complete the assessment as the majority of swallowing trials would have been invalid (e.g. peak clipping).

- Reduced task complexity: Although only patients with the cognitive ability to follow instructions were included in this study, the more complex skill learning assessment (no visual feedback of the sEMG waveform) was particularly challenging and could not be completed by four out of the six patients.

- Number of trials: All patients had difficulties eliciting a swallow every 30 s. Therefore, an increased number of trials were completed to achieve ten valid swallowing attempts per assessment. The percentage of successful swallowing trials out of total swallowing attempts was noted for each assessment block and each patient. Patients with trouble initiating a swallow were encouraged to keep trying to swallow, even if the sEMG waveform might have already passed the target. This ensured that patients kept trying to initiate one swallow on each screen. All adaptations that were made throughout the study procedure were individually noted and are explicitly mentioned in the results section.

12.3.3 Single case analyses by stimulation condition

Anodal tDCS

Patient 103

Behavioural swallowing examination: Patient 103 presented with mild impairments of CN VII and CN XII resulting in left side facial weakness and mild impairments of tongue lateralisation movements in the initial behavioural swallowing assessment. Furthermore, a weak volitional cough was noted, suggesting some impairment of CN X. The function of all other CNs were not impaired. When drinking 100 ml of water for the TWST, he achieved a smaller volume per swallow and swallowing capacity compared to the average performance of age and gender-matched healthy individuals (Hughes & Wiles, 1996; Table 9). He needed more than double the time for task completion of the TOMASS and performed a greater number of masticatory cycles compared to norms (Huckabee et al., 2017) and coughed at the end of the test. In contrast, he performed less bites and swallows per cracker compared to the age-matched healthy individuals (Huckabee et al., 2017) and presented with oral residues following the trial. His initial EAT-10 score of 19 (out of 40) indicated a moderate to severe swallowing impairment (Belafsky et al., 2008).

One and seven days post-treatment, his CN examination resulted in marginal changes of the impaired functions. The amount of water that he took per swallow during the TWST was still outside the range of normal and even more reduced (Hughes & Wiles, 1996). He required fewer masticatory cycles and more swallows and bites when eating the cracker in the TOMASS evaluation compared to his first assessment; these scores were in the range of normal behaviour (Huckabee et al., 2017). His EAT-10 score went down to 13 on day one and to seven on day seven post-treatment which reflected improvements in the patient's perception of his swallowing disorder from pre- to post-intervention.

Table 9. Results of the TOMASS and TWST for patient 103.

Timepoint	TWST			TOMASS			
	V/S (ml)	T/S (s)	V/T (ml/s)	Chews	Bites	Swallows	Time (s)
Baseline	10.6*	5.3*	2.1*	78**	1**	1**	82.2**
Follow-up 1	9.0*	4.3*	2.3*	67	4**	2	90.8**
Follow-up 2	7.3*	4.3*	1.8*	66	3	1**	82.2**

*Note: * Outside the range of normal based on the Timed Water Swallowing Test (TWST) norms (Hughes & Wiles, 1996); ** Outside the range of normal based on the Test of Masticating and Swallowing Solids (TOMASS) norms (Huckabee et al., 2017); V/S = Volume per Swallow in millilitre (ml); T/S = Time per Swallow in seconds (s); V/T = Volume per Time in millilitre per second (ml/s); Time in seconds (s); Follow-up 1 = one day post-treatment; Follow-up 2 = one week post-treatment.*

Swallowing skill assessment: During the initial swallowing skill assessment, the patient had difficulties initiating swallowing. He was therefore not able to complete 10 valid trials with a swallowing frequency of one swallow per one 30 s screen. The patient required 33 trials to initiate six swallows in the retention test at baseline. The assessment was terminated by the patient following 33 trials due to complaints of exhaustion and concentration. The transfer test without visual feedback could not be performed due to these difficulties and the patient's loss of concentration.

Accuracy in the timing and magnitude of submental muscle activation during swallowing in the retention test assessing swallowing performance changed only marginally over time (Figure 33). However, there was a noticeable increase in swallowing performance in the amplitude error one week post intervention compared to baseline (Figure 34). Furthermore, the patient was able to reduce the number of swallowing trials needed to produce at least six valid swallowing trials over time compared to baseline (Follow-up one day: 9/24 trials; Follow-up one week: 10/14 trials). The patient was not able to complete the swallowing skill training due to complaints of fatigue but he increased the number of trials performed during training from 15 trials in the first session up to 20 trials in the second session.

Figure 33. Individual results of the relative temporal target error for motor skill learning and performance across all timepoints. Different colours denote the stimulation condition. StimGroup = Stimulation Condition. x-axis: 2 = Baseline; 3 = Post-treatment day 1; 4 = Pre-treatment day 2; 5 = Post-treatment day 2; 6 = Follow-up one day; 7 = Follow-up one week post-treatment.

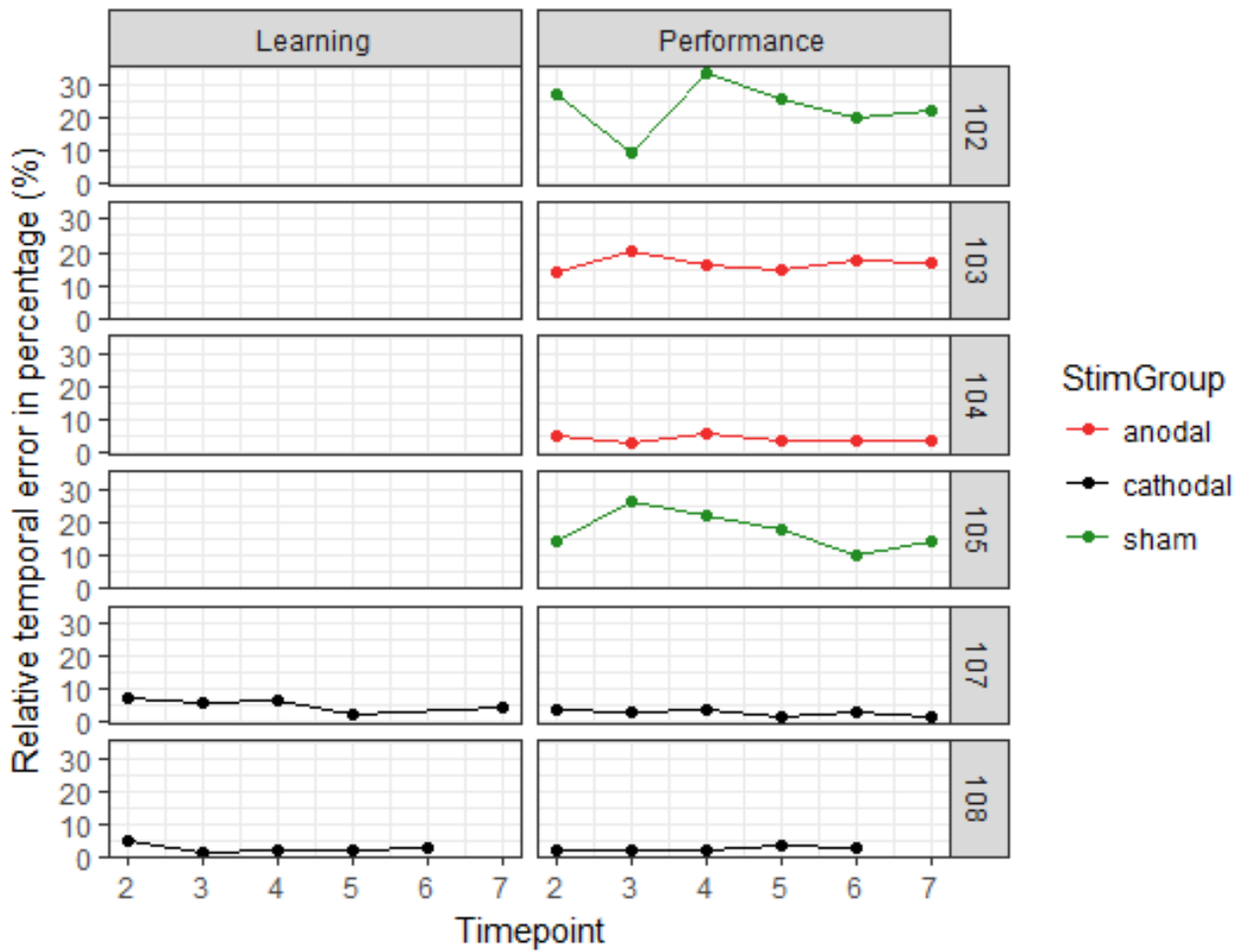*Figure 34.* Individual results on the relative amplitude target error for motor skill learning and performance over time. Different colours denote the stimulation condition. StimGroup = Stimulation Condition. x-axis: 2 = Baseline; 3 = Post-treatment day 1; 4 = Pre-treatment day 2; 5 = Post-treatment day 2; 6 = Follow-up one day; 7 = Follow-up one week post-treatment.
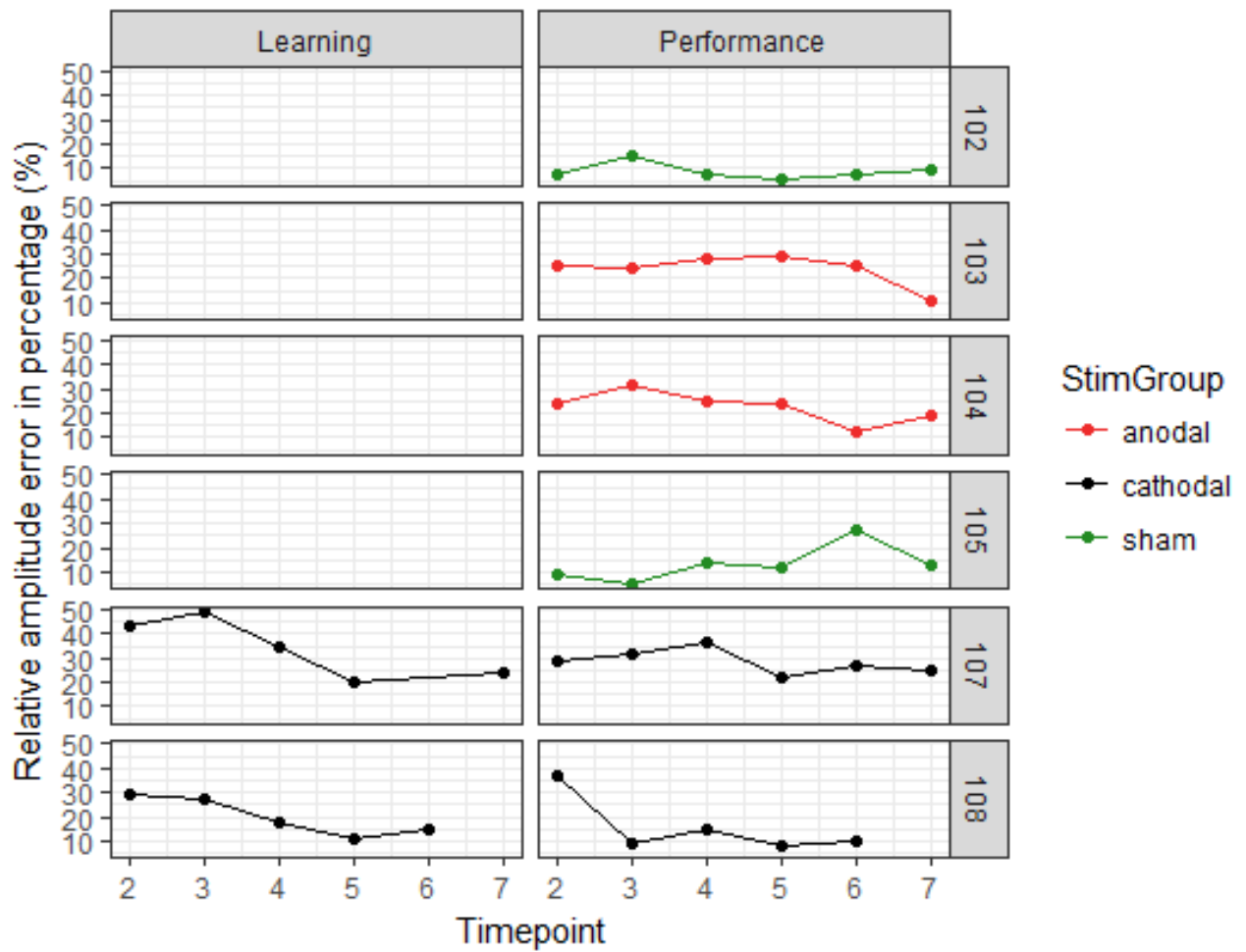
Patient 104

