Transcranial Direct Current Stimulation (tDCS) as an Adjunct Intervention in Stroke Rehabilitation

Claire Allman

Green Templeton College

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FMRIB Centre

Nuffield Department of Clinical Neurosciences

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Abstract

Stroke is the leading cause of physical disability worldwide and patients are often left with substantial impairments in motor function. Intensive physiotherapy is the most widely used rehabilitative intervention following a stroke, but the potential for motor recovery with physiotherapy alone is limited by the activity of the residual cortex. Consequently there is increasing interest in combining traditional rehabilitation techniques with adjunct interventions to improve outcomes. Transcranial direct current stimulation (tDCS) is a promising tool for rehabilitation. Anodal stimulation of primary motor cortex increases cortical excitability and in stroke patients this translates into transient behavioural improvements after a single session of stimulation. This thesis aimed to investigate whether repeated sessions of tDCS delivered in conjunction with an intensive motor training program would result in long-lasting improvement of motor abilities in chronic stroke patients.

Twenty-four patients received motor training for two weeks for the paretic upper limb; patients were randomised to receive anodal or sham tDCS and were then examined over the subsequent three months to investigate behavioural improvements. All patients experienced a significant improvement in motor function following the motor training intervention. Patients who had received anodal tDCS experienced additional behavioural improvements that were more enduring over time. Baseline characteristics such as the presence of motor evoked potentials in the paretic hand and lesion volume were found to be significantly associated with initial motor impairment, and the degree of initial impairment and allocation to the anodal tDCS condition was found to predict therapy-mediated improvements in motor function.

The results presented in this thesis provide important information regarding the improvement in motor abilities associated with repeated sessions of tDCS, and in particular they suggest that tDCS may be utilised as a clinically useful tool for motor rehabilitation in chronic stroke. Furthermore, they suggest that baseline characteristics could be used to target interventions to patients most likely to benefit.

This thesis contains approximately 27,000 words.

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i. Abbreviations

ADL	Activities of Daily Living
ARAT	Action Research Arm Test
CIMT	Constraint Induced Movement Therapy
CSF	Cerebrospinal Fluid
CST	Corticospinal Tract
DTI	Diffusion Tensor Imaging
EEG	Electroencephalography
FAST	FMRIB's Automated Segmentation Tool
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional Magnetic Resonance Imaging
FMRIB	Centre for Functional MRI of the Brain
FNIRT	FMRIB's Non-linear Image Registration Tool
FSL	FMRIB Software Library
GABA	γ-amino butyric acid
GRASP	Graded Repetitive Arm Supplementary Program
IHI	Interhemispheric Inhibition

JTT	Jebsen Taylor Test of Hand Function
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MPRAGE	Magnetization Prepared Rapid Gradient Echo
MRI	Magnetic Resonance Imaging
MRP	Motor Relearning Program
NIHSS	National Institute of Health Stroke Scale
NMDA	*N*-methyl-D-asparate
SMA	Supplementary Motor Area
tDCS	Transcranial Direct Current Stimulation
TIA	Transient Ischemic Attack
TMS	Transcranial Magnetic Stimulation
UE-FM	Upper Extremity Fugl-Meyer Assessment
WMFT	Wolf Motor Function Test

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iv. Acknowledgements

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1 INTRODUCTION

In this thesis, I aim to investigate whether multiple, consecutive sessions of transcranial direct current stimulation (tDCS), when delivered in conjunction with a two-week motor training program, will result in the long-lasting enhancement of motor and functional abilities in chronic stroke patients. This introductory chapter will discuss current approaches to stroke rehabilitation, the plasticity of the brain and how this changes in response to learning, and how these elements may be combined in a way that is useful for chronic stroke patients. In addition to summarising relevant background literature, I will also discuss how this has shaped the approaches taken in the subsequent experimental chapters.

A stroke occurs as a result of a sudden interruption in the blood supply of the brain and may fall into one of three main categories: ischemic stroke (accounting for up to 90% of cases), intracerebral haemorrhage, or subarachnoid haemorrhage. Ischemic stroke occurs after the occlusion of an artery within the brain, whereas both intracerebral and subarachnoid haemorrhage occurs after the rupture of a blood vessel. Whatever the cause, a stroke is characterised by the rapid onset of clinical symptoms due to the abrupt interruption of blood flow. Symptoms vary depending on the site and size of the lesion, but commonly include numbness or weakness resulting in paralysis of one side of the body, dizziness and communication problems, problems with balance and coordination or severe headache. Further complications may also occur, including but not limited to recurrent stroke,

seizure, infection, thromboembolism, pain, and psychological symptoms including depression and anxiety (Langhorne et al., 2000).

Not all episodes of ischemia result in permanent symptoms of stroke. A transient ischemic attack, or TIA, is a brief period of interrupted blood flow to an area of the brain that may result in the same symptoms as a major stroke, but by definition symptoms resolve completely within 24 hours. However, up to 20% of people who experience TIA symptoms go on to suffer a major stroke within three months (Easton et al., 2009).

The recent and widespread development of acute stroke care has led to a substantial decline in post-stroke mortality (Saver et al., 2009); however, declining mortality does mean that a significant number of stroke survivors now face life with some residual degree of disability, often severe, which may significantly impact on daily activities and functional independence. Indeed, stroke is the leading cause of disability throughout Europe and the United States (Floel & Cohen, 2010); between 50% and 70% of stroke survivors may regain some degree of functional independence, but 15% to 30% are left permanently or severely disabled and 20% require long-term institutional care (Roger et al., 2011). Early rehabilitation can be effective in increasing the recovery of motor abilities above that which can be achieved by natural recovery alone (Maulden, Gassaway, Horn, Smout, & DeJong, 2005); however, even after completing conventional motor rehabilitation, a substantial proportion of patients still experience physical impairment.

1.1 The Natural Course of Clinical Recovery after Stroke

The natural course of clinical recovery after stroke varies significantly from patient to patient, reflecting the ability of an individual's neuronal networks to adapt after injury. However, the exact mechanisms relating to recovery remain elusive. The degree to which a patient recovers is dependent on factors relating to the stroke, for example location and size of infarct, and factors relating to the patient, for example their age (Gadidi, Katz-Leurer, Carmeli, & Bornstein, 2011). At stroke onset, approximately 90% of patients exhibit some degree of motor impairment at a population level. This is equally distributed between mild, moderate and severe paresis. Paresis refers to a condition of paralysis affecting the side of the body contralateral to the lesion, ranging from weakness (mild paresis) to total paralysis (severe paresis). At a population level, the mean initial motor deficit in the acute phase is approximately half of the maximum score of most motor ability scales. Motor function improves on average to three quarters of the scale's maximum at follow-up in the chronic stage, defined as the time after which the direct and secondary consequences of the infarct have subsided and plastic processes become increasingly fixed (Kreisel, Hennerici, & Bazner, 2007). For the purposes of most research studies, including this one, the chronic phase is said to begin at six months post-infarct.

The most dynamic period of natural recovery occurs after the initial 48 hours after infarct (during which the direct consequences of ischemia are most prominent) within the acute (up to four days after onset) and sub-acute stages of recovery (lasting up to two or three weeks after onset) (Kreisel et al., 2007); here, patients reach at least half of their individual maximum best scores on

motor scales. On average, recovery progresses from the sub-acute phase into a period whereby neurofunctional alterations decrease (continuing up to no more than two to three months after onset); this continues to slow substantially as time passes in the majority of cases until patients reach the chronic stages of stroke. In most cases, recovery from paresis will have levelled off substantially at this point (usually three to six months after stroke onset). Notwithstanding the fact that motor deficits remain relatively unchanged thereafter, functional compensation may significantly influence the degree of impairment in the long run (Kreisel et al., 2007). These principles of motor recovery can be summarised in figure 1.1.

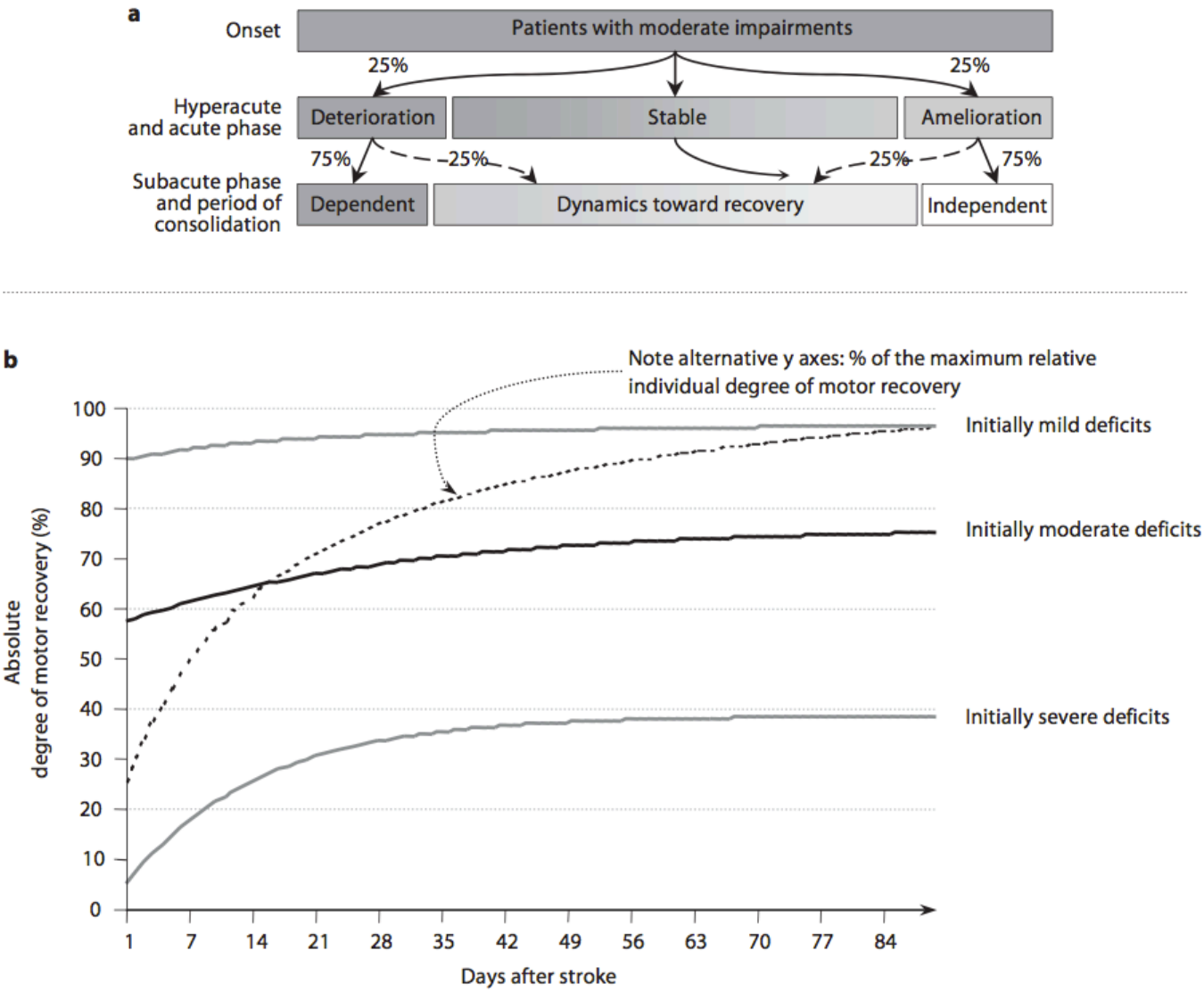


Fig. 1.1. a. Schematically delineated motor recovery pattern of stroke patients with initially moderate impairments, showing the percentages of those deteriorating or recuperating in each phase of recovery. **b** Recovery from paresis, up to an absolute best (i.e. completely restored muscle innervation = 100% on the majority of motor scales), stratified according to onset severity versus the timeline after onset. The dashed curve reflects the relative degree of motor recovery (i.e. 100% = best possible individual recovery), being more or less independent of initial severity. (Figure taken from Kreisel et al., 2007).

Consequently, the timeline of natural clinical recovery following infarct highlights the fact that the most dynamic period of recovery lies in the initial weeks after ischemia. The best individually achievable neurological outcome (referring to neural recovery via neurofunctional adaptation in areas directly affected by ischemic injury) is usually reached in 80% of patients within four to five weeks, with the same degree of functional disability recovery (referring to

motor activity as a correlate of the degree of paresis) occurring within six weeks after stroke (Kreisel et al., 2007). In the majority of cases of natural recovery, little further change is detected starting three months after onset.

1.2 Motor Rehabilitation

Motor rehabilitation post-stroke was traditionally based on conventional physiotherapy approaches such as the Bobath concept, one of the most long-established and widely used approaches in the rehabilitation of hemiparetic patients (Paci, 2003). The Bobath concept places emphasis on the reduction of enhanced muscle tone in the paretic limb; movement of this limb is discouraged while there is a risk of reinforcing abnormal tone. However, despite nearly 50 years of clinical use its effectiveness is questionable, with results from clinical trials showing no evidence of its efficacy (Paci, 2003). Instead, techniques that aim to encourage movement of the affected limb have received more attention; indeed, interventions utilising a higher intensity of training for the paretic limb have reported greater improvements in motor and functional ability in patients with both acute and chronic deficits caused by stroke. One randomised controlled study compared the effectiveness of a Motor Relearning Program (MRP) with the more traditional Bobath technique for patients with acute stroke (Langhammer, 2000). The MRP is an approach that encourages active repetition of a movement of the affected limb and practice of functional skills. Patients treated with this approach stayed fewer days in hospital and had significantly more improved motor function than those receiving more traditional physiotherapy (Langhammer, 2000).

Effects of stroke rehabilitation are cumulative; greater intensity and duration of training leads to greater recovery (Bohannon, 2007; Krakauer, 2006; Wallace et al., 2010). Consequently, techniques have more recently been employed that aim to increase the amount of time patients engage in use of their paretic arm, and the amount of effort they use. An intensive rehabilitation technique that has had some success in motor rehabilitation post-stroke is Constraint Induced Movement Therapy (CIMT). CIMT combines restraint of the unaffected limb and intensive use of the paretic limb. This typically consists of placing a mitt or glove on the unaffected hand and subsequently forcing the use of the affected limb with the goal of promoting purposeful movements when performing functional tasks.

Recent studies of CIMT's effectiveness have been promising; repetitive training of functional movements with this technique have improved the outcome of motor rehabilitation of the paretic hand in both acute and chronic stroke (Winstein et al., 2004). Similarly, others found that chronic stroke patients who received CIMT made greater gains in function of the paretic upper limb than patients who received a less intense control therapy (Wittenberg et al., 2003). However, despite such evidence for its success, rehabilitation techniques such as CIMT are only appropriate for a proportion of stroke patients; these techniques rely on patients having a certain degree of movement and are not appropriate for those patients who experience a high degree of spasticity post-stroke or for those with a fixed hand or fingers.