Behavioural swallowing examination: Patient 104 presented with left-sided weakness when opening and closing his lips to resistance, the remaining motor functions of CN VII were within an adequate range. The patient had a hoarse voice and a weak volitional cough, implying impairment of CN X. All other CNs presented with no clinical signs of impairment. His volume per swallow of thin liquids in the TWST and his swallowing capacity were reduced compared to age and gender-matched healthy individuals (Hughes & Wiles, 1996; Table 10). He also took more time per swallow when drinking water in the TWST compared to norms (Hughes & Wiles, 1996). The results of the TOMASS were close to or within the normal range of the results from age and gender-matched controls (Huckabee et al., 2017) at baseline. One exception, however, was that he only used one swallow to clear the oral cavity without any residues which is less than the usual three swallows needed by healthy controls. His EAT-10 score was five (out of 40), indicating a mildly perceived swallowing impairment.

Post-treatment, function of the CNs did not change. The volume per swallow, swallowing capacity and time per swallow further decreased in the TWST. The number of swallows increased and was within the normal range for the TOMASS. However, it took him longer to finish eating the cracker. His EAT-10 score reduced to three at the first follow-up, one day post intervention and he scored a nine at the one week follow-up assessment.

Table 10. Results of the TOMASS and TWST for patient 104.

Timepoint	TWST			TOMASS			
	V/S (ml)	T/S (s)	V/T (ml/s)	Chews	Bites	Swallows	Time (s)
Baseline	12.1*	2.3*	9.3*	46**	3	1**	59.0
Follow-up 1	9.4*	2.1*	8.0*	50**	3	4	60.0
Follow-up 2	7.8*	1.0*	8.1*	58	3	3	80.0**

*Note: * Outside the range of normal based on the Timed Water Swallowing Test TWST norms* (Hughes & Wiles, 1996)*; ** Outside the range of normal based on the Test of Masticating and Swallowing Solids TOMASS norms* (Huckabee et al., 2017)*; V/S = Volume per Swallow in millilitre (ml); T/S = Time per Swallow in seconds (s); V/T = Volume per Time in millilitre per second (ml/s); Time in seconds (s); Follow-up 1 = one day post-treatment; Follow-up 2 = one week post-treatment.*

Swallowing skill assessment: The patient initiated 10 swallows out of 17 trials in the retention test assessing swallowing skill performance at baseline. His difficulties with the initiation of swallowing made it impossible for him to participate in the transfer test without visual feedback. At baseline but also throughout the study, his performance on temporal skill (Figure 33, page 151) was more accurate than his accuracy in swallowing magnitude (Figure 33, page 152). His timing continued to be very precise at all assessments. His performance in controlling swallowing magnitude got worse after the first session. From there he steadily improved until the follow-up one day after the treatment and performed slightly worse again in the second follow-up after one week. He reduced the number of swallowing trials needed to initiate 10 swallows (Baseline: 10/17; Follow-up one day: 10/10 trials; Follow-up one week: 10/11 trials).

Cathodal tDCS

Data of patients 107 and 108 were collected after the results of behavioural study one had been analysed. They were informed about the outcomes of this study, i.e. that both anodal and cathodal tDCS inhibited temporal motor skill learning, but elected to participate.

Patient 107

Patient 107 received two weeks of intensive dysphagia treatment prior to this study. The treatment included swallowing skill training using the BiSSkiT^{CE} software, Mendelsohn Manoeuvre trainings and Expiratory Muscle Strength Training (EMST). Although ongoing rehabilitation was set as an exclusion criteria for this study, an exception was made for the enrolment of this patient since he continued the EMST training during participation in this study.

Behavioural swallowing examination: CN XI and X were mildly impaired, which did not change throughout the duration of the study. His soft palate deviated to the left, he had a weak volitional cough and dysphonia. All other CNs did not show any clinical signs of impairment. He was non-oral and received nutrition via percutaneous endoscopic gastrostomy (PEG). The TWST and TOMASS could, therefore, not be completed. The severity of his swallowing impairment was also reflected in his EAT-10

score of 31 (out of 40) in the initial assessment and was perceived to be worse after the treatment, with a score of 32 one day and a score of 36 one week post-treatment.

Swallowing skill assessment: The patient was able to perform both types of swallowing skill assessment. He needed 13 trials to achieve 10 swallows in the initial retention test with visual feedback (swallowing performance) and nine out of 17 in the transfer test without visual feedback (swallowing skill learning). The patient achieved a reduction in the temporal error over time in both assessments (Figure 33, page 151). He also decreased the amplitude error in the retention and transfer tests, with the largest improvements during the second treatment session (Figure 34, page 152). He maintained the level of performance up to the one week follow-up assessment. Unfortunately, the second clinician mistakenly missed data collection for the assessment of learning on day one post-treatment. His ability to initiate swallowing at the frequency of 30 s improved slightly; he needed between 10 and 13 trial to initiate 10 swallows in both assessments in the follow-up sessions.

Patient 108

This patient was seen for a two week intensive dysphagia treatment protocol 24 months prior to inclusion in the study.

Behavioural swallowing examination: In the initial behavioural swallowing assessment, the patient showed impaired function of the pharyngeal plexus and CN XII. His tongue deviated to the left and his palate showed a slight deviation to the right. All other CNs that were tested presented with no clinical signs of impairment. He was slightly below all norm values in completing the TWST prior to the training and performed within the normal range for most TOMASS measures, but took more bites to eat the cracker compared to norms (Hughes & Wiles, 1996; Huckabee et al., 2017; Table 11). He scored 17 (out of 40) in the initial EAT-10 evaluation and reported that his pleasure of eating is affected by coughing and regurgitation during meals.

On day one post-treatment, his CN function was not different compared to the pre-treatment. Of note, the follow-up session one week post-treatment was performed via an online video call. Therefore, no CN examination could be performed. He increased the volume per swallow and swallowing capacity when drinking water in the TWST and

fell within the normal range at both follow-up assessments (Hughes & Wiles, 1996). Although he reduced the number of bites in the first follow-up session in the TOMASS, he increased the number of bites and swallows again at the one week follow-up, so they were outside the norm again (Huckabee et al., 2017). His EAT-10 scores reduced from 15 one day to 14 one week post-treatment.

Swallowing skill assessment: Patient 108 was able to complete both swallowing skill assessments and had no problems initiating swallows at the frequency of 30 s. The patient's performance on the timing of swallowing was stable across all timepoints, except for a small decrease of the temporal error in the transfer test in the first session (Figure 33, page 151). He was able to decrease the amplitude error in both skill assessments, with the largest decrease in amplitude error in the retention test (swallowing performance) also during the first session (Figure 34, page 152). The patient was unavailable for the swallowing skill assessment at the one week follow-up.

Table 11. Results of the TOMASS and TWST for patient 108.

Timepoint	TWST			TOMASS			
	V/S (ml)	T/S (s)	V/T (ml/s)	Chews	Bites	Swallows	Time (s)
Baseline	16.7*	1.5*	10.9*	70	6**	3	69.0**
Follow-up 1	30.0	1.7*	18.1	73**	2	4	66.0**
Follow-up 2	30.0	1.9*	16.1	76**	5**	7**	67.3**

*Note: * Outside the range of normal based on the Timed Water Swallowing Test (TWST) norms (Hughes & Wiles, 1996); ** Outside the range of normal based on the Test of Masticating and Swallowing Solids (TOMASS) norms (Huckabee et al., 2017); V/S = Volume per Swallow in millilitre (ml); T/S = Time per Swallow in seconds (s); V/T = Volume per Time in millilitre per second (ml/s); Time in seconds (s); Follow-up 1 = one day post-treatment; Follow-up 2 = one week post-treatment.*

Sham condition

Patient 102

Behavioural swallowing examination: This patient presented with right-sided facial weakness, with drooping of the mouth to the affected side and she reported drooling on the same side. Wet dysphonia was present constantly and the volitional cough of the patient was noted as weak. All other CNs that were tested presented with no clinical signs of impairment. The patient was NPO and received nutrition via a PEG. She was not able to complete oral trial assessments throughout the study. Her perception of her swallowing impairment improved slightly as revealed by the results of the EAT-10 with a score of 22 at baseline, and 20 one day and 19 one week post-treatment.

Swallowing skill assessment: This patient had difficulties in differentiating between normal and effortful swallowing, the screen range had to be adapted to 200% of the calibration value. Despite this, the patient had severe difficulties initiating dry swallows and was therefore unable to participate in the skill assessment of learning without feedback. She needed 24 trials to initiate nine swallows in the initial retention test.

Her performance on the timing of swallowing improved from pre to post-treatment session but was worse at the beginning of session two compared to baseline (Figure 33, page 151). Her control of submental muscle activation magnitude got worse from pre to post-treatment session one, but improved in the second session (Figure 34, page 152). She only needed 11 and 14 trials in the follow-up assessment to initiate 10 swallows in the retention test.

Patient 105

Behavioural swallowing examination: This patient presented with left-sided facial sensory (CN V) and motor (CN VII) deficits. Her lingual lateralisation movements to the left were impaired and he had a weak volitional cough. No clinical signs of impairment were identified at the other CNs. Although her swallowing capacity was within the norm on the TWST, it took him much longer to drink 150 ml of water compared to age and gender-matched controls (Hughes & Wiles, 1996; Table 12). This was mainly because she paused in between swallows and did not drink the water sequentially, despite verbal cues. She also took longer than the norm for the completion

of the TOMASS and used less swallows (Huckabee et al., 2017). Her EAT-10 score at the initial assessment was four.