One motor rehabilitation package that has experienced some success in increasing functional and movement abilities in stroke patients is the Graded Repetitive Arm Supplementary Program (GRASP). The GRASP was originally developed as a self-directed arm and hand exercise program to be completed independently by the patient (Eng, 2009). Research evidence behind the GRASP program supports its use with both acute and chronic stroke patients. Individuals who have used this program were found to demonstrate significant improvements over a control group who received conventional physiotherapy and occupational therapy in important spheres of upper limb function, including the ability to use the paretic limb in activities of daily living, and the ability to reach and grasp objects as assessed by the Action Research Arm Test (ARAT) (Eng, 2009; Harris, Eng, Miller, & Dawson, 2009; 2010). Indeed, when tested four months after the completion of training, the GRASP group still maintained a significantly greater score on measures of motor function than did a control group who received conventional stroke therapy but not the GRASP program (Eng, 2009). In addition, participants were found to report high levels of satisfaction with the GRASP protocol, and no adverse side effects were recorded (Harris et al., 2009; 2010). One of the major factors behind the success of the GRASP program is that it is specifically tailored to each patient's functional ability; the GRASP consists of three different training levels, each designed for a specific range of post-stroke impairments in the upper limb. This is important; for a rehabilitative therapy to be successful it is vital that it suitably addresses individual patients and their level of functional impairment. Therapy intensity can therefore be set accordingly.

1.2.1 Therapy Intensity

It is well accepted that, when learning a new skill, acquiring and maintaining a good performance follows a dose-response relationship: the more time dedicated to learning a specific skill, the more performance improves (Kwakkel et al., 2004). Post-stroke rehabilitation can be regarded as a process in which patients are taught to accomplish complex motor and functional tasks, and therefore it may be inferred that intensive training by stroke patients will follow the same pattern. Research indicates this to be the case. Evidence suggests that intensive motor rehabilitation has a positive impact on motor recovery in stroke patients (Schaechter, 2004). Meta-analyses and reviews have concluded that the greater the intensity of the therapy, the better the functional outcome for patients (Kwakkel, 2006). Consequently, definitions of treatment intensity in post-stroke therapies commonly focus on the duration of the therapy (Kwakkel, 2006; Kwakkel et al., 2004). Through this approach, increases in intensity have been shown to result in improved outcomes, and therefore treatment time seems to be an important variable in rehabilitation. However, equal duration of therapy between different approaches may not accurately reflect equal intensity of treatment, as differences in the content of therapies may be present.

As yet, the optimal "dose" for different forms of upper limb therapy is not known. Recent studies and rehabilitation packages, such as the GRASP, have aimed to resolve this problem by defining rehabilitation intensity by adjusting the level of exercise to the ability of the individual at baseline, rather than solely on time engaged in therapy (Wallace et al., 2010). Such an approach is taken in the studies presented in this thesis. In this way, rehabilitation approaches ensure

that patients are consistently working at a level that challenges their physical ability even when progression is achieved. Such approaches have found statistically significant relationships between specified therapy intensities and rehabilitative success in chronic stroke patients (Wallace et al., 2010).

Rehabilitative success for chronic stroke patients, particularly for those patients who have experienced a plateauing in functional movements, may take some time to become evident during therapy. However, a recent study indicated that motor training delivered over a period of just 10 days led to statistically significant changes in motor and functional ability in chronic stroke patients (Wallace et al., 2010). Furthermore, implementation of this motor training protocol was found to be straightforward in this group, and a therapy compliance rate of 100% was achieved; the authors suggested this high compliance rate was achieved for a number of reasons, but primarily because patients felt subjectively that they were benefitting from an intensive, daily intervention. Indeed, the delivery of therapy daily for 10 days in this instance encouraged therapy compliance and attendance, as participants were found to devote the whole two week period to therapy and arrange other engagements outside this time (Wallace et al., 2010). The study presented in this thesis followed a similar two-week regime.

1.2.2 Time from Stroke to Rehabilitation

Neurofunctional change in relation to injury is likely to depend on the time elapsed after the lesion. In experimental animal studies, some researchers have seen a clear negative dependency between time after lesion onset and probable

effects of rehabilitation; in rodents, rehabilitative training initiated after day one is more effective than training started a week after experimentally induced ischemia (Kreisel et al., 2007). Similarly, if rodents receive rehabilitative training within five days after onset, clinical outcome is significantly better compared with those who received specific training starting 14 days after ischemia (Kreisel et al., 2007). It appears that the longer one waits, the smaller the chance to therapeutically manipulate rehabilitative outcome, which appears to be true of human rehabilitation too (Kreisel et al., 2007; Kreisel, Bazner, & Hennerici, 2006; Yang, Wang, & Wang, 2003). In human research, many studies report a benefit if training is started earlier; patients receiving rehabilitation within the first three weeks after stroke, compared to those who received treatment between three and six weeks, have a greater chance of benefitting from therapy. In turn, the latter group perform better than those starting rehabilitation even later (Biernaskie, Chernenko, & Corbett, 2004). Furthermore, patients receiving more specialised treatment packages early on in their recovery tend to benefit more than those patients receiving non-specialised treatment within the same timeframe (Kreisel et al., 2006; 2007); it appears that in addition to early rehabilitation, certain levels of skill learning must be present to promote cortical plasticity. Additionally, early active participation in rehabilitation and voluntary motor control is more effective in eliciting network alterations than early passive movement (Kreisel et al., 2007; Zhuang et al., 1998).

However, early onset motor training after infarct is not always found to be beneficial (Johansson, 2000). There is some animal data indicating that overtraining of the lesioned forelimb induced by immobilization of the intact

forelimb can expand the volume of cortical lesions. In one study, researchers started training animals five days after inducing a lesion (Nudo, Wise, SiFuentes, & Milliken, 1996); whilst housing these animals in an enriched environment with the opportunity to perform various activities but no specific training significantly improved functional outcome without increasing tissue loss, if combined with more specific and intensive training, an increased tissue loss was observed (Nudo et al., 1996). This increased tissue loss might make the brain more vulnerable to additional insults or aging (Johansson, 2000). In humans, excessive motor activity in the vulnerable acute stage of stroke may also lead to increased tissue loss; one possible explanation for this loss may be that hyper-excitability of local tissue in the early post-ischemic period makes the surrounding neurons vulnerable to excitation. Excessive motor activity may stimulate the release of glutamate and catecholamines (hormones produced under times of physical stress). In the presence of excitatory and toxic substances from the ischemic tissue, an additional release of excitatory substances induced by motor activity may be harmful in the early post-ischemic stage (Johansson, 2000).

Consequently, whilst it is a common consensus that for a rehabilitative approach to be successful it should be specific to the individual's level of functional ability, there is still some uncertainty about how soon after infarct motor rehabilitation should be commenced with any great intensity. The majority of research studies, including those presented in this thesis, have been conducted in stroke patients with chronic (greater than six months) motor impairments. This study design element strengthens the internal validity of such research because motor

function of chronic patients is typically relatively stable at this point (Schaechter, 2004). Therefore, changes in brain function in chronic stroke patients associated with therapy-induced gains in motor function would be more likely due to the effects of the therapy than to spontaneous recovery. Furthermore, by conducting rehabilitation in patients with chronic deficits, it can be ensured that the process will not negatively interfere with stages of acute neural recovery, which may take some time to effectively stabilize. In addition, the physical demands of intense and specialized rehabilitation techniques are likely to be too physically and emotionally demanding for patients still in the acute phases of post-stroke recovery.

1.2.3 Outcome Measures used in Stroke Research

An important consideration in clinical trials that aim to evaluate the effect of rehabilitative interventions for the paretic extremity is the choice of valid, reliable and sensitive outcome measures. Choice of outcome measures should be informed by the need to measure both muscle strength and functional ability. In addition, choice of outcome measures may be influenced by a desire to use assessment techniques employed in other published trials of upper extremity stroke rehabilitation, in order to make effective comparisons.

Outcome measures that focus purely on independence in activities of daily living (ADL) are not always specific for the motor function of the paretic arm, because in some circumstances independence can be achieved using only one arm (van der Lee, Beckerman, Lankhorst, & Bouter, 2001a). Indeed, impaired arm function can be alleviated to some extent by compensation with the contralateral

upper limb. Consequently, assessment scales focusing only on independence lack sensitivity to change in the function of the stroke-affected arm itself. Assessment measures need to be sensitive to true, clinically meaningful change (Guyatt, Kirshner, & Jaeschke, 1992; Guyatt, Walter, & Norman, 1987; Stratford & Binkley, 1996).

There are a number of commonly used outcome measures in stroke research. The three measures used in this thesis are the Upper Extremity Fugl-Meyer Assessment (UE-FM), the Action Research Arm Test (ARAT) and the Wolf Motor Function Test (WMFT).

The UE-FM is considered to be one of the most comprehensive, quantitative measures of motor impairment following stroke (Fugl-Meyer, Jääskö, & Leyman, 1975), and its use as a primary outcome measure in stroke rehabilitation trials has been frequently recommended (Fugl-Meyer et al., 1975; Gladstone, Danells, & Black, 2002; Pang, Harris, & Eng, 2006). Indeed, the UE-FM is one of the most established and commonly used primary outcome measures in stroke rehabilitation trials (Page, Fulk, & Boyne, 2012); in a study designed to compare the psychometric properties of clinical measures used to assess motor function in patients with chronic stroke, the UE-FM was found to assess a wider spectrum of upper extremity motor function and was more discriminative for patients with either very good or very poor motor function than were other measures used (Lin et al., 2009). In fact, the UE-FM was the only measure assessed that did not exhibit obvious floor-effects at various different assessments (Lin et al., 2009).

The UE-FM is readily applicable for multiple assessments during rehabilitation to monitor progress. Excellent intra-rater (0.95-1.0) and inter-rater (0.99) reliability have been demonstrated for the scale (Sullivan et al., 2011). The scale's validity is supported by numerous studies (Gladstone et al., 2002; Page et al., 2012; Pang et al., 2006; Sullivan et al., 2011). The UE-FM has been found to have particular value in clinical trials designed to evaluate changes in motor impairment following stroke, although some limitations should be kept in mind; the scale is strictly an impairment index, and as with most scales its use as a measurement of recovery for patients with mild motor impairment is limited by a ceiling effect (Gladstone et al., 2002). Thus, other outcome measures may be used in combination with the UE-FM for a more comprehensive assessment of motor impairment.

The ARAT is a standardized ordinal scale that was designed to measure upper extremity disability through the assessment of four subtests comprising 19 movements (Lyle, 1981; van der Lee, de Groot, Beckerman, Wagenaar, Lankhorst, et al., 2001b). Test materials consist of a wooden box placed on the table in front of the patient, containing blocks and objects of different sizes (figure 1.2). In three subtests (Grasp, Grip and Pinch), the ability to grasp, move and release objects differing in size, weight and shape is tested. Objects must be picked up and moved vertically or horizontally to a standardized location. Two items in the Grip subtest not only require horizontal movements but also a certain degree of vertical movement and pronation (pouring water from one glass to another) or supination (turning a washer) (van der Lee, de Groot,

Beckerman, Wagenaar, Lankhorst, et al., 2001b). The fourth subtest consists of three gross movements.

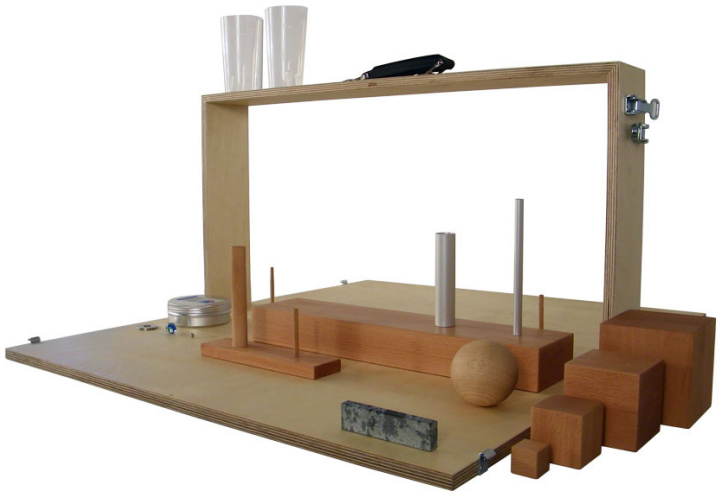


Fig 1.2. Photograph of the ARAT kit and its equipment. Taken from http://www.reha-stim.de/.

A number of previous studies have indicated that the UE-FM and the ARAT are equally sensitive to changes during stroke rehabilitation (De Weerdt, 2009; Lin et al., 2009; Rabadi, 2006). Others have shown the ARAT to be more responsive to improvements in paretic arm function than the UE-FM in people with chronic stroke (van der Lee, Beckerman, Lankhorst, & Bouter, 2001a), and Lin et al. (2009) found that of four measures of functional assessment, the ARAT showed the highest responsiveness at different recovery stages. However, in the same study the ARAT was also found to have significant floor and ceiling effects in comparison with other measures, including the UE-FM (Lin et al., 2009).

The WMFT quantifies upper extremity movement ability through single or multiple joint movements and functional tasks (Wolf et al., 2001). Tasks are

arranged in order of complexity, progressing from proximal to distal joint involvement, testing total extremity movement and movement speed.

The reliability and validity of the WMFT have been ascertained in patients with chronic stroke (Wolf, 2005). The scale has high inter-rater reliability (0.97-0.88) among patients and correlates with performance on the UE-FM (0.88-0.96) (Wolf, 2005; Wolf et al., 2001). The scale is also able to successfully discriminate patient scores by functional ability to a reasonable level (Whitall, Savin, Harris-Love, & Waller, 2006; Wolf, 2005). Consequently, the WMFT is commonly used as a measure of functional ability in stroke research.

1.3 Processes Mediating Motor Recovery: Fundamentals of Plasticity

A clear understanding of the processes underlying brain plasticity is crucial for the development of successful rehabilitative strategies for patients with a diverse range of post-stroke impairments. This includes understanding the neural processes underlying recovery of function, and how these may contribute to spontaneous or rehabilitation-mediated recovery (Ward, 2005). The brain's potential for plastic reorganisation has been confirmed in both sensory and motor areas of the adult cortex as a consequence of trauma, pathology, manipulation of sensory experience or learning (Rioult-Pedotti, 2000).

Plasticity may be considered as the capacity of the brain to change, or as an inherent property of the human nervous system that persists throughout one's lifespan. In the adult brain, plasticity is associated with learning and occurs with

the learning of new motor skills (Doyon & Benali, 2005). Therefore, plasticity in this context carries important behavioural implications over time (Ward, 2005). Plasticity occurs over different scales in the brain, from cellular and synaptic changes to network level changes, as described in detail below. These processes result in long-term change in the use of connections between one part of the central nervous system and another (Rothwell, 2010).

1.3.1 Synaptic Plasticity

Synaptic plasticity refers to the ability of the connection, or synapse, between two neurons to change in strength in response to use or disuse. Thus, the basis of functional plasticity within the brain is that the strength of connection between separate neurons can change in response to their firing patterns (Hebb, 1949). This is known as use-dependent plasticity (Rosenzweig & Bennett, 1996). Use-dependent changes in synaptic communications have been proposed to play an important role in the capacity of the brain to translate transient experiences into more enduring patterns of behaviour or learning (Davis, Charney, Coyle, & Nemeroff, 2002). Synaptic transmission can either be enhanced or depressed by activity, and these alterations span timeframes ranging from milliseconds to more permanent modifications that may potentially persist for days, weeks, or even longer.

Transient forms of synaptic plasticity have been associated with short-term sensory adaptations, temporary changes in behaviour, and transient forms of memory (Malenka, 1999). In the short term, alterations in firing patterns of pre- and post-synaptic neurons lead to changes in the ability of a synapse to fire again

(Malenka & Bear, 2004). More enduring changes are believed to play an important role in the construction of neural circuits, both during phases of development and with long-term forms of memory and learning in the mature nervous system.

Two of the most fundamental aspects of long-term synaptic plasticity include long-term potentiation (LTP; referring to the long-lasting enhancement of synaptic transmission) and long-term depression (LTD; involving the weakening of a neuronal synapse). LTP allows for the modulation of synaptic strength that stabilises for enduring periods of time, and has therefore been suggested as a potential mechanism for learning (Butler & Wolf, 2007). LTP-like plasticity has been observed at virtually every excitatory synapse in the brain, including the motor and somatosensory cortex (Davis et al., 2002).

LTP can be induced within the primary motor cortex (M1) through a variety of manipulations (Monfils & Plautz, 2005). Initial work in the cat M1 showed that stimulation of the somatic sensory cortex produces synaptic potentiation in M1 (Sakamoto, Porter, & Asanuma, 1987). In terms of motor learning and motor control, more compelling evidence for the involvement of synaptic strengthening during learning came from a variety of experiments on rats that had been trained to produce a certain movement using one forelimb (Rioult-Pedotti, 2000). After training, the rats were sacrificed and the primary M1 in each hemisphere (trained vs. untrained) was examined to test the induction of synaptic plasticity through intracortical pathways (Rothwell, 2010). It was more difficult to produce LTP in the trained hemisphere than in the untrained hemisphere,

suggesting that the process of motor learning alters synaptic plasticity (Rioult-Pedotti, 2000). Consequently, it would seem that motor skill learning itself is accompanied by changes in the strength of connections within the primary M1 (Ward, 2005). Due to these processes, the adult brain retains the capacity to reorganise throughout the lifespan.

1.3.1.1 Neurochemistry underlying synaptic plasticity

Within the human cortex, significant changes in synaptic strength are caused by functional changes in the excitatory neurotransmitter glutamate and the inhibitory γ-amino butyric acid (GABA). These neurotransmitters are tightly integrated with normal brain functioning and are closely related biochemically.

Glutamate is the major excitatory neurotransmitter in the human brain, and alterations in density are vital to the induction of LTP and LTD (Mackenzie & Erickson, 2004). LTP is primarily dependent on changes in glutamatergic transmission (Johnston, Williams, Jaffe, & Gray, 1992). GABA receptor antagonists also serve to facilitate LTP induction (Hess & Donoghue, 1994; Hess, Jacobs, & Donoghue, 1994; Monfils & Plautz, 2005). GABA is the major inhibitory neurotransmitter. Modulation of inhibitory activity may play a critical role in motor learning. Long-term increases in cortical plasticity and development of new synaptic connections is associated with decreases in the number of postsynaptic GABA receptors, as evidenced by magnetic resonance spectroscopy (Floyer-Lea, Wylezinska, Kincses, & Matthews, 2006). Reduction in GABA inhibition is thought to facilitate LTP-like activity in M1 (Castro-Alamancos &

Connors, 1996).

1.3.2 Motor Map Changes

In the motor system, changes in synaptic strength can be seen in changes occurring in motor cortical maps. Since the discovery of motor maps within M1, numerous experiments have shown that changes in motor representations occur in response to a variety of manipulations, including learning (Kleim, 2004; Sanes & Donoghue, 2000). The capacity for motor map reorganisation exists to support the acquisition of skilled movements achieved through learning, and these motor-learning dependent changes in movement representations have been demonstrated in human and non-human research (Kleim, 2004; Nudo & Milliken, 1996). For example, experiments in healthy rodents using a skilled forelimb-reaching task demonstrate that two weeks of learning to retrieve a pellet induces an expansion of distal forelimb (wrist/digit) movement representations within the caudal forelimb area (figure 1.3). The finding that motor map topography reflects skilled movement capacity confirms that maps can adapt in response to motor learning (Monfils & Plautz, 2005).

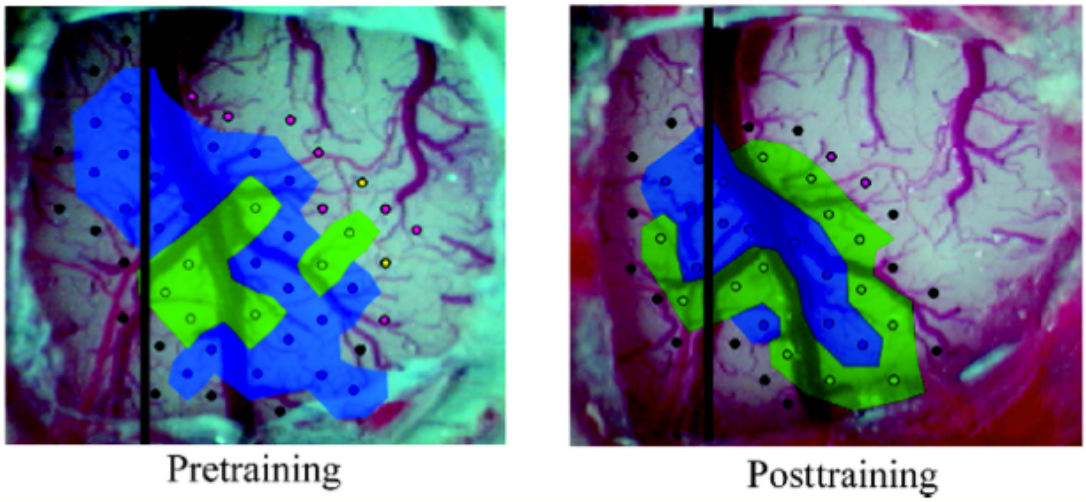


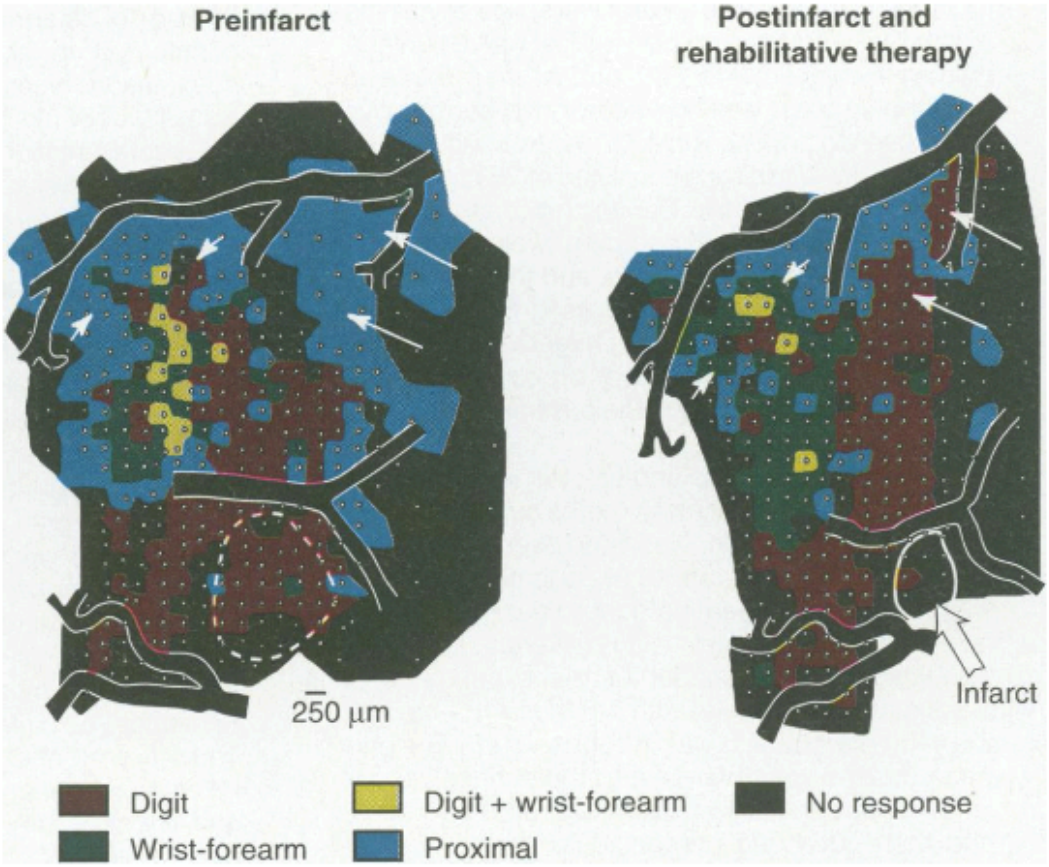
Fig 1.3. Example of motor map reorganisation within the rat motor cortex after two weeks of training on a skilled reaching task. Digit and wrist representations (green) can be seen to expand into elbow/shoulder representations (blue). (Figure adapted from Monfils et al., 2005).

Learning-dependent changes in motor map topography have also been observed in human subjects. The M1 contralateral to the dominant hand of skilled racquetball players exhibits larger hand representations and enhanced motor evoked potential (MEP) amplitude to transcranial magnetic stimulation (TMS) in comparison to less skilled players and non-playing controls (Pearce, Thickbroom, Byrnes, & Mastaglia, 2000). Similarly, subjects trained to produce skilled digit movement sequences on a piano for five days exhibit an increase in area of digit representation corresponding to the trained hand and a decrease in activation threshold, as measured by TMS mapping. The changes are limited to the cortical representation of the trained hand and do not occur in control subjects (Pascual-Leone et al., 1995).

Cortical motor maps are maintained at least in part by GABA (Boroojerdi, 2002; Conner, Culberson, Packowski, Chiba, & Tuszynski, 2003; Jacobs & Donoghue, 1991), but they can be deliberately altered through pharmacological

manipulations, or distorted unintentionally by lesions (Ward, 2005). Indeed, as with learning, synaptic plasticity and reorganisation of cortical representations also occur with recovery after brain injury, which can be thought of as a 'relearning' of impaired functions. For example, lesions induced in the hand area of the monkey cortex have been found to be accompanied by a shift in the representation of the hand into adjacent areas of the cortex (Nudo, 2003). This was found to occur when animals were given 'therapy' to encourage them to use the affected hand, but not when the animals didn't receive this intervention. Thus, functional recovery in this case appeared to depend on reorganisation of the cortex (Nudo, 2003; Rothwell, 2010). Similarly, Nudo and colleagues have shown that rehabilitation in experimentally lesioned squirrel monkeys causes an expansion of digit, hand and forearm representations (Nudo & Milliken, 1996) (figure 1.4).

a.



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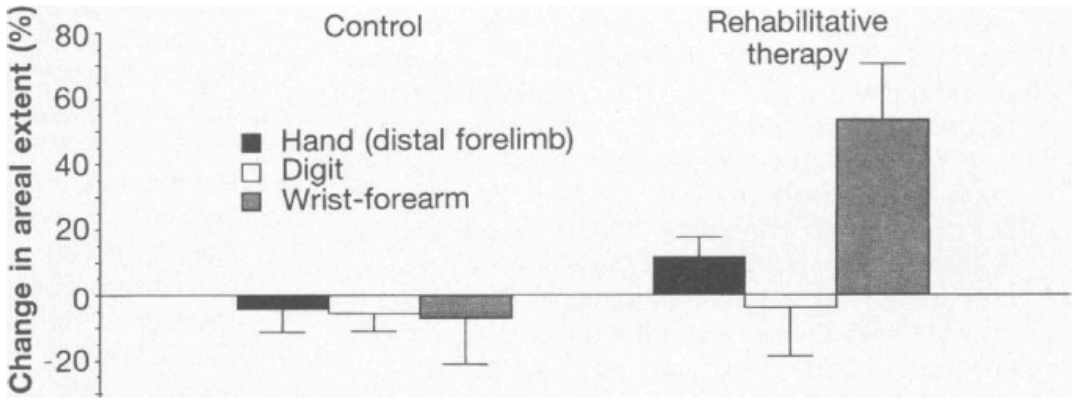


Fig 1.4. a. Reorganization of hand representations in the primary M1 before infarct (left) and after a focal ischemic infarct and rehabilitative training (right). At each lesion site (small white circles), intracortical micro-stimulation techniques were used to define movements evoked by near-threshold electrical stimulation. In this animal, the infarct destroyed 21.6% of digit and 4.1% of wrist-forearm representation. After rehabilitative training, the spared digit representational area increased by 14.9% and the spared wrist-forearm representational area increased by 58.5%. The dashed circle in the pre-infarct map encompasses cortical territory targeted for ischemic infarct. The large white arrow in the post-infarct map indicates the

infracted region. The reduction in size of the infracted zone is attributable to tissue necrosis during the rehabilitation period. Long thin arrows point to adjacent, undamaged cortex in which digit representations (red) appear to have invaded regions formerly occupied by representations of the elbow and shoulder (blue). Short thin arrows point to wrist-forearm representations (green) that appear to have invaded digit, elbow, and shoulder representations. **b.** Changes in the areal extent of hand representations in the control group and rehabilitative training group. (Figure adapted from Nudo et al.,1996).

Modifications in motor map location have also been observed in response to rehabilitation in stroke patients, and there is a strong positive correlation between the magnitude of this map shift and improvements in grip strength in the paretic hand (Monfils & Plautz, 2005; Thickbroom, Byrnes, Archer, & Mastaglia, 2004). Finally, direct electrical stimulation of the motor cortex in a manner that causes motor map expansion in animals with experimental strokes also enhances motor recovery after stroke in human patients (Brown, Lutsep, Cramer, & Weinand, 2003).

1.3.3 Widespread Cortical Changes occur with Recovery

In addition to localized changes observed in motor maps, changes occur across more distributed networks involved in motor control and there has been a particular focus on the balance of activity between the two hemispheres. Homologous regions of the primary motor cortices are connected via transcallosal fibres (Wahl et al., 2007); these connections are of an inhibitory nature (though excitatory connections also exist) and can be measured in order to assess interhemispheric inhibition (IHI) (Avanzino, Bassolino, & Pozzo, 2011). Interhemispheric communication between the motor cortices plays a major role in control of unimanual hand movements, as balanced interhemispheric interactions are required to generate proper voluntary movements (Murase,

Duque, Mazzocchio, & Cohen, 2004). Stroke may affect the balance of transcallosal inhibitory circuits between the motor areas in both the ipsilesional and contralesional hemisphere (Nowak, Grefkes, Ameli, & Fink, 2009).

Longitudinal functional magnetic resonance imaging (fMRI) studies reveal that, early after infarct, neural activity is frequently enhanced in motor-related areas in both hemispheres (figure 1.5) (Grefkes & Fink, 2011); over the first initial months post-stroke, this then returns to levels similar to those observed in healthy controls, and in particular in patients with good motor recovery (Rehme, Fink, & Cramon, 2011; Tombari et al., 2004; Ward, Brown, Thompson, & Frackowiak, 2003).