The results of the CN examination, the TWST and the TOMASS showed only small changes post-treatment. In the TWST, the time per swallow and the volume per time decreased and increased respectively and were, therefore, closer to the norm one day following the treatment. However, these two measures went into the opposite direction again at the one week follow-up. The time needed for the completion of the cracker in the TOMASS increased by 30 s at the one week follow-up compared to baseline and was, therefore, twice as long as the average norm. The EAT-10 score increased by one point and resulted in the score five for both follow-up assessments.

Swallowing skill assessment: The patient had severe difficulties with the initiation and control of swallowing. The calibration value needed to be adjusted to 200% and it was not possible for the patient to perform the transfer test without feedback. Although this patient was able to initiate 10 out of 10 swallows in the retention test at baseline, only five swallows could be initiated out of 12 trials one day post-treatment and 20 trials were needed to initiate 10 swallows in the assessment one week post-treatment. Her timing of swallowing initiation got worse from pre to post training session one but she improved her performance in the second session (Figure 33, page 151). However, this patient did not perform better than at baseline following the second treatment session. The best performance of timing was one day following the treatment and she performed similar to baseline level at the one week follow-up. The opposite development of performance was seen for the amplitude error (Figure 34, page 152). Although this patient achieved improvements in the accuracy of swallowing magnitude during the first two sessions, the performance over swallowing amplitude got worse during the follow-up assessments.

Table 12. Results of the TOMASS and TWST for patient 105.

Timepoint	TWST			TOMASS			
	V/S (ml)	T/S (s)	V/T (ml/s)	Chews	Bites	Swallows	Time (s)
Baseline	17.0	11.7*	2.6*	58	4	2**	105.0**
Follow-up 1	18.8	5.5*	6.8*	67	6**	2**	112.0**
Follow-up 2	13.6	17.0*	0.8*	58	4	3	131.0**

*Note: * Outside the range of normal based on the Timed Water Swallowing Test (TWST) norms* (Hughes & Wiles, 1996)*; ** Outside the range of normal based on the Test of Masticating and Swallowing Solids (TOMASS) norms* (Huckabee et al., 2017). *V/S = Volume per Swallow in millilitre (ml); T/S = Time per Swallow in seconds (s); V/T = Volume per Time in millilitre per second (ml/s); Time in seconds (s); Follow-up 1 = one day post-treatment; Follow-up 2 = one week post-treatment.*

tDCS tolerability:

All patients tolerated the 20 min of cerebellar stimulation well. Only two patients − patient 107 (cathodal tDCS) and patient 104 (anodal tDCS) - reported mild discomfort, mild tingling or a mild itching sensation on the cheek.

12.4 Discussion

Swallowing skill learning

This study explored the effects of cerebellar tDCS on motor skill learning in swallowing in patients with oropharyngeal dysphagia. Although all patients were able to participate in the performance assessment of swallowing skill, only two out of the six patients were able to perform the assessment without visual feedback for the assessment of swallowing skill learning. Both of these patients received cathodal stimulation. They showed improvements in controlling the timing and even greater improvements in the control of magnitude of submental muscle contraction during swallowing, from pre-treatment to immediately post-treatment. However, it cannot be concluded that these effects were facilitated or diminished by the cathodal stimulation, as comparisons to the effects of anodal stimulation or controls on swallowing skill learning is not possible.

Clinical outcome measures for the patient who received cathodal tDCS and who was able to complete oral swallowing trials showed improvements on two measures on the

TWST, whereas patients in the anodal or sham condition did not improve in these measures. In contrast, the performance on the TOMASS got worse for the patient who received cathodal tDCS, especially at the follow-up one week post-treatment. This is contrary to the patient results in the anodal and sham condition, who were more often within the norm at the post-treatment timepoint. For the patients that received cathodal tDCS, the EAT-10 scores of one patient improved whereas the other patient's scores suggested a deterioration. This was also the pattern for the other two conditions; i.e. one patient improved and the other one perceived the swallowing impairment as worse over time. Therefore, the EAT-10 scores do not indicate different effects of the three stimulation conditions. Interestingly, however, although the EAT-10 scores of the two patients that revceived cathodal tDCS were higher compared to the patients in the other two conditions, they were the only patients that were able to perform the swallowing skill assessment without visual feedback. This raises questions about the comparison of EAT-10 scores. However, assuming that EAT-10 scores can be compared, the results of the current study may indicate that a more severe swallowing impairment as perceived by the patient, does not necessarily exclude participation in a more challenging swallowing assessment. More so, the ability to initiate and control the timing of swallowing may be important prerequisites for participation in this more challenging task. On a side note, these two patients were enrolled towards the end of the data collection process. By this time, the results of the effects of cerebellar tDCS in swallowing motor skill learning in healthy volunteers were analysed. For ethical reasons, both patients were informed about the results of the Behavioural study I prior to enrolment in this study.

Swallowing skill performance

Contrary to the hypotheses, neither patients who received anodal tDCS showed enhanced swallowing skill performance nor did patients who received cathodal tDCS showed diminished swallowing skill performance compared to sham. Furthermore, swallowing performance of the patients of all conditions remained relatively stable from baseline to post-treatment and at the follow-up measures. Functional measures of swallowing of the patients who received anodal and cathodal tDCS showed small improvements, i.e. more values on the TWST and TOMASS were within the norm of healthy controls, especially at the one week follow-up compared to baseline. The patient who received cathodal tDCS and who was able to perform oral trails improved

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in the TWST but performed worse in the follow-up after one week on the TOMASS after initial improvement in the first follow-up. The small treatment effects from baseline to post-treatment may be the consequence of the short duration of the treatment – only two days. More intensive swallowing skill training, e.g. over two weeks as used by Athukorala et al. (2014), has been demonstrated to result in greater changes of swallowing performance on the skill training task as well as functional swallowing changes. This protocol was based on the principles of neural plasticity from Kleim and Jones (2008) since neuroplastic change requires high-intensity training with many repetitions. For comparison reasons with Behavioural study I, a shorter duration of treatment was chosen for this proof-of-concept study. However, using a two week training protocol, patients would have more time to practice the control of submental muscle contraction and new neural pathways may be established. Future research is required to clarify if the training of submental muscle contraction using swallowing skill training changes swallowing function or the neurophysiology of swallowing in patients with swallowing impairments post stroke.

Multiple sessions, i.e. more than two, of swallowing skill training with additional tDCS, might also be hypothesised to intensify possible effects of the stimulation. Given that at least two consecutive sessions of cortical tDCS are necessary to evoke long-lasting changes in cerebral plasticity of healthy volunteers (Monte-Silva et al., 2013), multiple sessions of tDCS may have a cumulative effect. Especially, when tDCS is used for neurorehabilitation of impaired brain function, multiple repetitions might be needed to induce reliable long-term effects (Brunoni et al., 2012). Daily repetition has been found to be more effective than weekly sessions (Boggio et al., 2007). It needs to be considered that these findings are based on studies using tDCS over the motor cortex. Research investigating plasticity mechanisms of cerebellar tDCS are required to identify the optimal dosage to facilitate neurorehabilitative therapy approaches.

Overall, patients that received anodal and cathodal tDCS demonstrated smaller temporal than amplitude errors, whereas patients in the sham condition achieved smaller amplitude than temporal errors. However, these differences already existed prior to the intervention and are therefore not the result of the stimulation. Furthermore, the results on this proof-of-concept study can only be understood as observations of possible effects for future investigation because of the small sample size and the

heterogeneity of patients. Not only was there heterogeneity between patients across conditions but also for the two patients within a single condition. There was one severely and one mildly swallowing impaired patient each, in the sham and anodal tDCS condition, whereas the two patients that received cathodal tDCS were more severely impaired, based on their EAT-10 scores. Counterbalancing patients for stimulation condition could have been used to reduce heterogeneity, however, the random assignment of patients to the three conditions was the preferred methodology to eliminate accidental bias of the researchers for a specific type of stimulation.

Methodology

Some patients had difficulties differentiating between normal and effortful swallowing. Since this differentiation was required for the calibration of the BiSSkiT[CE] software to use skill training, it is important to explore the capabilities of healthy and swallowing impaired individuals to manipulate the sEMG amplitude during swallowing. This information can then be used to develop a calibration protocol that will enable swallowing impaired patients the best potential for using the swallowing skill training using submental sEMG. A recent study demonstrated that healthy volunteers were able to produce significantly different amplitudes of the submental sEMG signal when swallowing with effort compared to normal swallowing (Ng, Jones, Erfmann, & Huckabee, 2017). However, it is unknown if patients with swallowing impairment following stroke are also able to produce this distinct difference in sEMG amplitudes of submental muscle activity when swallowing with normal effort compared to effortful.

Although the assessment of skill learning in this study was a suitable approach for healthy individuals with unimpaired swallowing, not all patients with dysphagia were able to participate in this assessment. In particular, the cognitive complexity of the task using a disappearing waveform was too challenging for most stroke patients. Although the frequency to swallow once every 30 s was found to be appropriate for patients with Parkinson's Disease (Athukorala et al., 2014), most stroke patients in this study were not able to voluntarily initiate a swallow at this frequency, regardless of the underlying swallowing pathophysiology. An increased duration of up to 45 or 60 s per trial might be necessary to meet the patients' needs for accomplishing this task. Furthermore, incorporating an assessment to test the frequency of volitional swallowing initiation might be a valuable parameter to include in the calibration process. The patient might

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be asked to initiate three swallows in a row, as quickly as comfortably possible. The average time taken between swallows could then inform about the duration needed for each swallowing trial. This measure could also be used as assessment to measure changes in voluntary swallowing initiation.

Since several patients had problems initiating dry swallows (approximately once every 30 s), water spray was used to moisten the oral cavity of three patients. Even though only a very small amount of water was provided (just enough to moisten the oral cavity) it may have increased the sensory input to NTS and therefore facilitated the initiation of swallowing. Therefore, these patients may have had an advantage in achieving better temporal control over their swallowing compared to patients that struggled with the initiation of swallowing but did not request the water spray. However, these patients did not perform better in their temporal control compared to the patients that did not receive water spray and were spread out evenly across stimulation conditions (one each per condition).

Interestingly, the patients' ability to initiate a swallow could have influenced the ability to participate in the skill learning assessment itself. It was noticed that patients with higher precision in the timing of swallowing were able to participate in the skill learning condition (with an exception of patient 104). This leaves unanswered if the task was too challenging or if it was the result of the specific swallowing impairment of the patients in this study. As this was a pilot study, the inclusion criteria were very broad, e.g. no further specification was made to account for different types of stroke, time post-onset or swallowing impairment. Additionally, for those recruited to the study, instrumental assessment was not completed to diagnose the nature of swallowing impairment as this was not the scope of this research programme. However, this should be included in future studies.

Although different treatment approaches for different types of swallowing impairment have been suggested, e.g. specific treatment for strength and skill impaired patients, assessments do not currently exist for this type of differential diagnosis. Increased diagnostic specificity is needed to identify the underlying issue of the swallowing impairment, i.e. skill versus strength impaired patients. The next steps would be to identify which type of patients (or which pathophysiology or signs and symptoms of

their swallowing impairment) may benefit from swallowing skill training and if skill training using submental sEMG results in functional changes of swallowing in patients with stroke, similar to what has been found in other patient populations (Athukorala et al., 2014).

Tolerability of tDCS

All stroke patients in this study tolerated the application of tDCS with an intensity of 2 mA for 20 min well. Minor side effects reported by two patients, including mild discomfort, mild tingling and a mild itching sensation on the cheek, are within the normal range of reported side effects (Brunoni et al., 2011). However, possible side effects will need to be monitored closely if the duration and frequency of tDCS applications would be increased.

Conclusion

This exploratory study was conducted to evaluate the feasibility of tDCS and skill training in patients following neurological injury. All patients were able to complete the swallowing skill training and the assessment of skill performance (with visual feedback), suggesting that the treatment approach may be viable for patients with dysphagia following stroke. However, the majority of stroke patients (four out of six) were unable to perform the swallowing skill assessment of learning (without visual feedback). Therefore, the effects of cerebellar tDCS on motor skill learning could not be compared between stimulation conditions. The two patients who were able to perform the assessment received cathodal stimulation. They demonstrated small changes on temporal error measures and larger improvements for error measures of magnitude on skill learning over time, particularly after the second treatment sessions and one week post-treatment. The outcomes in the assessment of motor performance did not change considerably from baseline for any patient over time regardless of stimulation condition. This is in contrast to the results of healthy participants in Behavioural study I where one session of swallowing skill training was sufficient to significantly improve swallowing skill performance which persisted through the non-treatment period. This indicates that two days of treatment may not be sufficient to increase swallowing performance. Hence, neurologically impaired patients may need multiple skill training sessions to increase volitional control over swallowing behaviours.

13. Methodological study: The effects of midline cerebellar tDCS on motor evoked potentials from corticospinal and corticobulbar projections.

13.1 Introduction

The majority of studies investigating the effects of cerebellar tDCS have been performed in the corticospinal motor system utilising a mono-hemispheric electrode placement, as originally reported by Galea et al. (2009). In their protocol, one electrode was placed over the right cerebellar hemisphere and the electrode with the opposite polarity was placed over the right cheek muscle (buccinators). This has since become a well-established electrode montage in the corticospinal literature (Galea et al, 2011; Hamada et al., 2012; Sandicka et al., 2013; Zuchowski et al., 2014; Herzfeld et al., 2014). However, unlike limb motor control, swallowing involves midline structures that are bilaterally innervated by the corticobulbar motor system. Therefore, the typically used unilateral electrode placement is likely not appropriate for investigations of these midline, bilaterally innervated tasks. A *midline* placement of the tDCS electrode over the cerebellum and a placement of the two electrodes of the opposite polarity in a bilateral set-up, one over the buccinator muscles on each side, allows an equal distribution of the electrical current across both cerebellar hemispheres. In order to expand the application of tDCS technology to corticobulbar behaviours, e.g. as proposed in the behavioural studies of this research programme, careful scrutiny of the methods used in corticospinal motor control research warranted.

This proof-of-concept study evaluated if midline cerebellar tDCS would evoke changes in cerebellar excitability in healthy individuals. Paired-pulse TMS over the cerebellum and motor cortex was used to assess changes in the inhibitory cerebellar-cortical connections, known as cerebellar-brain inhibition, or CBI, as previously demonstrated for the corticospinal system (Galea et al., 2009; Pinto & Chen, 2001; Ugawa et al., 1995). MEPs were measured from the FDI of the dominant hand. The assessment of effects in the corticospinal tract was used as baseline measurement for changes in CBI by replicating previous findings using a unilateral placement for cerebellar tDCS (Galea et al., 2009). However, since a midline placement of the cerebellar tDCS electrode was

proposed to target midline functions, MEPs were measured at midline from the submental muscles using a novel adapted version of the paired-pulse TMS protocol. In addition, this protocol considered facilitatory cerebellar-cortical connections (cerebellar-brain facilitation = CBF) in the corticobulbar system (Jayasekeran et al., 2011). The outcomes of this proof-of-concept study contribute to current cerebellar tDCS research, as they may broaden the application of tDCS as a novel assessment and intervention procedure for more proximal motor behaviours.

13.2 Methods

This study was conducted at Flinders University in Adelaide, Australia, and additionally supervised by Dr Sebastian Doeltgen, who provided access to the required equipment to perform the paired-pulse TMS assessments. This study was approved by the Adelaide Clinical Human Research Ethics Committee (Application Number: 351.15).

13.2.1 Participants

Fifteen right-handed healthy individuals (5 male; mean = 25 years, age-range: 20-33 years) participated in this study. They provided written consent and reported no swallowing problems, history of epilepsy, alcoholism, pregnancy, cardiac pacemaker, metal in the head, or the use of medication known to lower seizure threshold (e.g. propofol or penicillins). All participants were screened prior to inclusion by a registered medical professional to ensure their safety for participation in this study.

13.2.2 Equipment

sEMG recordings: Disposable electrodes (Ambu® Blue Sensor N; Ref.: N-00-S/25) were used for the electromyographic recordings. A velcro strap ground electrode (1.5 cm wide) was soaked in saline-solution (0.9% sodium chloride solution) and used as a ground electrode. EMG signals were sampled at 5.0 kHz, amplified (x1000), and bandpass filtered (20 Hz – 1 kHz) (Cambridge Electronic Design 1401/1902, Cambridge, UK). Data were analysed off-line using SIGNAL software V4.08

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(Cambridge Electronic Design, Cambridge, UK). Alcohol wipes and fine sandpaper (600 grit size) were used for skin preparation.

TMS: TMS was delivered using two 70-mm diameter figure-of-eight coils with a maximal output of 2.2 Tesla connected to two magnetic stimulators (Magstim Ltd., Whitland, Wales).

tDCS: TDCS was delivered using the research version of the Transcranial Stimulation Kit (TCT Research Limited). Three rectangular rubber electrodes in sponge covers (5 cm × 5 cm) were soaked in a 0.9% sodium chloride solution and held in place using a soft neoprene montage set for the application of tDCS. This was the same equipment as used for Behavioural studies I and II.

13.2.3 Study protocol

This study was performed using a within-subject, repeated-measures design. All participants attended two double-blind sessions, separated by at least six days. Participants were randomly assigned to start with either cathodal tDCS or sham, the other condition was consequently applied during the second session. Only cathodal tDCS, not anodal tDCS, was tested for two reasons. First, previous research by Galea et al. (2009) demonstrated smaller effects of anodal tDCS on CBI compared to cathodal tDCS. Secondly, considering that anodal tDCS increases cerebellar excitability, maximum CBI recruitment with no additional inhibition of M1 would theoretically result in a ceiling effect and could not be assessed without performing a CBI recruitment curve (Galea et al., 2009). Changes in the excitability of cerebellar cortical connections were assessed, measuring CBI or CBF, using a paired-pulse TMS assessment for the corticospinal and corticobulbar system respectively (Figure 35). The assessment was performed before and after cathodal tDCS or sham tDCS, and 10 and 20 min post-intervention.
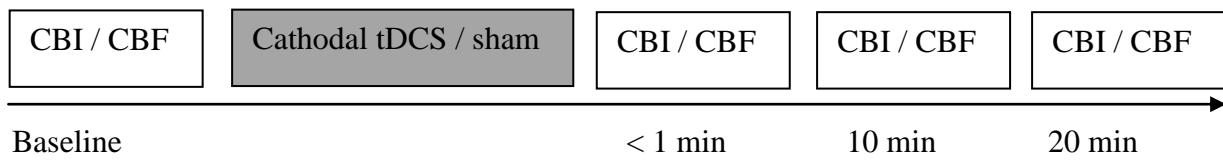
CBI / CBF	Cathodal tDCS / sham	CBI / CBF	CBI / CBF	CBI / CBF

Baseline   < 1 min   10 min   20 min

Figure 35. Study design of the Methodological study, assessing the effects of cathodal tDCS on excitability changes in inhibitory (CBI) and facilitatory (CBF) cerebellar-cortical connections across four timepoints in a within-subject, repeated-measures design.

13.2.4 Experimental procedure

In each session, the participants sat in a comfortable chair with arms resting on the armrests. Prior to placement of the recording electrodes, the skin was prepared using medical sandpaper and alcohol wipes. One set of electrodes was placed over the muscle belly of the FDI and the metacarpophalangeal joint and the other set was placed at midline over the submental muscle group in the bipolar belly-tendon montage (Figure 36). The reference electrode was situated around the wrist for the measurements from the FDI muscle, and around the forehead for measurements from the submental muscle group.
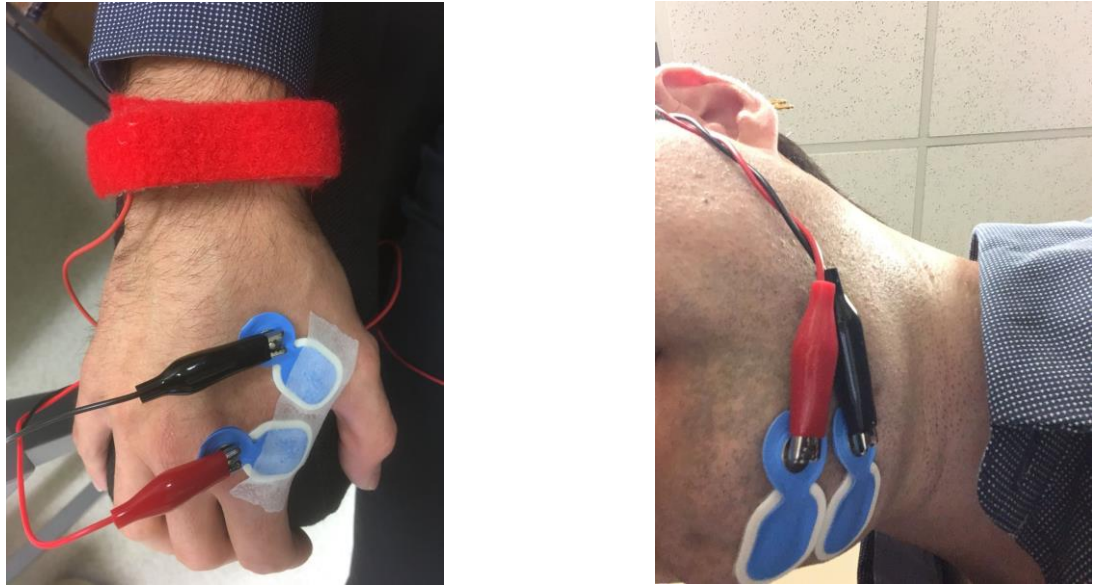
Figure 36. Left: Electrode placement over the muscle belly of the FDI and the metacarpophalangeal joint for the collection of MEP measures on changes in CBI. *Right:* Electrode placement at midline over the submental muscles in the bipolar belly-tendon montage for the collection of MEP measures on changes in CBF.