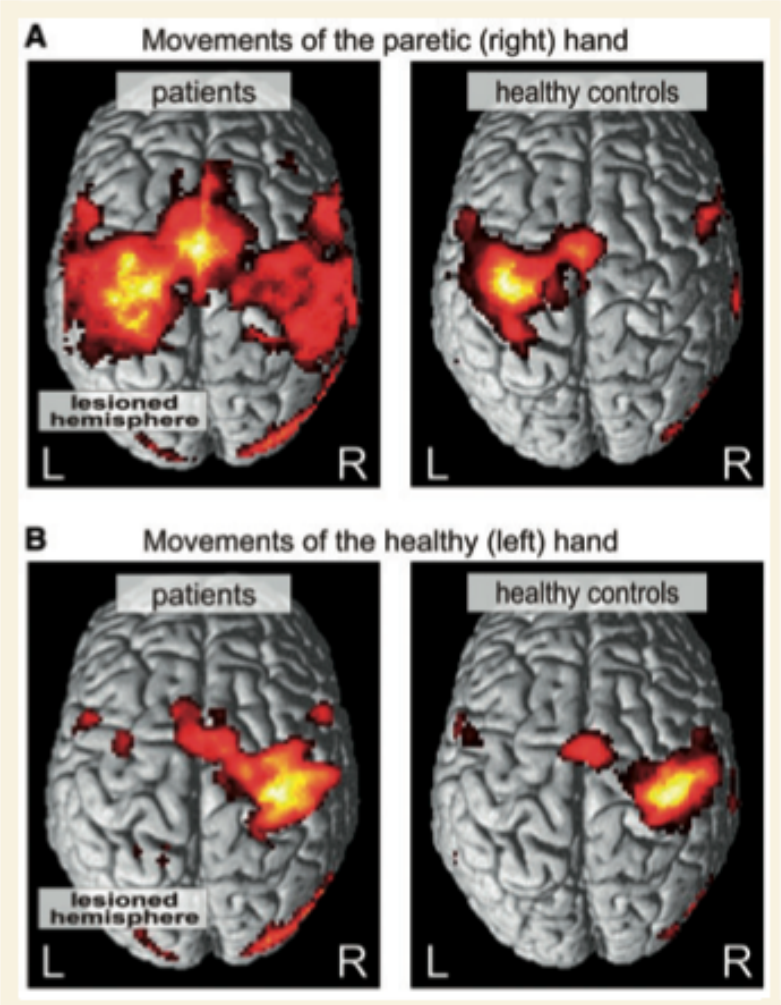


Fig 1.5. Neural activity during movement of the left or right hand in healthy controls and stroke patients with left sided lesions. In patients, movements of the paretic hand have been associated with significant activations in ipsilateral (contralesional) motor areas, which are absent in controls (**A**) or when moving the unaffected hand (**B**). (Figure taken from Grefkes et al., 2008).

Furthermore, the balance of activity across the hemispheres may normalise over time with increasing motor recovery, from initial widespread bilateral activation to a more focused activation contralateral to the moved hand (referring to increased sensorimotor activity within the stroke-affected hemisphere) (Calautti, Leroy, Guincestre, & Baron, 2001; Carey, Abbott, Egan, Bernhardt, & Donnan, 2005; Feydy et al., 2002; Tombari et al., 2004). More bilateral activity patterns that persist into the chronic stages post stroke are associated with

worse impairments, as these indicate poorer recovery and reorganization within the damaged hemisphere (Johansen-Berg, Dawes, et al., 2002a).

Normalization of hemispheric balance may underlie the functional gains observed in stroke patients after rehabilitative techniques such as CIMT (Schaechter, 2004). For example, studies have found that before therapy, the size of the representation within M1 in the ipsilesional hemisphere was smaller for the paretic hand in comparison to that observed for the unaffected hand (Liepert, Bauder, Miltner, & Taub, 2000; Liepert et al., 1998). After therapy, when functional use of the paretic upper limb had improved, the size of the paretic hand representation was enlarged when visualized with imaging techniques (whereas that of the unaffected hand remained unchanged), which represented a return to the normal balance of excitability of the two hemispheres, as assessed further by brain stimulation techniques such as transcranial magnetic stimulation (TMS).

Changes in the balance of activity across the hemisphere may involve change in the contralesional as well as the ipsilesional hemisphere, though the role of the contralesional M1 for motor recovery remains controversial (Grefkes & Fink, 2011).

Some evidence suggests that increased contralesional activity is greatest in patients who have achieved a poorer recovery (Johansen-Berg, Rushworth, et al., 2002b; Ward et al., 2003), leading to suggestions that this pattern of activity is maladaptive. Consistent with this, research has demonstrated that inhibition of

contralesional M1 excitability may lead to improved motor performance of the paretic hand during both acute and chronic phases after stroke (Mansur et al., 2005; Nowak et al., 2008; Takeuchi, Chuma, Matsuo, Watanabe, & Ikoma, 2005). A combined TMS and fMRI study indicated that stroke patients might benefit from contralesional M1 inhibition, which shows movement-related over-activity in the contralesional precentral gyrus, i.e. the cortex below the repetitive TMS stimulation site (Grefkes & Fink, 2011; Nowak et al., 2008). Thus, enhanced activity in contralesional M1 might exert a negative influence on the motor network controlling the paretic hand and may thereby even impair recovery of function.

In addition, some of the most convincing evidence regarding increased control over motor movements from techniques such as CIMT has come from studies investigating inhibitory interactions between the hemispheres (Wolf, 2007). Information from brain stimulation studies has shown that when M1 in one hemisphere is activated, it produces interhemispheric inhibition of the contralateral M1 (figure 1.6). In patients recovering from stroke, increased excitability is found in the homotopic M1 of the contralesional hemisphere, presumably due to reduced interhemispheric inhibition from the ipsilesional M1 (Butefisch, 2003). This increased excitability of the contralesional M1 in turn results in greater inhibition of the ipsilesional M1, which is especially prevalent during attempts to initiate movement with the impaired hand (Wolf, 2007).

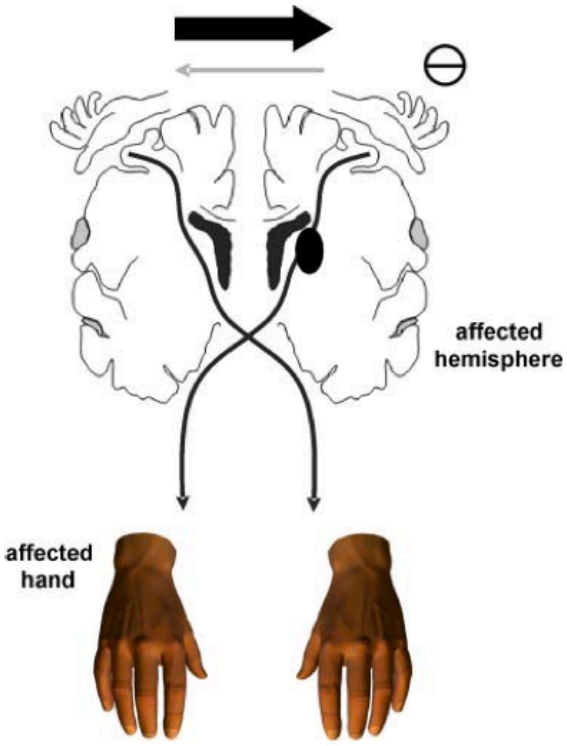


Fig 1.6. Interhemispheric competition following stroke. Following a stroke in the left hemisphere resulting in sensorimotor deficits in the right hand, the primary M1 of the unaffected hemisphere is disinhibited and exerts enhanced transcallosal inhibition of the primary M1 of the affected hemisphere. Enhanced inhibition of the M1 of the affected hemisphere can hamper recovery of the affected hand. (Figure taken from Nowak et al., 2009).

Consequently, these observations support the possibility that increased excitation of the contralesional hemisphere, and increased inhibition of the ipsilesional hemisphere, may play a substantial role in impaired motor function in patients with stroke.

However, evidence is currently inconsistent as to whether the increased contralesional activity is maladaptive in all stroke patients. Although relative over activity in the contralesional hemisphere is generally associated with poor outcome (Johansen-Berg, Rushworth, et al., 2002b; Murase et al., 2004; Ward et al., 2003), this increased activity of the contralesional motor areas may nevertheless be supportive of motor function rather than maladaptive. In

support of this possibility, disruption of activity in contralesional motor or premotor cortex using pulses of TMS can impair motor response in patients in the chronic stages of recovery (Johansen-Berg, Rushworth, et al., 2002b; Lotze, Markert, Sauseng, & Hoppe, 2006). It is likely that individual patient characteristics will determine what level of activity in the contralesional hemisphere will prove adaptive or not. In those patients who are able to rely to some extent on ipsilesional cortical activity for the majority of motor functions, contralesional over-activity or inhibition is likely to be maladaptive. However, in those patients with little residual function of the ipsilesional M1, increased contralesional activity may be vital in preserving function.

1.3.4 Conclusions regarding the physiological Processes Underlying Plasticity and Change

As described above, the physiological processes that underlie changes within the brain may be classified as acute, post-acute or chronic (Ween & Shutter, 2002). Acute changes may refer to the recovery of ischemic tissue, while post-acute changes refer to the recruitment of neighbouring or remote tissue to facilitate functionally important processes. This neuronal reorganization proceeds over weeks or months, so it is reasonable to predict that any functional recovery occurring post-stroke (either spontaneously or as a result of rehabilitative interventions) will be greatest during this period. Significant neuronal reorganization in the chronic stage is considered unlikely (Ween & Shutter, 2002); however, some change may still occur, in that established networks may be modified and strengthened to achieve specific functional activities. This is

precisely what the application of brain stimulation aims to target when used in conjunction with rehabilitative techniques.

1.4 Predicting Functional Recovery in Chronic Stroke Patients

The ability to provide an accurate prognosis for recovery following stroke is critical for treatment planning, realistic goal-setting and efficient resource allocation by clinicians and patients (Stinear, 2010; Ween & Shutter, 2002). To understand recovery from stroke and in order to be able to make accurate predictions with regards to motor recovery, a number of different variables should be considered. These include an understanding of the physiological processes that underlie changes within the brain, and numerous factors associated with the infarct itself, including lesion characteristics such as size and location. The degree of presenting motor impairment should also be considered, as should those techniques that allow clinicians to clearly measure the integrity of systems relating to movement and functional ability.

1.4.1 Degree of motor impairment

The degree of motor impairment post-stroke is one of the simplest prognostic indicators, with greater initial impairment frequently predicting worse functional recovery (Stinear, 2010). Voluntary shoulder and finger movements seven days after stroke have been strongly related to subsequent recovery of upper limb function (Beebe & Lang, 2009), and more generally the degree of initial motor impairment in the arm is frequently found to be the most important determinant of both motor and functional recovery (Feys, Hetebrij, Wilms, Dom,

& De Weerdt, 2000a). However, there is often noteworthy inter-individual variability in the relationship between initial impairment post-stroke and subsequent recovery of motor function, meaning that accurate predictions for each patient remains difficult.

1.4.2 Residual Corticospinal Output

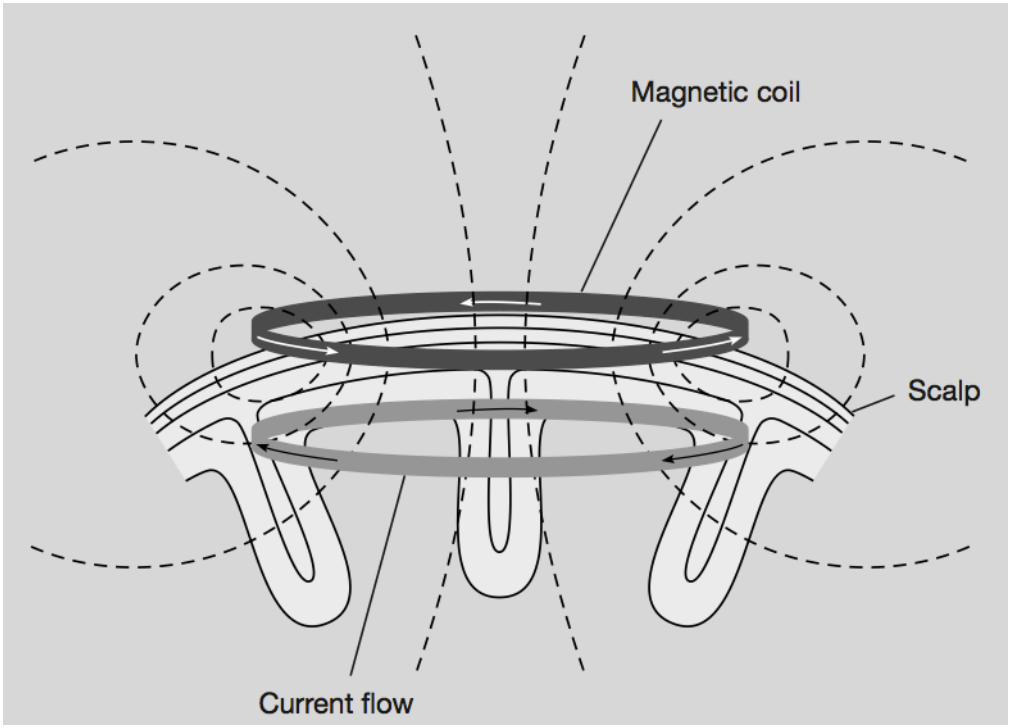
The functional integrity of the corticospinal tract (CST) may be a useful predictor of recovery. The CST conducts impulses from the brain to the spinal cord, and contains mostly motor axons. It is concerned specifically with voluntary skilled movements, such as accurate movement of the fingers. CST functional integrity can be readily assessed using TMS. TMS was introduced in 1985 as a technique that allowed for stimulation of both nerves and the brain (Baker, 1985). It is now commonly used in clinical neurology as a powerful, non-invasive tool for mapping cortical motor representations in normal and pathological cases (Butler & Wolf, 2007; Hallett, 2001). Electromagnetic induction allows for a current to be directed through a handheld copper stimulation coil. This produces a transient magnetic field which, when positioned over the scalp, induces a small electric current in the underlying brain tissue (figure 1.7). This electric current produces a depolarization of nerve cells subsequently resulting in either the stimulation or disruption of cortical activity, depending on the frequency and intensity of the stimulation.

When applied over M1, single-pulse TMS is thought to stimulate the CST indirectly (trans-synaptically) through horizontal fibre depolarization (Butler &

Wolf, 2007). The results can be recorded as MEPs elicited through the contralateral upper limb, or can be simply viewed as a muscle twitch within the paretic hand or fingers. The threshold for producing an MEP in a resting muscle reflects the excitability of a central core of neurons, which arises from the excitability of each individual neuron and their local density (Hallett, 2001); this threshold represents neuronal excitability.