TMS: Data collection using TMS was performed with the targeted muscles at rest since cerebellar inhibition is markedly reduced and difficult to demonstrate when the target muscle is active (Pinto & Chen, 2001). Data collection started, in all cases, with measurements from the FDI muscle, as this procedure has been demonstrated to produce strong and reliable measures of CBI in previous research (Galea et al., 2009).

First, the optimal stimulation locations ('hot spots') for the motor cortical stimuli were determined by delivering single magnetic impulses over the approximate target areas and monitoring the sEMG for MEP activity. This assessment was started at an intensity of 30% of the maximum stimulator output (MSO) and was slowly increased in steps of 2% until suprathreshold stimulus intensity was reached. This suprathreshold stimulus intensity was kept at a constant level, while the figure-of-eight coil was systematically moved in 1 cm steps over the approximate target areas. The coil placement for each muscle group that elicited reliable MEPs (five consecutive) with maximum peak to peak amplitude at suprathreshold intensity was marked on the scalp with a removable marker.

For the identification of the FDI hotspot, one TMS coil was placed laterally over the left scalp (corresponding to the dominant right hand) in the area overlying the cortical motor representation of the right FDI muscle area, the coil handle pointed backward at an approximate angle of 45° to the sagittal plane (Galea et al., 2009). To identify the motor cortical hotspot of the submental musculature, the coil was placed overlying the representation of the submental musculature – approximately 5 cm lateral along the interaural line from the vertex and 1 cm anterior – with the coil handle oriented parallel to the mid-sagittal plane (Plowman-Prine et al., 2008). This procedure was started over the left side also, and followed by an assessment over the right side, in order to identify the hemisphere where greater and more reliable MEP responses could be elicited. This was initially trialled in the submental musculature at rest. However, if no MEPs could be elicited at rest, using a stimulation intensity of 80% of the MSO, pre-activation of the musculature was used to identify the dominant hemisphere. Data collection of submental muscle MEP responses was terminated, if the maximal MSO of 80% was exceeded since higher intensities can cause discomfort for the participants and MEPs of the submental muscles at rest were previously identified using $73 \pm 3\%$ MSO (Gallas, Marie, Leroi, & Verin, 2009). An assessment using pre-activation of the muscle group was not used for data collection, as CBI has been demonstrated to be reduced with voluntary muscle contraction (Pinto & Chen, 2001).

Two slightly different paired-pulse TMS protocols for FDI and submental muscle group representations were used for the assessment of excitability changes in cerebellar-cortical connections. In both paired-pulse TMS assessments, a conditioning stimulus (CS) was applied over the cerebellum using one TMS coil, followed by a test stimulus (TS) over the previously identified motor hotspots of the primary motor cortex. The coil delivering the CS was placed over the cerebellar hemisphere contralateral to the TS and positioned 3 cm lateral and 1 cm inferior to the inion with the handle pointing upwards (Daskalakis et al., 2004; Galea et al., 2009; Pinto & Chen, 2001; Ugawa et al., 1995).

The intensity of the TS was set to the intensity required to produce a half-maximal MEP response for each muscle group (Bradnam et al., 2015). The intensity to produce a maximum MEP response was determined by increasing the intensity until a plateau in the MEP response was reached. From there, the intensity was gradually lowered by 2% until a consistent MEP response (three consecutive responses), approximately half the

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size of the maximum MEP response amplitude was observed. This was set as the TS intensity. The stimulation intensity for the CS over the cerebellum was set to the percentage of MSO required to elicit an MEP of around 50 µV in at least 50% of the trials (Rossini et al., 1994). This intensity was chosen to be a subthreshold stimulus for both conditions to avoid antidromic pyramidal tract co-activation as a result of a higher resting motor threshold within the corticobulbar system (Groiss & Ugawa, 2012). In line with previous studies, the inter-stimulus interval (ISI) was set to 5 ms for corticospinal measurements (FDI) (Ugawa et al, 1995; Saito et al. 1995; Werhahn et al. 1996; Daskalakis et al. 2004; Pinto and Chen 2001) and 50 ms for corticobulbar measurements (submental) (Jayasekeran et al, 2011).

Thirty single (TS only) and paired-pulse (CS and TS) MEP responses were collected in a randomised order at baseline and immediately, 10 and 20 min following tDCS. The participant was asked to relax the targeted muscle group for the assessment and to keep their eyes open and look straight ahead during assessment to avoid falling asleep.

tDCS: The same stimulation parameters and electrode set-up used in the behavioural studies were used here, with the stimulating electrode centred at midpoint, 1 cm below the inion and the return electrodes split, with one electrode placed over each buccinator muscle. Both the participant and the researcher recording the outcome measures were blinded to the stimulation type delivered (cathodal tDCS or sham) in each session. Participants were informed that they may, or may not, perceive the electrical current. The electric current was delivered at an intensity of 2 mA for 20 min using 5 cm x 5 cm electrodes (equating to a current density of 0.08 mA/cm^2). The current was ramped over a period of 30 s. No current was delivered during the sham condition.

13.2.5 Data analysis

Conditioned (paired-pulse) and unconditioned (single pulse) MEP amplitudes were measured from peak to peak (mV) and averaged over the fifteen trials per condition (cathodal tDCS, sham) for each individual at each timepoint (baseline and at 0 min, 10 min and 20 min post tDCS). The CBI ratio was calculated, dividing the conditioned MEP response by the non-conditioned MEP response.

Linear mixed effects models analyses were performed using R studio version 3.2.5 including the packages lme4 and lmeTest (Bates, Maechler, & Bolker, 2012). Linear fixed effects for the CBI ratio were predicted by a session by stimulation interaction using the functions "plot" and "qqnorm" and resulted in a linear distribution of the residues. Timepoint and stimulation type were set as the fixed effects and the variable subject was set as random effect.

13.3 Results

No MEPs could be collected from the submental musculature at rest; hence only the data pertaining to the FDI musculature are reported here. The average resting motor threshold was 49% of stimulator output (range $40 - 66\%$) for the assessment of the FDI for both conditions. The average stimulator output for the TS was 65% (range $51 - 77\%$) for the stimulation condition and 64% (range $49 - 79\%$) for the sham condition. An initial analysis of the effects of stimulation and timepoint on CBI ratio for measures of the FDI revealed no effect of timepoint χ^2 (3) = 6.72, p = 0.08 or interaction χ^2 (3) = 4.06, p = 0.26, and only a significant main effect of stimulation condition χ^2 (1) = 7.69, $p > 0.005$. Figure 37 displays the CBI ratios over time for both tested conditions. A CBI ratio of less than one implies an inhibition of the motor cortex by cerebellar stimulation.
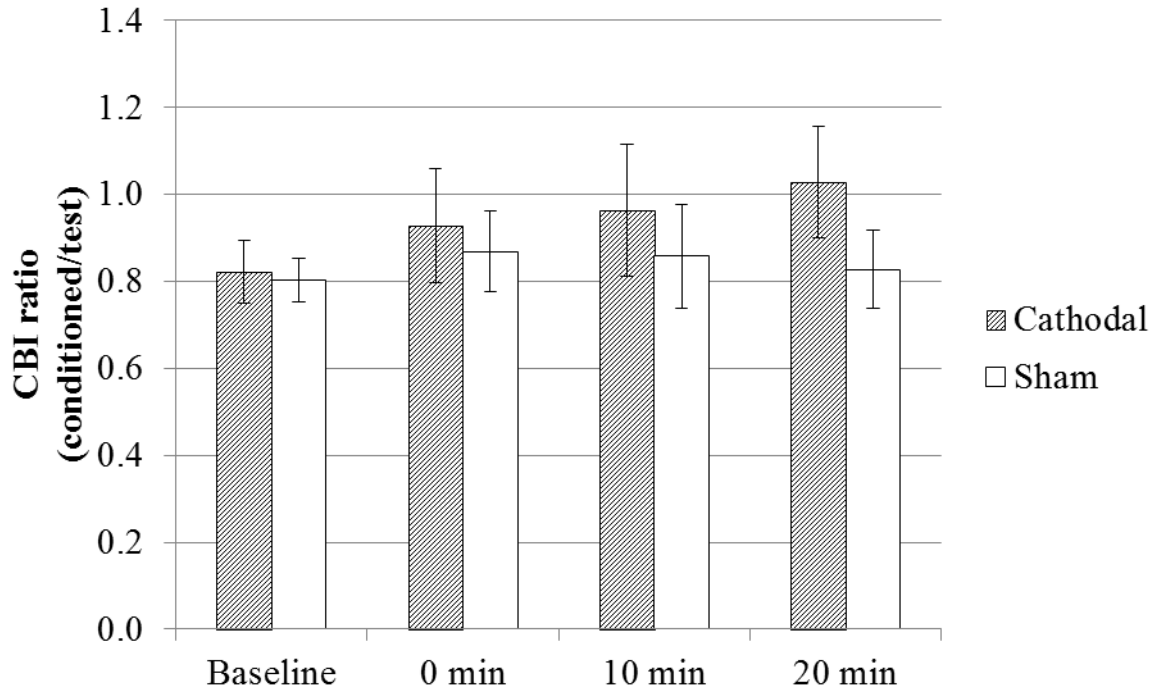
Figure 37. Cerebellar brain inhibition (CBI) ratios assessed from the FDI at baseline, immediately following cathodal tDCS or sham, and at 10 min and 20 min post. Data means with 95% confidence intervals.

13.4 Discussion

The effects of cerebellar tDCS using a midline cerebellar electrode montage on cerebellar-cortical connections from corticospinal (FDI) projections were assessed. Although attempted, no reliable MEPs from corticobulbar (submental) projections at rest could be collected at a reasonable stimulator output (below 80% maximal stimulator output). When evaluating tDCS effects of the cerebellum on FDI motor circuits over time, there was no statistically significant difference between the active tDCS and sham groups. It may suggest that a midline electrode placement for cerebellar tDCS of 2 mA applied over 20 min is not sufficient to induce neurophysiological changes within the corticospinal system. However, the lack of difference at a group level is likely due to the large inter-individual response variability, since behavioural inhibition using these stimulation parameters was demonstrated in Behaviuoral study I.

This hypothesis is supported by findings in Behavioural study I, where behavioural inhibition was demonstrated using the same electrode placement.

tDCS effects on corticobulbar projections (submental muscles – CBF)

The electrode placement at midline for cerebellar tDCS was purposefully chosen to target bilateral motor behaviours. In particular, the assessment of CBF measuring MEPs from the submental muscles was attempted, as this was the targeted muscle group for behavioural changes for the behavioural studies of this research programme. Although reliable MEPs from submental muscles at rest have been reported in previous research (Gallas et al., 2009), no reliable MEP responses from this muscle group could be collected in healthy volunteers in the current study. Data collection of MEPs for this muscle group at rest was terminated, when no discernible or reliable cortical MEPs from the previously identified dominant hemisphere (from the pre-activated muscle) could be recorded. There are two common ways to increase the MEP amplitude to evoke more discernible MEPs (Cruccu, Berardelli, Inghilleri, & Manfredi, 1990; McMillan, Watson, & Walshaw, 1998). First, pre-activation of the musculature can be used to increase the magnitude of MEPs and is in most cases necessary to even detect and record them from facial muscles (Cruccu et al., 1990; Doeltgen, 2009). Pre-activation of the submental muscles was not used for data collection in the current study since CBI has been demonstrated to be reduced with voluntary muscle contraction (Pinto & Chen, 2001).

The second option of using a higher TMS intensity was trialled in the current study. In participants for whom submental MEPs could be identified using muscle pre-activation, a substantial increase in the stimulation intensity was necessary to identify MEPs at rest. However, data collection was terminated for the submental muscle group if a stimulator intensity of 80% or above was necessary to elicited discernible MEPs. Very early motor responses akin to direct nerve root stimulation were noted and would have confounded the recordings. Hamdy and colleagues demonstrated that MEP responses of conditioned cerebellar TMS can be measured by an intraluminal catheter within the pharynx at rest (Jayasekeran et al., 2011). Although this method has limitations, e.g. specificity of the assessed muscle group, this experimental protocol might be an alternative to assess the effects of cerebellar tDCS on cerebellar excitability in a bilaterally innervated, proximal muscle group relevant for swallowing. Another option

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would be to substitute the figure-of-eight coil for the CS in the current protocol a cone-shaped coil, to increase the strength of stimulation. This way the stimulation intensity required to elicited MEPs in the submental musculature might be kept at a lower level.

tDCS effects on corticospinal projections (FDI – CBI)

There was no statistically significant difference between the active and sham groups when evaluating tDCS effects on CBI of FDI motor circuits. This is likely due to the significant inter-individual response variability that is reflected in the relatively large confidence intervals. Although this trend was not statistically significant, quantitatively, there was a 23% increase in CBI ratio (i.e. cerebellum is less inhibitory) from baseline to the 20 min follow-up assessment for the active tDCS condition. Clinically, it may indicate that if variance can be minimised, this change in the CBI ratio might be sufficient to evoke behavioural changes for motor behaviours, as suggested in previous research. For example, a 25% increase in the CBI ratio from baseline in the assessment of healthy volunteers following a locomotor task (Jayaram et al., 2011) and a estimated 23% increase in CBI ratio following a visuomotor task with the dominant upper limb (Schlerf et al., 2012), are sufficient to evoke changes in motor behaviour. Both studies reported no change in the magnitude of CBI relative to baseline for a control task where no learning was required. In addition, Jayaram and colleagues (2011) noted a correlation between achievements in the locomotor task and changes in the CBI ratio (i.e. more learning correlated with a greater reduction in CBI). These findings from the corticospinal system suggest that the observed increase in CBI ratio for the active condition in the current study could, therefore, result in behavioural changes, although not statistically significant. This hypothesis would need to be confirmed for a corticobulbar function such as swallowing in future research on a larger sample to account for inter-individual response variability.

In contrast to the statistically non-significant findings of group differences over time in the current study, previous research using similar methods demonstrated a significant increase in CBI following 25 min of cerebellar tDCS (Galea et al., 2009). Contrary to the current study, they employed unilateral electrode placement over the cerebellum for tDCS but applied the same stimulation intensity of 2 mA. In Galea's study, CBI was significantly reduced for up to 30 min following cathodal tDCS compared to the sham condition. Interestingly, the changes in CBI ratio decreased over time in the Galea

study, whereas the longevity effects in the current study demonstrate a gradual increase in CBI ratio over time. Although the current study partly replicated that of Galea and colleagues (2009), two main methodological differences may have contributed to non-statistically significant effect in the present study.

One possible explanation may be found in the stimulation parameters and electrode set-ups of tDCS. Instead of a unilateral placement, a midline placement of the electrode over the cerebellum was utilised in the current study. The effectively lower stimulation intensity (2 mA split between two return electrodes) due to this setup, or the shorter stimulation duration (20 min vs. 25 min) may account for the lack of difference in the current study. In order to increase the stimulation intensity per cerebellar hemisphere up to 2 mA for each side, a stimulation intensity of 4 mA at the active electrode would be required. Such high intensities for cerebellar tDCS have not been explored for potential side effects and were considered inappropriate for this proof-of-concept study. However, bilateral cerebellar cathodal tDCS applied symmetrically at 1 mA per hemisphere resulted in a greater estimated reduction in CBI than it has been reported for 1 mA cathodal tDCS applied to a single cerebellar hemisphere (Galea et al., 2009). Simultaneous stimulation of both cerebellar hemispheres via midline electrode placement thus may increase the magnitude of tDCS effects compared to unilateral cerebellar tDCS. Bilateral assessment of MEP responses from the FDI muscles would be necessary to confirm that the current flow equally affects both cerebellar hemispheres with this electrode placement.

A second difference can be found in the slightly different paired-pulse TMS assessment protocol of CBI. In particular, a different type of coil over the cerebellum as well as different protocols for setting the test and conditioning stimulus intensity were used. Even though using a cone coil for stimulation over the cerebellum has been discussed to be a reliable tool in the assessment of MEP responses for CBI (Hardwick et al., 2014), the results of previous (Bradnam et al., 2015; Doeltgen, Young, et al., 2015) and the current study demonstrated that a figure-of-eight coil might be an appropriate alternative for conditioning stimulation over the cerebellum. This was achieved in the current study by demonstrating repeatedly measurable CBI. The results of the current study showed a less pronounced CBI at baseline with higher variability ~ 0.80 mV (SD +/-0.20) compared to MEP responses of ~ 0.65 mV (SD +/- 0.06) reported by

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Galea. However, the less pronounced CBI ratio and the higher variability might not be explained by the difference in coils. Studies using the same equipment (cone coil) and experimental protocol as Galea also demonstrated baseline CBI ratio measures close to 0.80 mV (Jayaram et al., 2011) and a higher variability ~ 0.22 mV (Schlerf et al., 2012). Assuming that cerebellar tDCS may have influenced cerebellar excitability, the combination of both factors (smaller inhibition at baseline and variability of the MEP measures) may have been the cause for non-statistically significant results in the small sample size of the current study. Data collection from a bigger sample or refined methodology to reduce the variability of MEP measures would be necessary to answer this question.

Conclusion

No reliable MEPs could be collected from corticobulbar (submental) projections at rest in the current study; it is possible that other assessment protocols would need to be tested, e.g. pharyngeal MEP measures, or developed to evaluate the effects of cerebellar tDCS at midline for swallowing. There was no statistically significant difference between the active tDCS and the sham groups when evaluating the effects cerebellar tDCS at midline on the FDI over time. High variability in the TMS response measurements may have contributed to the lack of difference between the two groups. Future studies using a refined methodology to reduce the variability of MEP measures and data collection from a bigger sample are required to answer the question of current study. Lastly, the functional relevance in relation to the magnitude of the change in CBI for proximal movements such as swallowing remains to be investigated.

PART III: CONCLUSION

Conclusion

This programme of research evaluated the effects of a cerebellar tDCS protocol on motor skill learning in swallowing, using a midline electrode placement. The effects of tDCS using this protocol were further tested to evaluate their viability as a new approach for neurorehabilitation in patients with dysphagia following stroke. In contrast to the hypotheses and previously reported findings in the limb literature, the results of this programme of research revealed *inhibitory* effects of anodal cerebellar tDCS on temporal aspects of motor skill learning in swallowing. This raises interesting questions about the underlying neurophysiological processes that are responsible for this outcome and the differences between the effects of tDCS on motor learning in corticospinal and corticobulbar motor functions.

Cerebellar tDCS has been proposed to affect cerebellar-cortical connections by changing the excitability of cerebellar output neurons, the Purkinje cells, arising from the dentate nucleus (Galea et al., 2009). In contrast to inhibitory cerebellar-cortical connections that have been identified in the corticospinal system (Galea et al., 2009), facilitatory cerebellar-cortical connections from corticobulbar projections in the swallowing motor system were reported (Jayasekeran et al., 2011). This neurophysiological difference may explain the unexpected results of anodal cerebellar tDCS in this programme of research. The inhibitory results of anodal cerebellar tDCS in the current study may provide behavioural data that support the existence of a facilitatory connection between the cerebellum and the cortex. Further research exploring the differences and similarities between the corticospinal and corticobulbar system will help to identify neural control mechanisms of different types of human motor functions. This knowledge is mandatory to guide the development of stimulation protocols for treatment of impaired motor functions post-stroke, particularly when tDCS protocols are adapted from one domain to another.

Another important finding of this research programme is that swallowing skill training *without* tDCS improved motor performance and motor skill learning in swallowing. It was demonstrated that cortical manipulation of the semi-reflexive swallowing response, controlling the timing and magnitude of the submental muscle activity during swallowing, can be learned and maintained following two days of training. This finding

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also provides indirect evidence of swallowing skill training to evoke lasting neuroplastic changes in corticomotor pathways, given the effects endured for up to one week discontinuation of treatment. Previous research identified a strong relationship between behavioural changes in swallowing skill following one hour of skill training and changes in corticobulbar excitability (Caruana, 2015). However, neurophysiological changes following multiple days of swallowing skill training and long-term effects of skill training still need to be determined. The swallowing skill training and assessment protocols, as used in the behavioural studies of this research programme, could further be used to investigate the effectiveness of motor skill learning in swallowing or physiological changes in swallowing function following skill training.

The inhibitory effects of cerebellar tDCS in the behavioural study with healthy volunteers indicated that it may be contraindicated to use the tested cerebellar tDCS protocol in combination with swallowing skill training in patients with dysphagia following stroke. Unfortunately, this hypothesis could not be fully answered in the small, proof-of-concept study of this research programme, since the majority of stroke patients were unable to participate in the swallowing skill assessment of learning (without visual feedback). Only the patients in the cathodal group were able to complete the assessment, which hinders comparisons between the three conditions in this study. Future studies are required to develop alternate measures of swallowing skill learning.

Nevertheless, all patients in Behavioural study II were able to complete the assessment of swallowing skill performance. At least two conclusions can be drawn from this study on stroke patients, in comparison to the study on healthy volunteers. First, the outcomes in the assessment of motor performance did not change considerably from baseline in any patient over time, regardless of stimulation condition. In line with the results of healthy individuals, this suggests that stimulation may not have influenced swallowing skill performance over time, compared to the no stimulation condition. It needs to be considered that this comparison is based on a small sample in the patient study and needs a larger sample study to be confirmed.

Second, two days of skill training with additional cerebellar tDCS may have been sufficient to strengthen existing neural connections within a healthy neural network,

however, the results of Behavioural study II suggest that it may have not been sufficient to establish new neural pathways or to produce lasting neurophysiological changes in an impaired brain. However, this hypothesis would need to be tested using neurophysiological measures. Study protocols with increased duration of the treatment intervention may be needed, to explore the effects of swallowing skill training or tDCS in a neurologically impaired population.