In recent years, TMS has been utilised to investigate those potential mechanisms that underlie both spontaneous and rehabilitation-induced motor recovery after stroke; specifically, TMS can be used to measure a variety of parameters within M1, which allows for the assessment of different aspects of cortical excitability. Reduced corticospinal excitability from the lesioned hemisphere reflects damage to the corticospinal connections (Swayne, Rothwell, Ward, & Greenwood, 2008). Therefore, it is generally agreed that, in the acute phase after infarct, the inability to elicit MEPs or a visible muscle twitch following focal stimulation of the affected hemisphere correlates with poor functional outcome (Hendricks, Hageman, & van Limbeek, 1997; Pennisi et al., 1999). Conversely, the preservation of MEPs evoked by TMS in the early period after stroke is predictive of good functional recovery (Ziemann, 2000).

a.


Magnetic coil
Scalp
Current flow

b.

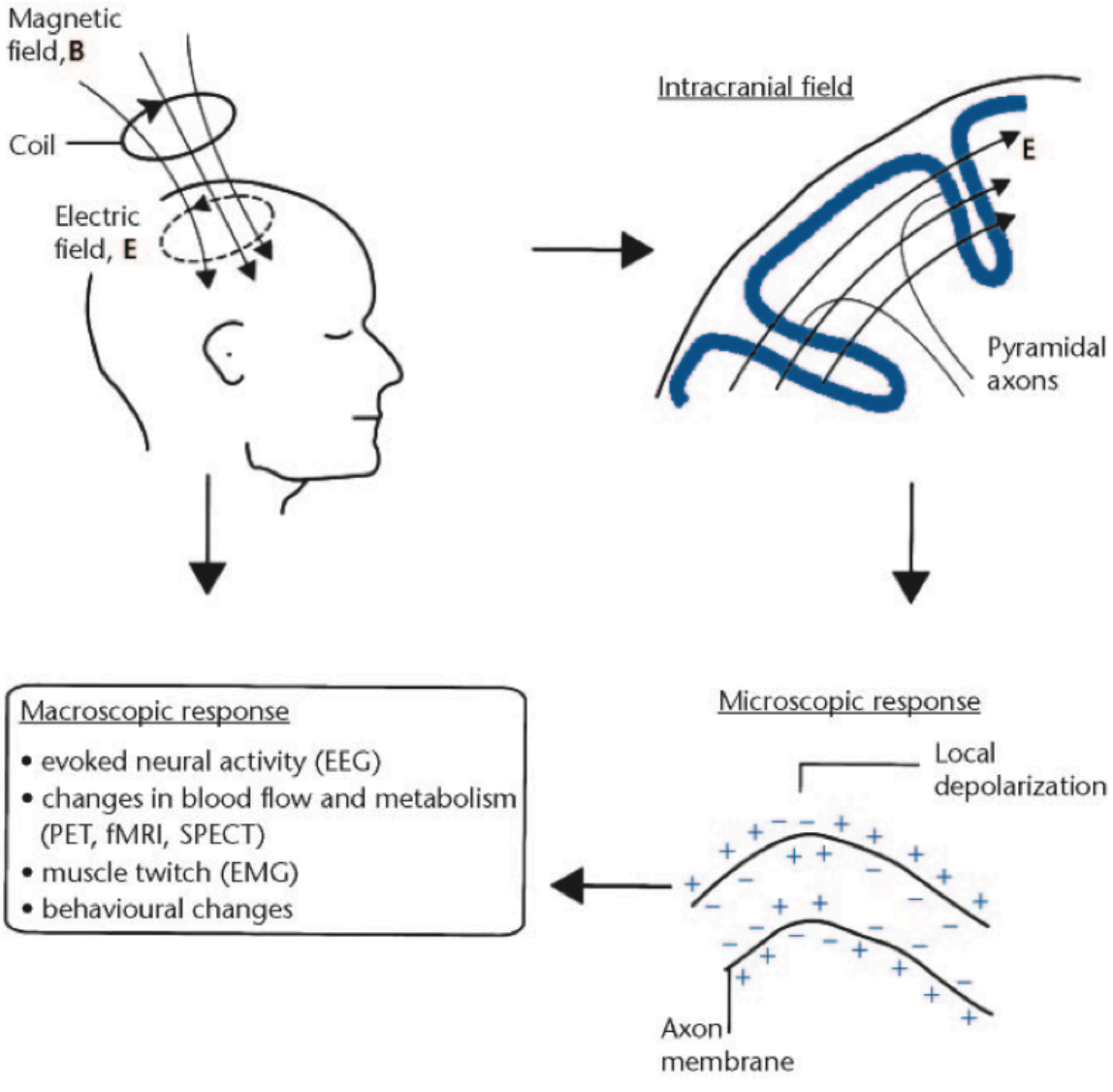

Magnetic field, B
Coil
Electric field, E
Intracranial field
E
Pyramidal axons
Macroscopic response
• evoked neural activity (EEG)
• changes in blood flow and metabolism (PET, fMRI, SPECT)
• muscle twitch (EMG)
• behavioural changes
Microscopic response
Local depolarization
Axon membrane

Fig 1.7. a. A brief, high-current pulse is produced in a coil of wire, the magnetic coil, which is placed above the scalp. A magnetic field is produced, and an electrical field is induced perpendicularly to the magnetic field. **b.** The current in the coil generates a changing magnetic field (B) that induces an electric field (E) in the brain. The electrical field affects the transmembrane potentials and may lead to local membrane depolarization and subsequent neural activation. Macroscopic responses can be detected with functional neuroimaging tools or as behavioural changes. (Figure adapted from Butler et al., 2007).

Other research has reported relationships between recovery and changes in the length of time for conduction from cortex to muscle, MEP latency, excitability threshold, and MEP amplitude. The latency of the elicited MEP response is typically prolonged in the acute phase and subsequently shortens in a manner that is highly correlated with muscle strength and hand function test scores as a patient recovers function (Heald, Bates, Cartlidge, French, & Miller, 1993). By contrast, a long duration of MEP latency, and an extended conduction time (in comparison to healthy controls) in the early period after injury are strongly predictive of reduced hand motor function recovery (Butler & Wolf, 2007).

MEP presence as assessed by TMS may also be a useful measure for patients in the later stages of recovery. One study examined MEPs in chronic stroke patients before and after a 30 day motor practice program, in which patients engaged in 30 minutes of daily exercise with their stroke affected arm (Stinear, Barber, Smale, & Coxon, 2007). The presence or absence of MEPs in the paretic arm strongly predicted UE-FM score at study inception; chronic stroke patients with MEPs had better upper limb function than those without MEPs. MEP presence was also used to predict functional potential, defined as the change in UE-FM score from baseline to follow-up. For patients with MEPs, functional potential declined with increasing time since stroke, but was greatest in those patients with a baseline UE-FM score of 20 or more (Stinear et al., 2007). For

patients without MEPs, functional potential depended on the extent of damage to M1; patients with damage to M1 made no functional gains.

Other studies have similarly found that the ability of TMS to predict arm recovery may be more useful when used in conjunction with clinical measurements than when used alone. Feys et al (2000) found that the best predictor of long-term outcome from the acute phase post-stroke was obtained by a combination of motor performance as measured by the UE-FM, and measurement of MEPs produced by TMS; long-term prognosis (12 months) could be made at two months after stroke based on the combination of these techniques. Application of this strategy led to an explained variance in motor recovery of 81% (Feys, Van Hees, & Bruyninckx, 2000b). Using motor scores alone explained a variance in recovery of only 49-75%.

1.4.3 Neuroimaging: Lesion Characteristics and Residual Brain Tissue

Neuroimaging plays a vital part in both the initial evaluation and later management of patients presenting with stroke. Although symptoms such as initial motor deficits and further neurological examinations can indicate different aspects related to disability, only neuroimaging can confirm distinct aspects related to infarct (Leary & Caplan, 2007).

Following stroke, MRI scans are frequently collected in clinical settings. This allows for the identification and assessment of stroke characteristics such as those described below. As previously discussed, these contain invaluable

information that allows clinicians to predict recovery of function after lesion. Most clinical scan protocols include as a matter of course a T1-weighted spin echo scan (referring to an MRI sequence that allows for good contrast between gray and white brain matter) that allows for structural information to be gained. In addition, diffusion scans can be acquired that allow for information to be collected regarding the integrity of the CST.

Two stroke characteristics that can be established from a T1 MRI scan that correlate strongly with impairment are lesion size and location, although a huge amount of variability in motor recovery relating to these variables remains unexplained (Zhu, Lindenberg, Alexander, & Schlaug, 2010). Some research suggests that lesion size correlates strongly with outcome (Kaczmarczyk, Wit, Krawczyk, Zaborski, & Gajewski, 2011), with lesions larger than 10% of intracranial volume leading to the poorest outcomes (Ganesan, Ng, Chong, Kirkham, & Connelly, 1999). In contrast, other studies have found no significant correlation between initial lesion size and recovery of upper-limb motor function (Binkofski, Seitz, & Hackländer, 2001; Kaczmarczyk et al., 2011) (Chen, Tang, Chen, Chung, & Wong, 2000). This may be due to substantial heterogeneity within the samples studied, or other factors such as patient age, time since stroke, or lesion location.

Lesion location may also influence recovery. Patients with cortical strokes have been found to have poorer functional scores on average than patients with exclusively subcortical lesions (Glymour et al., 2007). Right-sided lesions have been associated with poorer performance in activities of daily living, as

measured by the National Institute of Health Stroke Scale (NIHSS), than either left or bilateral lesions (Glymour et al., 2007). The relationship between lesion size and NIHSS score has also been found to differ for right- and left-hemisphere strokes; patients with small, right-sided lobar lesions (affecting less than half the lobe) have lower average NIHSS scores compared with patients with larger lobar lesions. For patients with left-hemisphere strokes, small lesions have been associated with substantially better NIHSS scores compared with patients with large lesions, suggesting that lesion volume has a larger effect on NIHSS in left-sided strokes than in right-sided strokes (Glymour et al., 2007).

An influence of lesion size on recovery has also been suggested for chronic stroke patients receiving rehabilitation interventions, with smaller infarct volumes associated with greater trial-related functional gains (Cramer et al., 2007). I have therefore tested for a relationship between lesion characteristics and both baseline clinical scores and response to intervention in the current thesis.

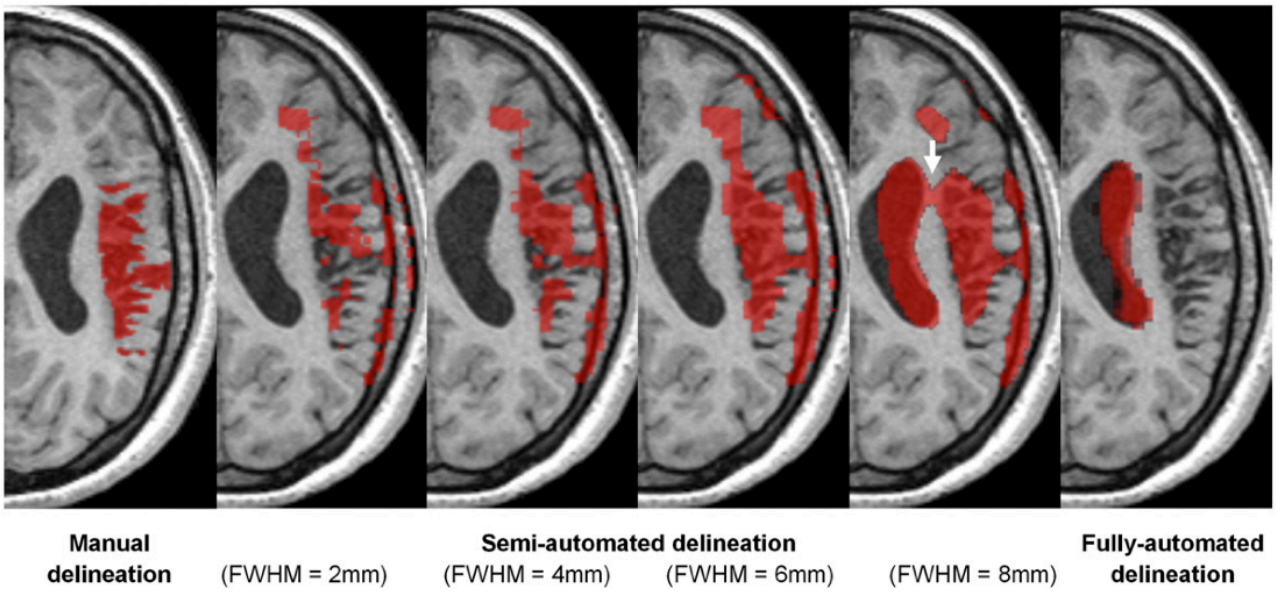
1.4.3.1 Lesion Characterization

A number of different approaches can be taken to quantify stroke lesion characteristics. A manual approach to lesion delineation is the most commonly used approach to date (Wilke, de Haan, Juenger, & Karnath, 2011), and although labour-intensive and time consuming the procedure allows for the delineation of exact regions of interest, particularly for chronic lesions. Also, chronic brain lesions may have a number of characteristics associated with them that lend

themselves to manual delineation approaches: lesions occupy space originally taken up with healthy brain tissue, and therefore healthy tissue will be destroyed or displaced. Effects associated with this displacement may be adjacent to the lesion or more remote from it. Manual tracing approaches may be better suited to these subtleties than are automated approaches, which may either be semi automated or fully automated.

Both types of automated approaches are associated with problems and are not always accurate; indeed, these approaches sometimes fail to identify lesions (Wilke et al., 2011), or identify non-lesioned areas incorrectly (figure 1.8), such as enlarged ventricles. In addition, automated approaches are often confounded by pre-existing disease, individual variances in brain shape, lesion location and severity of signal change (Farr & Wegener, 2010). Consequently, manual tracing of lesion boundaries on individual MRI scans must be considered the gold standard; in chronic lesions (such as those considered in this thesis), lesion boundaries are typically very clear and easily distinguishable from unimpaired tissue and non-brain areas and therefore can be traced with good accuracy.

a.



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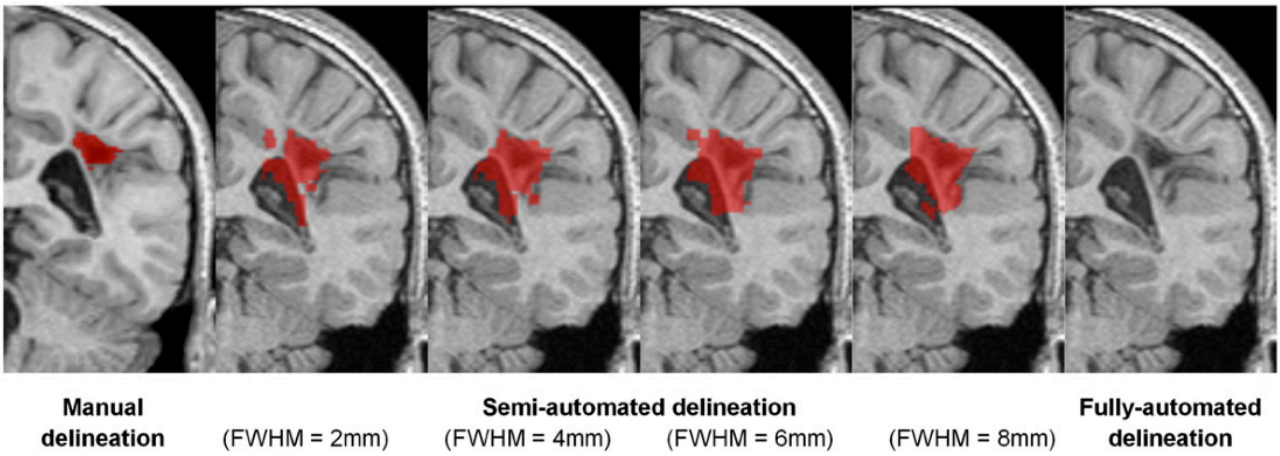


Fig 1.8. Examples of different lesion delineation techniques: manual, semi-automated and fully automated. **a.** Note difficult-to-describe inhomogeneous lesion and identification of indirect adjacent effect (enlarged ventricle) and larger smoothing (white arrow) in the semi-automated approach, and only identification of the indirect adjacent effect in the fully automated approach. **b.** Note inseparable identification of indirect adjacent lesion effect (enlarged ventricle) in the semi-automated approach. No lesion was detected in the fully automated approach. Images taken from Wilke et al. (2011).

When manual tracing of lesions is undertaken, there can sometimes be a notable difference in the perceptions of infarct extent among raters; this possibly reflects a lack of a common definition of a cerebral infarction (Neumann et al., 2009). It may also reflect tissue "at risk" of damage, or tissue damaged to a lesser extent

than other tissue following infarct. Essentially, the region of brain most deprived of cerebral blood flow is considered to be irreversibly damaged and destined for necrosis, or infarct (Farr & Wegener, 2010). The surrounding areas may retain some degree of blood flow and consequently represent a combination of tissue that might survive, or whose state is undecided depending on restoration of cerebral blood flow (penumbra) (figure 1.9).

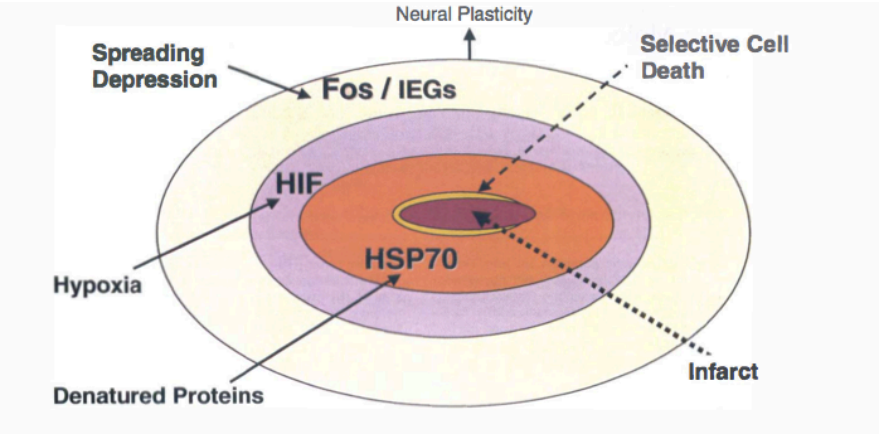


Fig 1.9. Molecular schematic of penumbras following a stroke. A zone of selective neuronal cell death borders the infarct. A zone of protein denuration (referring to the process in which proteins lose the structure present in their normal state, resulting in disruption of cell activity or cell death) extends outside of this and is demarcated by heat shock protein (HSP70), a protein expression in injured neurons. Hypoxia inducible factor (HIF) is induced in areas where blood flow is persistently decreased and oxygen delivery is impaired. Ischemia-induced spreading depression induces c-fos proteins (indirect markers of neuronal activity, the expression of which indicates cell death) and other immediate early genes (IEGs) at some distances from the infarct. Adapted from Sharp et al. (2000).

Many different descriptions and classifications of these regions exist, and there may be differing opinions about whether to include such tissue within measurements of stroke volume. In any case, such tissue is not considered to perform functionally as normal (Farr & Wegener, 2010), and is likely to

represent a rim of selective neuronal cell death (Sharp, Lu, Tang, & Millhorn, 2000). In such cases, allowing only one researcher to delineate lesions may have the advantage of involving the same outlining strategy for all patients. This was the case in this thesis, where the same researcher (CA) manually delineated all lesions. For the purposes of this thesis, penumbral tissue was included within stroke lesion volumes.

1.4.4 Corticospinal Tract Structural Integrity

Structural damage to the CST may influence motor recovery after stroke. In section 1.4.2, I discussed relationships between recovery and measures of the functional integrity of the CST; here I discuss the role of its structural integrity.

The degree of motor impairment after stroke may depend on the extent of structural CST damage caused by infarct (Jang, Yang, & Lee, 2009; Stinear et al., 2007). Some research studies have aimed to predict an individual's capacity for further functional or motor improvement at the chronic stages of recovery by investigating the structural integrity of the CST within the lesioned hemisphere (Grefkes & Fink, 2011; Zhu et al., 2010). The potential for motor recovery is thought to relate to how much of the CST has been impacted by the stroke (for example, see figure 1.10, (Riley et al., 2011)); the more damage inflicted to fibers originating from M1, the less likely a patient is to experience a successful or complete motor recovery, and the stronger the recruitment of higher motor areas such as the supplementary motor area (SMA) or pre-M1 to compensate for M1 deficiency (Newton et al., 2006; Stinear, 2010; Ward, 2006).

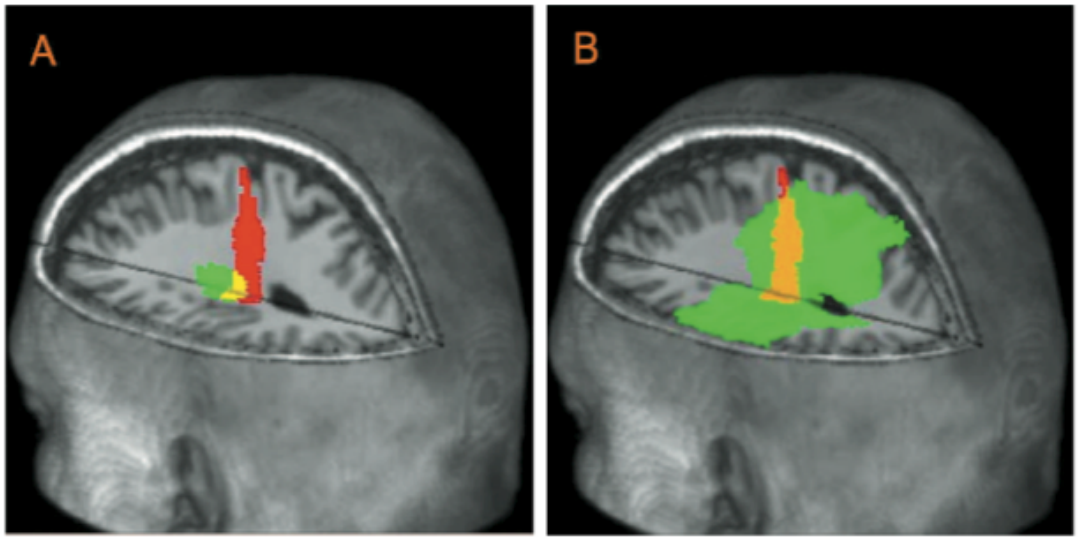


Fig 1.10. Examples of stroke injury to the tract descending from M1. A. This subject had 37.5% of the M1 tract injured by stroke and had a gain of 11 points on motor measures across a period of therapy. B. This subject had 93.4% of the M1 tract injured by stroke and had a gain of 1 point on the same motor scale across the period of therapy. Red: tract descending from M1, uninjured. Orange: tract descending from M1, injured. Green: stroke. (Figure adapted from Riley et al., 2011).

A recent trial investigated whether lesion load of the CST predicted motor impairment in chronic stroke. The authors overlaid lesion maps of 50 patients with a diffusion tensor imaging (DTI) derived probabilistic map of the CST and examined the relationship between volume of lesion-CST overlap and motor impairment after stroke (Zhu et al., 2010). Motor impairment correlated significantly with CST-lesion load, such that the greater the lesion load, the greater the level of functional impairment. Lesion size alone was not found to be a significant predictor of motor impairment, despite correlating with motor scores, such that when lesion size increased, motor impairments increased (figure 1.11).

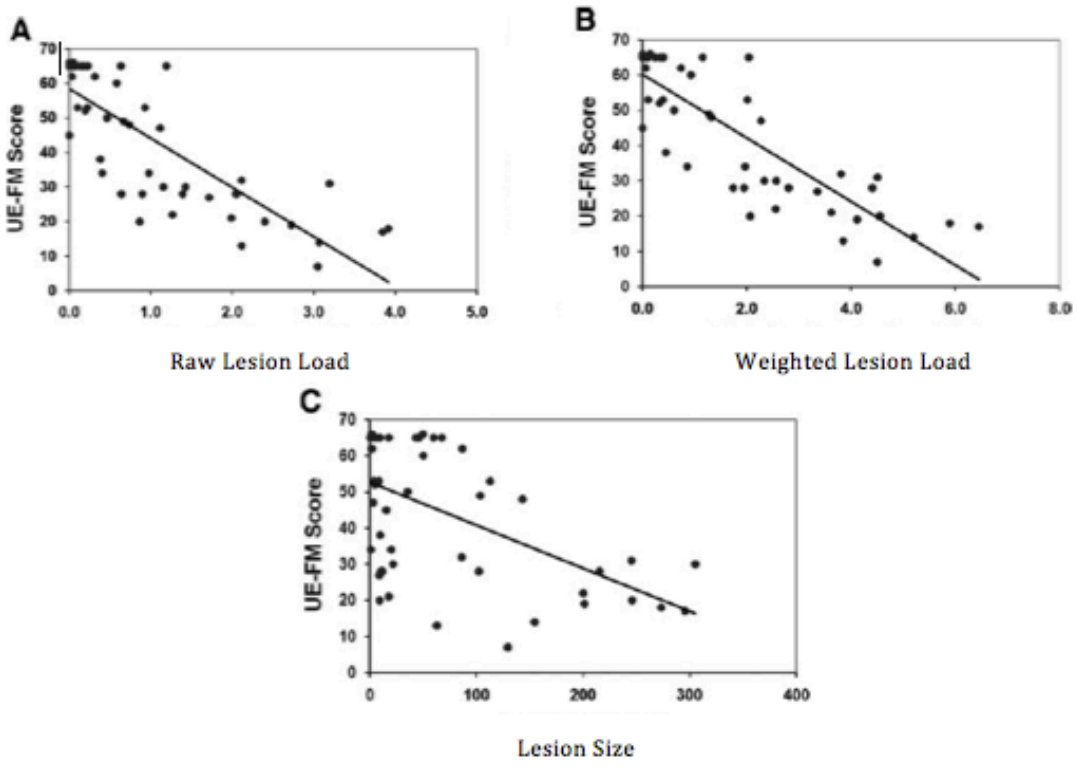


Fig 1.11. UE-FM scores correlated with (A) raw CST-lesion load, (B) weighted CST-lesion load, and (C) lesion size. (Figure adapted from Zhu et al., 2010).

Functional measurements of CST integrity as provided by TMS (as discussed in section 1.4.2) can complement data on anatomical integrity as provided MRI and DTI. Whilst paretic limb MEP presence can predict meaningful gains in chronic stroke patients receiving motor rehabilitation, within subgroups of patients in whom MEPs cannot be evoked in the affected extremity, functional outcome is poorer in patients with greater CST and neuronal fibre disruption, as measured by DTI (Dimyan & Cohen, 2010). Using these methods together can increase our ability to generate a more clinically relevant and accurate prognostic evaluation.

As previously discussed in section 1.4.2, one study has explored chronic stroke patient's functional potential by investigating the relationship between functional and structural measures and CST integrity, and their ability to predict both current impairment and improvement in upper limb function following a program of motor practice (Stinear et al., 2007). The presence or absence of MEPs in the affected limb was found to predict the change in functional ability following motor practice; decreased CST integrity was found to predict less functional potential. Interestingly, when patients in whom MEPs were elicited in the paretic hand were analysed separately, functional potential was no longer predicted by measures relating to the CST (Stinear et al., 2007). In this case, time since stroke was the greatest predictor, with greater time elapsed predicting less improvement in limb function. This study clearly demonstrates the complementary nature of combining both neurophysiological and imaging techniques in the prediction of functional potential and clinical outcomes. Another interesting finding of this research was that meaningful functional improvements were found to occur in chronic patients who were more than three years post-stroke; this is a considerably longer interval for recovery than has been commonly accepted.

Studies in this thesis will attempt to predict response to a brain stimulation and motor training intervention using baseline measures, including measures of structural damage to CST. Few studies have tested specifically for predicting response to brain stimulation interventions. One such trial examined whether DTI-derived measures of CST integrity collected at baseline predicted motor improvement in a neurorehabilitation trial involving brain stimulation in 15

chronic stroke patients (Lindenberg, Zhu, Rüber, & Schlaug, 2011); here, patients received bihemispheric brain stimulation in conjunction with physical and occupational therapy for five days. The authors found a distinct pattern of alterations in transcallosal and ipsilesional CSTs, with poorer tract integrity compared to a group of healthy controls. This pattern was consistent with chronic white matter degeneration. The authors concluded that these DTI-derived parameters predicted functional improvement in chronic stroke patients; the more the CST profiles resembled those seen in healthy controls, the greater their potential for functional recovery.

1.4.5 Conclusions Regarding the Prediction of Motor Recovery in Chronic Stroke Patients

There are a number of different factors that have to be taken into account when attempting to predict the course of motor recovery in chronic stroke patients. These include characteristics associated with the lesion, degree of motor impairment, and measures provided from TMS and MRI techniques that indicate the residual functional and structural integrity of the CST. In addition, time since stroke and patient age should be taken into account. Previous research has delivered conflicting findings as to how well each of these variables can predict recovery in the chronic phases of recovery, and specifically as to how well these variables can continue to predict response to rehabilitation in chronic stroke patients.

1.5 Developing Brain Stimulation for Stroke Rehabilitation

Currently, intensive physiotherapy is the most widely used therapeutic intervention for rehabilitation after stroke, within which an emphasis on higher intensity of motor training and task-specific practice within shorter durations has emerged. However, even with a move towards more intensive rehabilitation by physiotherapists, the potential for recovery with physiotherapy alone is inherently limited by the activity of the residual cortex. There is therefore increasing interest in combining physical therapy with other, adjunct interventions to extend the potential benefits of therapy to the patient in a more effective manner.