Although the tDCS parameters (20 min, 2 mA, midline cerebellar electrode placement) and timing of tDCS (prior to the skill training) did not facilitate motor skill learning in the current programme of research, it was demonstrated that cerebellar tDCS has the potential to modulate motor skill learning in a largely reflexive corticobulbar function like swallowing. Previous research on tDCS over the motor cortex demonstrated, that the effects of tDCS are sensitive to small changes in the protocol. For example, changing the stimulation intensity (Batsikadze et al., 2013) or the timing of the intervention (Cabral et al., 2015; Pirulli et al., 2013), could result in different behavioural or neurophysiological outcomes. This opens the arena for future research to explore different tDCS parameters and protocols for swallowing.

Neurophysiological effects of one adapted tDCS parameter, i.e. a change of the electrode placement, were investigated as part of this research programme. The methodological study of this programme was the first to assess a novel electrode placement developed to suit bilaterally innervated corticobulbar functions such as swallowing. The current flow in this electrode set-up was directed through both cerebellar hemispheres simultaneously. Although statistically significant differences between the active tDCS and the sham groups on changes in CBI using a mono-hemispheric electrode placement for cerebellar tDCS have been reported in previous research (Galea et al., 2009), no statistically significant difference were identified for cerebellar tDCS at midline on CBI in this study. Given the results of Behavioural study I, where cerebellar tDCS using a midline electrode placement resulted in changes of behavioural measures on motor learning in swallowing, neurophysiological evidence for this effect is still required. The results of the methodological study provide guidance on how to optimise the methodology of neurophysiological assessments, which may be required to answer this question.

In the future, different electrode placements for bilateral cerebellar stimulation could be explored to suit bilaterally innervated corticobulbar functions such as swallowing. Since no statistically significant effects of midline cerebellar stimulation were found in the methodological study, splitting the electrodes over the cerebellum, i.e. one electrode over each hemisphere, could be an alternative to increase the stimulation intensity bilaterally. The stimulation intensity of 2 mA would be kept the same per side. Unilateral cerebellar tDCS with the intensity of 2 mA evoked significant changes in motor skill learning in limb function (Cantarero et al., 2015). Applying higher stimulation intensities might not be advised. This could lead to decreased responsiveness of active neurons, as they are more frequent and longer in the refractory state, as demonstrated in animal research (Bikson et al., 2004). Furthermore, a linear relationship between the stimulation intensity and the behavioural effect was not found in previous research, following tDCS over the motor cortex (Batsikadze et al., 2013). The effects of increased stimulation intensity are, therefore, difficult to predict until further understanding of these effects has been gained. In addition, exploration of cerebellar stimulation and stimulation intensity has only been explored for motor cortical stimulation, and not for cerebellar tDCS yet.

Critique

The limitations of this programme of research need to be acknowledged, to understand the scientific significance and to improve the methodology of future studies investigating the effects of novel treatment approaches for dysphagia rehabilitation. As with many studies in swallowing research, this research programme produced Phase I research, exploring the effects of a proposed treatment, largely on healthy participants (Robey & Schultz, 1998). It was limited by small sample sizes relying on participants of mainly European descent. Future research should implement larger sample sizes, more varied ethnicities and adults of all ages to best reflect the patient population with dysphagia. A larger sample size might be required to reduce the variability of behavioural and neurophysiological measures since motor movements and neuroplasticity are highly variable from person to person and even within one person (Komar, Seifert, & Thouvarecq, 2015).

As with many behavioural studies, the studies investigating the effects of cerebellar tDCS on motor skill learning in healthy and patients with dysphagia, are limited by a

small number of training or treatment sessions. Despite the lack of data regarding optimal dose for dysphagia rehabilitation, positive clinical outcomes have been reported for intensive rehabilitation practices following the principles of neural plasticity (Kleim & Jones, 2008; Robbins, Butler, Daniels, Lazarus, & Mccabe, 2008). Extending the amount of sessions might be beneficial to determine to what extent tDCS in combination with swallowing skill training would influence volitional control over the precision of submental muscle contraction and the changes in swallowing function.

Follow-up measurements were performed on day one and following one week post-intervention in both behavioural studies to investigate if the changes were retained over time. Measures at these timepoints should be included in future research since long-term treatment effects are required to ensure the maintenance of the patient's ability to swallow. Although swallowing skill could be assessed in healthy individuals without complications, the assessment was too complex and required too much concentration for four of the six stroke patients. Changes in the assessment protocol would be required to assess swallowing skill in this population, e.g. generating a less challenging task or by using fewer swallowing trials for the assessment.

Although using a RCT had many advantages, e.g. controlling for an unbiased random allocation into different study conditions and its importance for blinding purposes, it had also disadvantages. Particularly for the study with patients, the groups were poorly balanced, for severity of swallowing disorders, lesion location of the stroke or the time post onset. Future studies would benefit from case-controlled matching these variables, rather than random group assignment.

Despite limitations, this research programme rationally translated evidence from related research areas that demonstrated the potential for cerebellar tDCS to enhance motor skill learning in limb function. Informed decision making was applied to achieve the best possible research outcomes without risking patient safety. The adapted protocol of cerebellar tDCS for swallowing in combination with swallowing skill training *inhibited* motor skill learning in this research programme. Therefore, cerebellar tDCS in combination with motor skill training, using the protocol as proposed in this research programme, may well be contraindicated for patients in swallowing rehabilitation using skill training. However, swallowing skill training *without* tDCS was demonstrated to

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improved motor performance and motor skill learning in swallowing. This provides a strong indication for future research into to the potential implementation of skill training in swallowing rehabilitation.

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Zhao, S., Dou, Z., Wei, X., Li, J., Dai, M., Wang, Y., … He, H. (2015). Task-concurrent anodal tDCS modulates bilateral plasticity in the human suprahyoid motor cortex. *Frontiers in Human Neuroscience*, *9*(June), 1–9. http://doi.org/10.3389/fnhum.2015.00370

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PART V: APPENDIX

Appendix I: Information Sheet and Consent Form

Participant Information Sheet
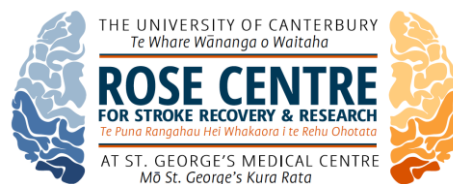
Study title: Brain stimulation in swallowing training

Locality: University of Canterbury Rose Centre for Stroke Recovery and Research
Ethics committee ref.: 15/STH/46 (Healthy)
Lead investigator: Kerstin Erfmann
Contact phone number: +64 (3) 364 2307

You are invited to take part in a study that evaluates the effect of non-invasive brain stimulation on swallowing. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it will not affect the care you receive. If you agree to take part, but change your mind, you withdraw from the study at any time.

This information sheet will help you decide if you would like to participate. It explains why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what happens after the study ends. We will go through the information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with others, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page. You will be given a copy of both the Information Sheet and the Consent Form to keep.

This document is 7 pages long, including the Consent Form. Please make sure you read and understand everything.

WHAT IS THE PURPOSE OF THE STUDY?

We are evaluating if people can learn a swallowing task better when a small electrical current is applied to their brain prior to the training. Furthermore, we want to know which type of stimulation is the most effective.

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People with swallowing problems need effective rehabilitation to safely consume food and drink. The results of this study will help us develop better treatment for people that are having trouble swallowing. This will improve their quality of life and will be more cost efficient for the national health system.

The supervisor of the study is Assoc. Prof. Maggie-Lee Huckabee. She has a Ph.D. in Speech Pathology. She has worked in the area of swallowing disorders for 29 years. She is an associate professor in the Department of Communication Disorders, and the Director of the University of Canterbury Rose Centre for Stroke Recovery and Research.

The main researcher is Kerstin Erfmann. She is a Ph.D. student at the University of Canterbury who has a Master of Science degree in Speech-Language Therapy. She has worked with patients in swallowing rehabilitation for nearly five years.

You can contact Kerstin Erfmann during work hours at (03) 364 2307 or anytime via email at kerstin.erfmann@pg.canterbury.ac.nz.

This study is funded by the University of Canterbury. Furthermore, a New Zealand Brain Research Institute Doctoral Scholarship has been awarded to the researching investigator. This study has been reviewed and approved by The Health and Disability Ethics Committees (HDECs). If you have any questions or concerns regarding the ethical aspects of this study please contact:

> The Health and Disability Ethics Committees (HDECs)
> Ministry of Health
> No 1 The Terrace
> PO Box 5013
> Wellington
> 0800 4 ETHICS (438 442)
> hdecs@moh.govt.nz

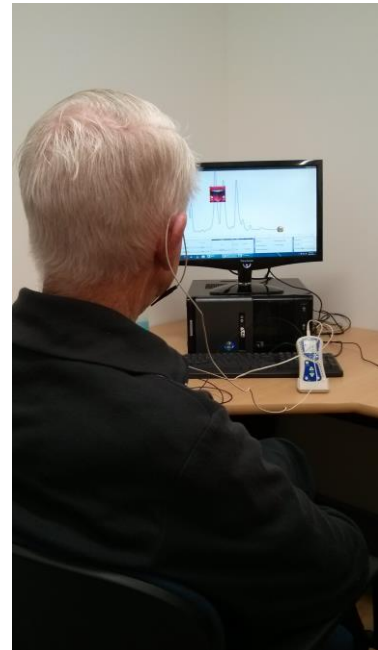## WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

If you agree to take part in this study, you will complete four sessions of swallowing training. Two sessions will be completed on two consecutive (about 60 min each). One and seven days later you will complete the other two sessions (about 10 min each).

For the swallowing training you will be seated in front of a computer monitor. A sticker will be attached to the skin under your chin. This sticker holds three electrodes that measure the activity of muscles that you use when you swallow.

The electrodes record muscle activity and do not put anything into the muscle. The recorded signal will be displayed by a moving line on the computer screen.

When you swallow, the movement of the line will change. This provides you with feedback about how well you control your swallowing movements. To complete the training, you will be asked to swallow in such a way that the peak of the moving line hits a target on the computer screen. The position of the target will change every 30 seconds. Drinking water will be provided for you before and after the training.

You will be assigned to one of three groups. This means you will either receive a positively charged current, a negatively charged current or no current at all. Neither you nor the main researcher will know which type of stimulation you will receive. The stimulation will be applied for 20 minutes prior the swallowing training.

To apply the stimulation, three sponge electrodes (5×5 cm) will be attached to your head. A sponge will be placed on each cheek; a third sponge will be placed over the lower portion of the back of your head. A soft bandage will hold the sponges in place.

At the end of each session you will be asked to fill in a questionnaire in which you rate and describe your experiences of the training.

WHO PAYS FOR THE STUDY?

This study will be paid for by the University of Canterbury. Furthermore, the New Zealand Brain Research Institute Doctoral Scholarship has been awarded to the Ph.D. student who is in charge of this study. You will receive one $20 petrol voucher to reimburse for travel costs.