A number of prospective therapeutic targets for adjunct therapies have been suggested for use with chronic stroke patients. As discussed in section 1.3.3.1 above, neuroimaging data suggests that patients with impaired hand movement after stroke demonstrate increased activity in M1 within the contralesional hemisphere when moving their paretic hand compared with controls (Calautti & Baron, 2003; Stagg, 2010). This activity is greater in patients who have made a poor recovery; those who have made a better recovery show a more lateralised activity pattern closer to that expected of healthy individuals (Johansen-Berg, Rushworth, et al., 2002b; Ward et al., 2003). Additionally, poorly recovered patients exhibit abnormally high levels of inter-hemispheric inhibition between the two motor cortices (Murase et al., 2004); this potentially indicates that the healthy hemisphere is exerting a pathological level of inhibition on the lesioned hemisphere. This concept has encouraged the use of non-invasive brain stimulation in such patients to try and 'rebalance' the hemispheres (figure 1.12),

either by increasing cortical activity within the lesioned hemisphere, or by decreasing activity in the contralesional hemisphere (Stagg, 2010). However, as previously discussed, the role of the contralesional hemisphere in promoting post-stroke recovery remains controversial, with some research suggesting that down-regulation of activity in this hemisphere could impair paretic hand use. Increases in contralesional M1 activity shortly after stroke have been correlated with motor improvement in more impaired patients, which suggests a supportive role of contralesional M1 for recovery of function after stroke in some individuals (Rehme et al., 2011). Furthermore, disrupting contralesional M1 activity by means of TMS has been found to cause a deterioration in motor performance of the stroke-affected hand of a sample of patients (Lotze et al., 2006). Accordingly, it is not yet clear which patients with which constellation of impairments might benefit from increased ipsilesional activity or decreased contralesional activity.

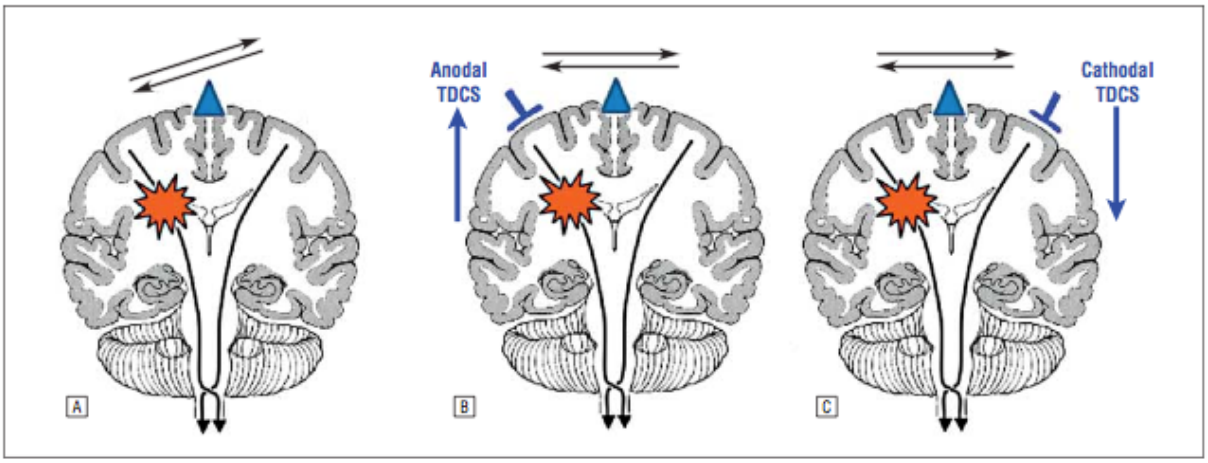


Fig 1.12. A proposed brain model of imbalanced interhemispheric inhibition and the therapeutic options to ameliorate this imbalance. The balance of IHI is disrupted post-stroke (A). This leaves the contralesional hemisphere in position where it could exert an imbalanced inhibitory effect on the lesioned hemisphere and possibly interfere with recovery processes. There are two possible ways to amend this imbalance: 1) up-regulation of excitability in the ipsilesional hemisphere (B) or down-regulation of excitability in the contralesional hemisphere (C). (Figure taken from Schlaug et al., 2008).

There are a number of possible therapies designed to target the effects associated with interhemispheric imbalance that are designed to increase a patient's response to physiotherapy by directly or indirectly modulating cortical excitability. Of these, transcranial direct current stimulation (tDCS) holds particular promise.

1.5.1 Transcranial Direct Current Stimulation (tDCS)

The concept of using therapeutic electricity in an attempt to modulate brain activity is not new; electric currents have been applied to patients for the medical relief of headache, epilepsy and other aliments for the past two thousand years (Stagg & Nitsche, 2011). Long before William Gilbert defined electricity in 1600, the ancient Egyptians and Greeks utilized the therapeutic value of naturally occurring electro-stimulation; the use of Nile catfish is displayed on wall reliefs of Egyptian tombs dating back thousands of years (Southworth, 1999). Similarly, Aristotle and Plato reference the black torpedo, an electric ray fish, which the physician Scribonius Largus prescribed for relieving headaches and gout in 46 A.D (Schlaug, Renga, & Nair, 2008).

More recently, the effects of direct currents on brain tissue in animals have encouraged investigators to develop a novel and non-invasive method of brain stimulation using weak direct currents. This technique has become known as tDCS. tDCS has attracted the attention of clinicians and scientists due to its influential effect on cortical functions. It involves the application of very low-

amplitude direct currents (2milliamps or less) via surface scalp electrodes (Bastani & Jaberzadeh, 2011). The application of electric current likely modifies the transmembrane neuronal potential and consequently influences and modifies the level of cortical excitability within brain tissue; this also has significant effects on neurotransmission. Stimulation can be targeted to the motor system by centring the active electrode over M1, with the reference electrode placed over the contralateral supraorbital ridge in a task neutral position (figure 1.13). Current flowing from the active electrode to the reference is described as anodal stimulation whereas current flowing in the opposite direction is described as cathodal stimulation (figure 1.13).

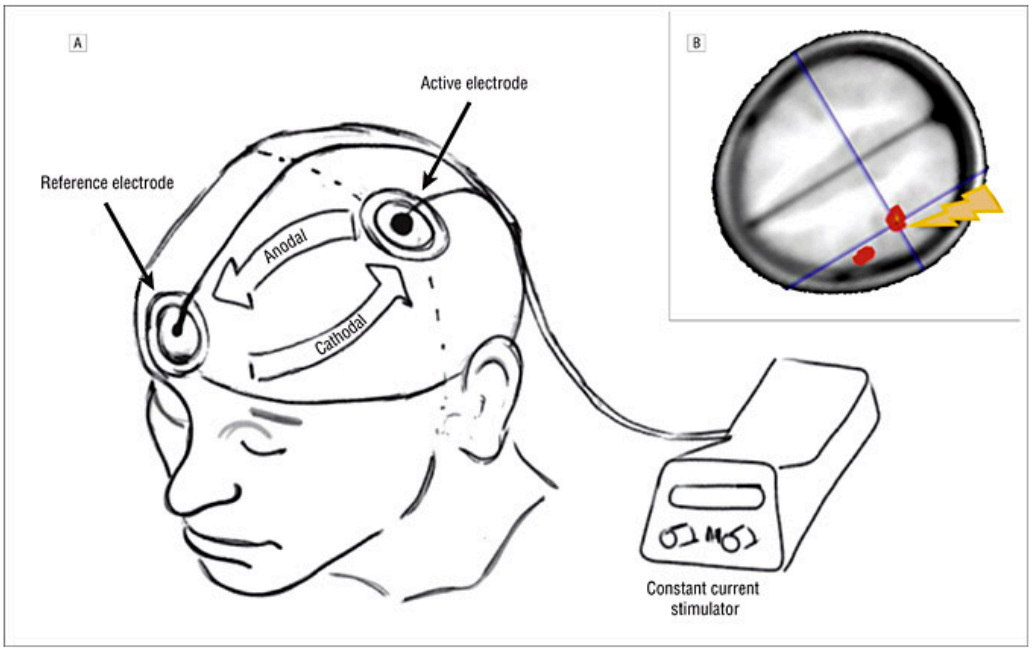


Fig 1.13. A. tDCS setup and montage. The setup using a mobile battery-operated direct current stimulator connected with 2 electrodes. One electrode (active) can be position over M1, and the reference electrode is positioned over the contralateral supraorbital region. If current flows from M1 to the supraorbital region, then the tissue underlying M1 is subjected to anodal stimulation. If the current is reversed, then the tissue underlying M1 is subjected to cathodal stimulation. **B.** Regional cerebral blood increases in the motor region underlying the electrode positioned over M1 after anodal stimulation. Regional cerebral blood can be determined using non-invasive imaging techniques. (Figure adapted from Schlaug et al., 2008).

1.5.1.1 Mechanisms of tDCS

The effects of tDCS on cortical excitability are polarity specific. Anodal tDCS increases cortical excitability and cathodal tDCS decreases cortical excitability by up to 40% as assessed by changes in the size of MEPs evoked by TMS to the stimulated M1 (Nitsche & Paulus, 2000) (figure 1.14). The effects on cortical excitability outlast the stimulation period by up to 90 minutes, depending on the duration of stimulation applied (Stagg, 2010). The mechanisms underlying these effects have been studied in some detail.

In the short-term, anodal tDCS has been found to increase cortical excitability through neuronal depolarization and the potentiation of glutamatergic *N*-methyl-D-asparate (NMDA) receptor efficacy (Nitsche & Paulus, 2000). After longer stimulation periods, the excitability effects of tDCS are mediated via the enhanced effectiveness of NMDA receptors. In addition, anodal stimulation leads to a significant decrease in GABA concentration within the cortex (Stagg et al., 2009). Indeed, this modulation of GABA has been suggested to play an important role in motor learning; following anodal stimulation to M1, subjects who demonstrated a greater subsequent decrease in GABA also showed faster short-term learning (Stagg, Bachtiar, & Johansen-Berg, 2011a). Therefore, LTP-like plasticity as modulated by the application of tDCS may be dependent on GABA modulation.

Cathodal tDCS has been found to decrease excitability (Vines, Nair, & Schlaug, 2006), via hyperpolarisation of neurons (Nitsche & Paulus, 2000), and changes in NMDA-dependent processes. In addition, inhibitory cathodal stimulation leads

to a significant decrease in glutamate (Stagg et al., 2009). Alterations in GABAergic tone had no effect on response to cathodal tDCS.

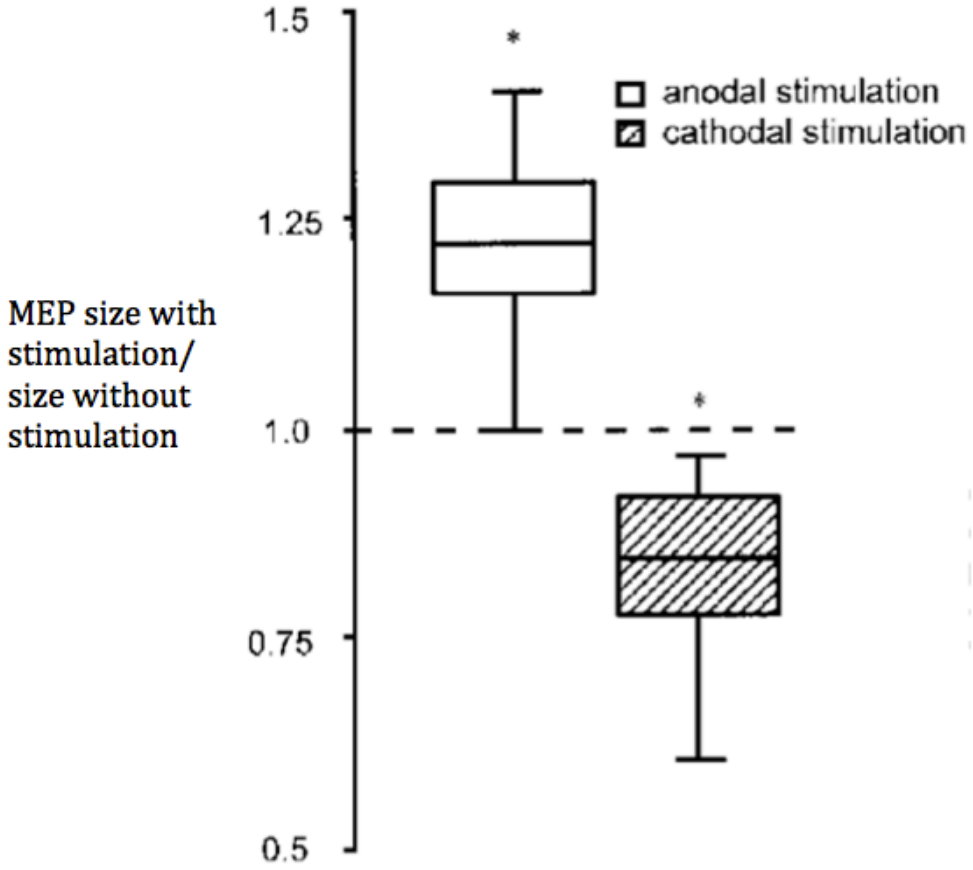


Fig 1.14. Changes in cortical excitability as indicated by neurophysiological measures following the termination of tDCS. Results are shown as MEP size post-stimulation as a proportion of baseline MEP size. (Figure adapted from Nitsche and Paulus, 2000).

1.5.1.2 tDCS and Neurorehabilitation in Chronic Stroke

Neurorehabilitation is a complex process that aims to aid recovery from a nervous system injury such as a stroke, and to minimize or compensate for any functional alterations resulting from it. In essence, neurorehabilitation refers to a particular form of motor learning. Whilst there are certainly differences between neurorehabilitation and motor learning in terms of the timescale over which these processes occur, it is likely that the underlying mechanisms are

similar (Matthews, Johansen-Berg, & Reddy, 2004). Indeed, it is reasonable to assume that rehabilitation interventions which attempt to induce changes in synaptic plasticity efficacy via LTP-like mechanisms (like those associated with learning) will also improve motor function in chronic stroke. Physiotherapy based rehabilitation methods assist patients in the re-learning of motor function. They act via increasing activity in the lesioned M1 through increasing activity in the paretic hand; techniques such as tDCS can additionally be used as an adjunct intervention to further increase cortical activity and promote synaptic plasticity. As previously discussed, the physiological effects associated with tDCS, such as increasing activity via LTP-like plasticity and decreasing GABA concentrations, are similar to those associated with motor learning (Stagg et al., 2009).

1.5.1.3 Practical Considerations and Safety

tDCS can be used successfully in blinded trials due to its effective sham condition; during both genuine and sham experiments, a direct current can be initially increased in a ramp-like fashion over several seconds until reaching a current density of choice. During genuine tDCS, stimulation will be constantly maintained for around 20 minutes. In sham sessions, the current will be terminated after 10 seconds; these parameters allow for perceived sensations of tingling on the skin during the initial ramping of the current (Gandiga, Hummel, & Cohen, 2006). Use of these parameters ensures that, during double or single blind trials, both subjects and raters can remain blinded to the intervention type.

The primary safety concern associated with brain stimulation techniques is the risk of any increased electrical activity inducing seizures. Both tDCS and TMS carry the potential to induce seizures, although the reporting of adverse events is very rare, as subjects who are at an increased risk of experiencing seizures are excluded from research studies (Rossi, Hallett, & Rossini, 2009). tDCS does not cause epileptic seizures or reduce the seizure threshold in animals, and consequently seizures are unlikely to be a risk for healthy subjects (Nitsche et al., 2008). However, this may not be true of patient populations, in whom cortical excitability may already be increased or disrupted. Safety studies of tDCS have not reported any seizure related adverse reactions, and some have reported no changes appearing on electroencephalography (EEG) after 20 minutes of treatment (Iyer et al., 2005; Nitsche et al., 2003; Poreisz, Boros, Antal, & Paulus, 2007).