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF THIS STUDY?

This study poses a low risk of side effects which include skin itching, slight tingling, headache, burning sensation, skin redness and/or discomfort. If these occur, they will subside almost immediately after the stimulation is turned off.

There will be no direct, personal benefit to you by participating in this study. However, you will help us to understand how brain stimulation affects swallowing training. This knowledge will be used to develop better rehabilitation for people with swallowing problems.

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WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, which is unlikely, you would be eligible for compensation from ACC just as you would be if you were injured in an accident at work or at home. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery. If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

WHAT ARE MY RIGHTS?

Your participation is voluntary. Whether or not you take part is your choice. If you decide not to take part, you don't have to give a reason. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

You have the right to access information about yourself collected as part of the study. You will be told of any new information about adverse or beneficial effects related to the study that may have an impact on your health. You will be given a code number so that your name and personal information will be removed from all paperwork. The data will be kept in locked storage at a research institute for 10 years.

WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

If you agree to take part in this study, you are free to withdraw at any time, without having to give a reason.

The data may be included in the investigator's Ph.D. thesis. With your permission, data from this study may be used in future related studies, which have been given approval from the Health and Disability Ethics Committees (HDECs). It is possible that data may be submitted for publication to a scientific journal. However, no material which could personally identify you will be used in any reports on this study.

Consent forms will be kept in a locked filing cabinet in the locked swallowing research laboratory or will be stored on password-protected laboratory computers. Research data will be stored for a period of 10 years after data collection, after which they will be destroyed.

You will be offered copies of the final manuscript or a summary. However, you should be aware that a long delay might occur between completion of data collection and the final report. Alternatively, or in addition, you can choose to have the results of the study discussed with you personally by the principal investigator.

If you need an interpreter, this can and will be provided.

If you have any questions, concerns or complaints about the study at any stage, you can contact:

> **Kerstin Erfmann**
> PhD Candidate
> The University of Canterbury
> Rose Centre for Stroke Recovery and Research
> Leinster Chambers, Level One
> Private Bag 4737
> 249 Papanui Road
> Christchurch 8140, New Zealand
> (03) 364 2307
> kerstin.erfmann@pg.canterbury.ac.nz
>
> **Maggie-Lee Huckabee**
> Director and Associate Professor
> The University of Canterbury
> Rose Centre for Stroke Recovery and Research
> Leinster Chambers, Level One
> Private Bag 4737
> 249 Papanui Road
> Christchurch 8140, New Zealand
> (03) 364 2042
> maggie-lee.huckabee@canterbury.ac.nz

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

> Phone: 0800 555 050
> Fax: 0800 2 SUPPORT (0800 2787 7678)
> Email: advocacy@hdc.org.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

> Phone: 0800 4 ETHICS
> Email: hdecs@moh.govt.nz

Consent Form

Please tick to indicate you consent to the following

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.	Yes ☐	No ☐
I have been given sufficient time to consider whether or not to participate in this study.	Yes ☐	No ☐
I have had the opportunity to use a legal representative, whānau/ family support or a friend to help me ask questions and understand the study.	Yes ☐	No ☐
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	Yes ☐	No ☐
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	Yes ☐	No ☐
I consent to the research staff collecting and processing my information, including information about my health.	Yes ☐	No ☐
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I consent to my GP or current provider being informed about my participation in the study and of any significant abnormal results obtained during the study.	Yes ☐	No ☐
I agree to an approved auditor appointed by the New Zealand Health and Disability Ethic Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.	Yes ☐	No ☐
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	Yes ☐	No ☐

I understand the compensation provisions in case of injury during the study.	Yes ☐	No ☐
I know who to contact if I have any questions about the study in general.	Yes ☐	No ☐
I understand my responsibilities as a study participant.	Yes ☐	No ☐
I wish to receive a summary of the results from the study.	Yes ☐	No ☐

Declaration by participant:

I hereby consent to take part in this study.

Participant's name: _____

Signature: _____ Date: _____

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: _____

Signature: _____ Date: _____

Participant Information Sheet

Study title: Brain stimulation in swallowing training

Locality: University of Canterbury Rose Centre for Stroke Recovery and Research
Ethics committee ref.: 15STH67 (Stroke)
Lead investigator: Kerstin Erfmann
Contact phone number: +64 (3) 364 2307

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> hdecs@moh.govt.nz

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

If you agree to take part, the researcher will ask for the contact details of your General Health Practitioner. This is to ensure that there are no risks involved in your participation. You will also fill in a questionnaire to ensure risks are identified.
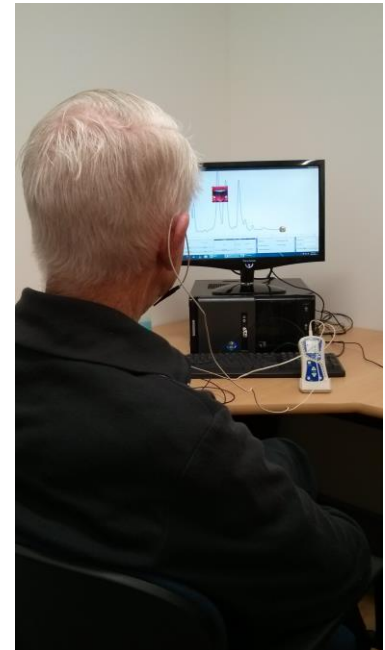
You will complete five sessions of swallowing training and assessment. The first four sessions will be completed on consecutive days (about 60 - 90 min each). There will be one assessment session before and after the two sessions of training. And another assessment session will be completed after one week.

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> (03) 364 2307
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If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

> Phone: 0800 555 050
> Fax: 0800 2 SUPPORT (0800 2787 7678)
> Email: advocacy@hdc.org.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

> Phone: 0800 4 ETHICS
> Email: hdecs@moh.govt.nz

Consent Form

Please tick to indicate you consent to the following

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.	Yes ☐	No ☐
I have been given sufficient time to consider whether or not to participate in this study.	Yes ☐	No ☐
I have had the opportunity to use a legal representative, whānau/ family support or a friend to help me ask questions and understand the study.	Yes ☐	No ☐
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	Yes ☐	No ☐
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	Yes ☐	No ☐
I consent to the research staff collecting and processing my information, including information about my health.	Yes ☐	No ☐
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I consent to my GP or current provider being informed about my participation in the study and of any significant abnormal results obtained during the study.	Yes ☐	No ☐
I agree to an approved auditor appointed by the New Zealand Health and Disability Ethic Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.	Yes ☐	No ☐
I agree to recording a photographic image and or audio or video of me for educational, academic, or research purposes.	Yes ☐	No ☐

I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	Yes ☐	No ☐
I understand the compensation provisions in case of injury during the study.	Yes ☐	No ☐
I know who to contact if I have any questions about the study in general.	Yes ☐	No ☐
I understand my responsibilities as a study participant.	Yes ☐	No ☐
I wish to receive a summary of the results from the study.	Yes ☐	No ☐

Declaration by participant:

I hereby consent to take part in this study.

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature: Date:

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Appendix II: Questionnaires for Non-invasive brain stimulation

Screening NIBS

Study title:
The influence of non-invasive brain stimulation on swallowing skill training

If you agree to take part in this study, please answer the following questions. The information you provide is for screening purposes only and will be kept completely confidential.

CIRCLE or CROSS OUT

Have you ever suffered from any neurological or psychiatric conditions? If YES please give details (nature of condition, duration, current medication, etc)	YES / NO
Have you ever suffered from epilepsy or febrile convulsions in infancy?	YES / NO
Have you ever fainted? If YES when did this (last) happen and what caused it:	YES / NO
Does anyone in your immediate or distant family suffer from epilepsy? If YES please state your relationship to the affected family member.	YES / NO
Do you suffer from migraine?	YES / NO
Have you ever undergone a neurosurgical procedure (including eye surgery)? If YES please give details.	YES / NO
Do you currently have any of the following fitted to your body? Heart pacemaker Cochlear implant Medication pump Surgical clips	YES / NO
Are you currently taking any unprescribed or prescribed medication? If YES please give details	YES / NO
Have you consumed more than 3 units of alcohol in the last 24 hours?	YES / NO
Have you consumed any alcohol today?	YES / NO

Have you had more than one cup of coffee, or other sources of caffeine, in the last hour?	YES / NO
Have you participated in any other brain stimulation experiment in the last 6 months?	YES / NO
Do you or did you in the past experience any swallowing problems?	YES / NO

Do you have any visual impairment?
    Dyschromatopsia (colour blindness)
    Glasses: Sphere
        Distance OD:_____ OS:_____
        ADD   OD:_____ OS:_____
	YES / NO

Date of birth: _____/_____/_____ Age:_____yrs	
Which of the following best represents your racial or ethnic heritage? ☐ New Zealand European ☐Tongan ☐Maori ☐Samoan ☐Niuean ☐Cook Island Maori ☐ Indian ☐Other	

Adapted from Pope et al., 2015

Signed: ....Date:.....................................

Name   (in   block   letters): ....………...

Signed: ....Date:.....................................

Name   (in   block   letters): ....………...

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Comfort Rating Scale

Study title:
The influence of non-invasive brain stimulation on swallowing skill training

Session _____   Date:_____   Participant #:_____

Did you experience any of the following symptoms or side-effects?	Enter a value (1–4) in the space below (1, absent; 2, mild; 3, moderate; 4, severe)	If present, do you think this is related to tDCS? (1, no; 2, possibly ; 3, probably; 4, definitely)
Headache		
Neck pain		
Scalp pain		
Tingling		
Itching		
Burning sensation		
Skin redness		
Sleepiness		
Trouble concentrating		
Acute mood change		
Others (specify)		

Adapted from Brunoni et al., 2011

Notes

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Appendix III: Behavioural Study I

Additional results

Temporal error

Skill learning: Percentage of change in the temporal error from baseline to each of the following timepoints for the two stimulation groups compared to the change from baseline to every other timepoint of the sham group. Post 1 = Post training session 1 assessment; Pre 2 = Pre session 2 assessment; Post 2 = Post training session 2 assessment; 95 % CI = 95 % confidence interval; * = statistically significant effect.

Stimulation condition and timepoint	% change from sham and baseline	Lower 95% CI	Upper 95 % CI	*p*-value
Anodal Post 1	10.90	-16.76	47.76	=0.045*
Anodal Pre 2	3.84	-22.06	38.35	=0.019*
Anodal Post 2	-10.68	-32.96	19.01	=0.042*
Anodal Follow-up 1 day	5.28	-20.98	40.27	<0.001*
Anodal Follow-up 1 week	-13.52	-35.09	15.23	=0.025*
Cathodal Post 1	-15.13	-36.3	13.08	=0.470
Cathodal Pre 2	-13.97	-35.43	14.62	=0.144
Cathodal Post 2	-22.59	-41.9	3.14	=0.175
Cathodal Follow-up 1 day	-22.14	-41.56	3.74	=0.021*
Cathodal Follow-up 1 week	-4.48	-28.31	27.27	=0.007*