The experiment in this thesis will use single pulse TMS to assess the ability to elicit a visible muscle twitch in the paretic hand at baseline. As with tDCS, a number of safety studies have also established working guidelines for single pulse TMS within which seizures have never been induced in neurologically normal subjects without a personal or family history of epilepsy (Rossi et al., 2009). There is a somewhat higher risk of inducing a seizure in a patient population, although this is still rare with single pulse TMS. Tharayil et al. (2005) reported a generalised tonic clonic seizure in a patient with bipolar depression using single pulse TMS during motor threshold assessment (Tharayil, Gangadhar, Thirthalli, & Anand, 2005). However, the patient suffered a family history of epilepsy, which may have increased the risk of seizures. In addition,

the patient was taking medications (chlorpromazine and lithium) which may have lowered his seizure threshold (Rossi et al., 2009). Very rarely, secondarily generalised or partial motor seizures have been induced by single-pulse TMS in several patients with acute stroke (Hömberg & Netz, 1989; Kandler, 1990). Epilepsy developed in one of these patients, presumably as a result of the underlying lesion (Wassermann, 1998).

1.5.2 Evidence of tDCS Efficacy from Single Session Studies

In healthy control experiments, a number of proof-of-principle studies have demonstrated that tDCS is capable of transiently modulating motor function. Anodal stimulation, which increases M1 excitability as discussed previously, transiently improves performance in tests of motor speed and dexterity (Hummel, Celnik, Giraux, Floel, & Wu, 2005; Nitsche et al., 2008). When delivered in conjunction with a motor learning task, anodal tDCS serves to improve the rate of learning of that task (Stagg et al., 2011b). However, timing is essential, as prior application of tDCS has been found to slow subsequent motor learning (Stagg et al., 2011b).

In a patient population, tDCS has continued to demonstrate its effectiveness in improving motor function (Hummel, 2006; Hummel et al., 2005). For example, anodal stimulation targeted to the lesioned hemisphere has been found to result in significant improvements in reaction times during performance with the paretic hand in chronic stroke patients (Stagg et al., 2011). This significant

response time decrease after anodal tDCS was found when compared to sham tDCS (figure 1.15).

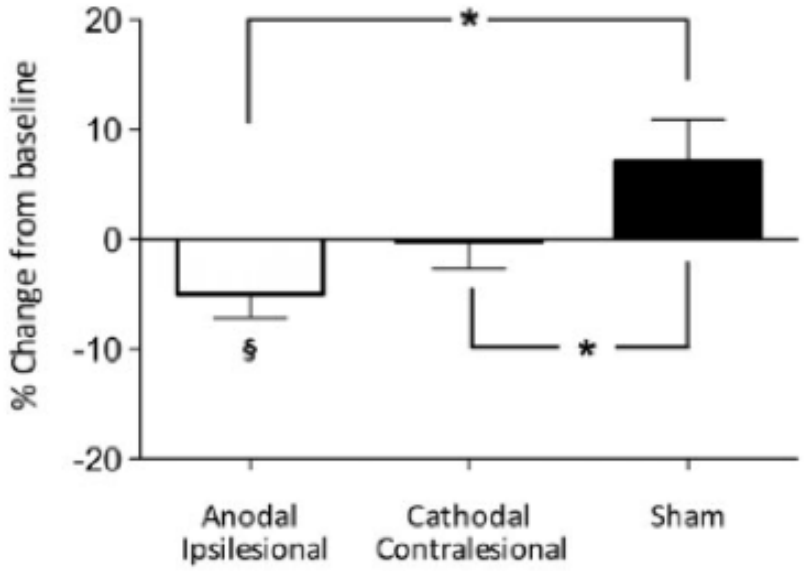


Fig 1.15. Behavioural effects of tDCS. Anodal tDCS to ipsilesional M1 leads to a significant decrease in response times. * shows significant differences (p<.05) between conditions; § shows significant differences (p<.05) within sessions. (Figure taken from Stagg et al., 2011).

Two primary approaches of brain stimulation techniques have been used to date: one is based on increasing cortical excitability within the lesioned hemisphere, and the other is based on a model of IHI or rivalry between the motor areas in the ipsilesional and contralesional hemisphere. Both approaches are based on the idea that post-stroke, motor deficits are due to reduced cortical activity in the lesioned hemisphere, and excessive inhibition of the affected hemisphere from the non-lesioned hemisphere (Talelli & Rothwell, 2006). Consequently, motor improvement might be possible by either increasing activity within the stroke-affected hemisphere, or by decreasing the overactive, inhibitory influence from

the intact hemisphere. Single-session studies have been utilised to test if a single application of tDCS causes a direct gain in an outcome measure.

In those patients who have made a reasonable recovery, motor control of the paretic limb comes mainly from the stroke-affected hemisphere. Therefore, facilitation of this hemisphere with the use of non-invasive stimulation may enhance motor control of the affected hand. Evidence suggests that this is the case: in a double-blind, sham controlled study, a 20 minute period of anodal tDCS applied to the affected M1 resulted in improvement in maximal pinch force and reaction times of the stroke-affected hand relative to sham stimulation in a group of chronic stroke patients (Hummel et al., 2006).

The alternative treatment approach involves reducing any possible interference with output from the lesioned hemisphere by reducing cortical activity within the intact hemisphere. This approach has also had some success: 20 minutes of cathodal stimulation delivered to the intact hemisphere reduced the time taken for stroke patients to perform the Jebsen Taylor Test of Hand Function (JTT) (Fregni et al., 2005). This effect was found to last for about 40 minutes following the termination of stimulation.

tDCS can be used to stimulate both M1s simultaneously. This has the effect of increasing cortical activity in one hemisphere whilst concurrently decreasing activity in the other. It is thought that this may serve to modulate interhemispheric interactions in a way that is particularly beneficial to stroke patients. Indeed, bilateral stimulation in healthy right-handed subjects, in which

the anode is positioned over the right, non-dominant hemisphere while the cathode is positioned over the left, dominant hemisphere, has been shown to result in significant improvements in the use of the non-dominant hand when compared to the performance of those individuals who receive sham stimulation (Williams, Pascual-Leone, & Fregni, 2010). In other studies using the same parameters, bilateral tDCS led to significantly greater improvements in finger-sequence performance for the non-dominant hand when compared to unilateral, anodal stimulation in healthy right-handed controls (Vines, Cerruti, & Schlaug, 2008). In patients with chronic stroke, bilateral tDCS (anodal stimulation delivered to the affected M1, and cathodal stimulation delivered to contralesional M1) has also been shown to be associated with improvements in motor function with the paretic hand: motor performance on the JTT was significantly improved post-stimulation (Mahmoudi et al., 2011).

However, other research has found conflicting evidence. One study found that bihemispheric tDCS, where anodal stimulation is delivered to ipsilesional M1 whilst cathodal stimulation is delivered to contralesional M1, provided no additional benefit on reaction time task performance over sham stimulation; in this study, unilateral, anodal stimulation to the affected M1 was found to be significantly beneficial to chronic stroke patients during task performance, where reaction times were shown to be significantly faster after stimulation (Bachtiar et al., unpublished data). In other research, bihemispheric stimulation was not found to differ significantly from unilateral, anodal stimulation in terms of induced implicit motor sequence learning in stroke patients (Kang & Paik, 2011). Indeed, some research has found that, when comparing anodal, cathodal

or bilateral stimulation paradigms in healthy subjects, only the anodal tDCS electrode montage (M1-contralateral supraorbital ridge) arrangement results in significant excitability changes (Nitsche & Paulus, 2000).

It might be that, in a chronic stroke population, unilateral anodal stimulation to the affected hemisphere is the most effective electrode montage available. Numerous imaging and stimulation studies have revealed irregular patterns of activation and excitability within the lesioned hemisphere immediately after infarct and during the process of recovery; these form the conceptual basis for excitability-enhancing procedures of cortical stimulation (Hummel et al., 2008). Evidence indicates that good functional recovery is paralleled by cortical activation patterns comparable to patterns in healthy controls, with lateralized activation contralateral to the moving hand. However, this is not the case in highly impaired patients (Hummel et al., 2008).

Based on this evidence, it appears most logical to attempt to enhance the reduced activity of M1 in the ipsilesional hemisphere in the incompletely recovered motor network after stroke. Consequently, M1 of the lesioned hemisphere is a prime target for cortical stimulation.

1.5.3 Evidence of tDCS Efficacy from Multiple Session Studies

The hypothesis underlying multiple session studies is that the transient benefits in motor abilities experienced after a single session may accumulate and thus eventually lead to a more permanent or enduring improvement in motor function (Talelli & Rothwell, 2006). Motor skills may take many months to

accurately acquire, and therefore strategies that serve to enhance skill acquisition or retention are of both clinical and practical interest, particularly in terms of stroke rehabilitation. Few studies have tested the assumptions underlying repeated tDCS sessions in chronic stroke patients, although data from healthy populations certainly indicates that prolonged enhancement after repeated sessions might be possible.

Reis et al (2009) investigated the effect of tDCS on an extended time course of learning an innovative and demanding task. Healthy subjects practiced a sequential visual isometric pinch task over five consecutive days whilst receiving simultaneous anodal or sham tDCS over M1 (figure 1.16). The authors assessed the impact of anodal relative to sham tDCS on both within- and between-day effects, and on the rate of forgetting during a three-month follow-up period. They found increased total skill acquisition (both within-day and between-day) with anodal tDCS compared to sham. Furthermore, performance on the skill measure remained significantly greater in those subjects who had received anodal tDCS three months later (Reis, Schambra, & Cohen, 2009).

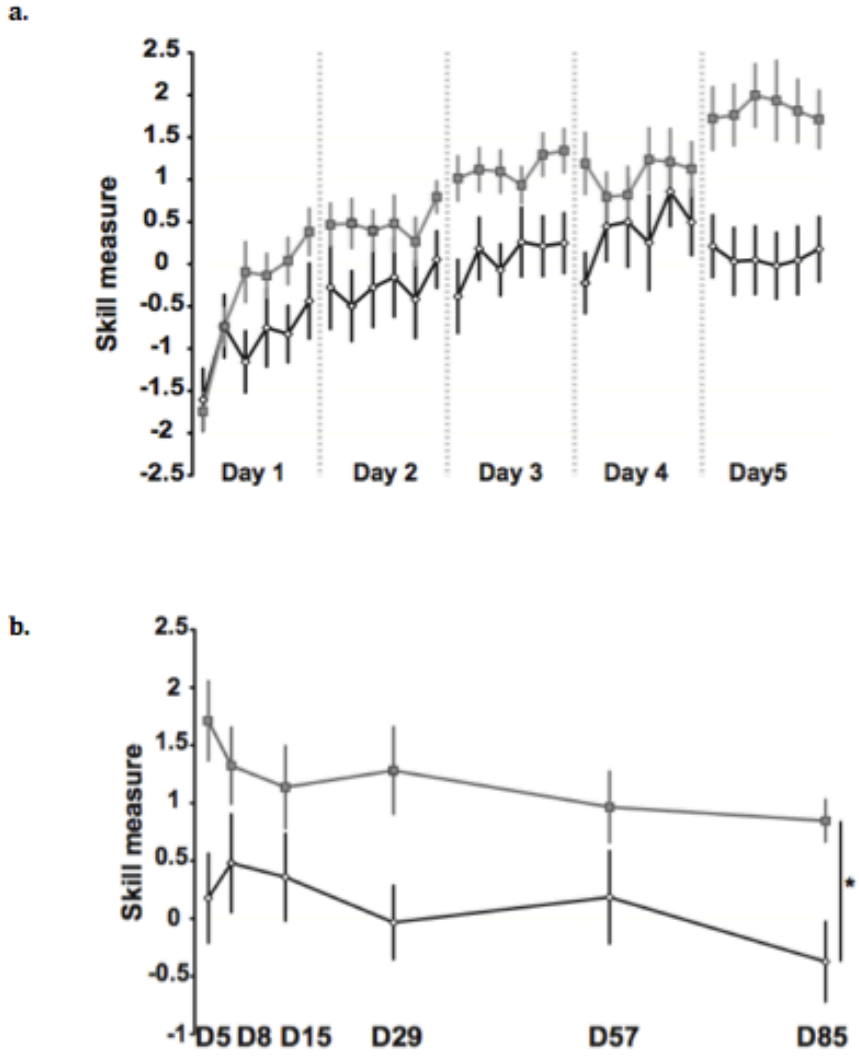


Fig. 1.16. a. The learning curve for the sham (white diamonds) and anodal (gray squares) tDCS groups for 30 training blocks over 5 days. Both groups started with comparable skills at day 1, but by day 5 the anodal tDCS group had acquired more skills than the sham tDCS group. **b.** Retention of skills at day 5, day 8, day 15, day 29, day 57 and day 85. Skill in the anodal tDCS group (gray squares) remained superior to the sham tDCS group (white diamonds) at all times, and were still significantly better at day 85; * $p<.01$. Figure adapted from Reis et al., 2009.

One recent study that has investigated the effects of repeated stimulation sessions in stroke patients used a bihemispheric electrode montage. Lindenberg et al (2010) randomly assigned chronic stroke patients to receive five consecutive sessions of either dual-hemisphere tDCS or sham stimulation with simultaneous physical and occupational therapy. Changes in motor impairment

and motor activity were assessed, while functional imaging parameters were used to investigate neural correlates of motor improvement. The authors found that improvement of motor function was significantly greater in the real stimulation group when compared to the sham group. Effects outlasted the stimulation period by one week; unfortunately, no longer follow-up period was observed so it is unclear as to whether the long-term effects of the stimulation were enduring. In addition, the effects of the dual hemispheric montage were not compared to the more conventional unilateral montage. Functional imaging showed that, in the real stimulation group, increased activation of intact ipsilesional motor regions during movement of the paretic limb were found post-intervention, whereas no significant activation changes were seen in the sham control group (Lindenberg, Renga, Zhu, Nair, & Schlaug, 2010).

1.6 Current Research Objectives

The primary objective of this research is to work towards developing tDCS as a clinically effective and practicable rehabilitation tool for chronic stroke patients. As is evident from the discussed research, tDCS has the potential to modulate cortical excitability post-stroke and potentially enhance the effects of motor training or physiotherapy when used as an adjunct tool. It is possible that combining tDCS and motor rehabilitation can potentiate relearning of motor skills to levels unattained by either intervention alone (Schlaug et al., 2008). The combination of stimulation and peripheral training may enhance skill acquisition or consolidation through mechanisms of LTP (Lindenberg et al., 2010), by

increasing inputs to the cortex while excitability is simultaneously modulated by tDCS.

tDCS is easily used and not associated with any adverse effects; therefore, if shown to be effective in increasing the benefits from physiotherapy, it could potentially be combined with more traditional rehabilitative techniques. Particularly in patients with chronic stroke in whom the likelihood of any spontaneous improvement in motor function is low, the use of tDCS as an adjunct therapy to motor rehabilitation might be of enormous functional benefit (Williams, Imamura, & Fregni, 2009).

Furthermore, this research aims to establish the effects and efficacy of using repeated sessions of anodal stimulation; previous research in healthy controls and patient populations has demonstrated increased benefit from multiple sessions of tDCS in comparison to those derived from single sessions. It remains to be seen what benefits can be associated from combining unilateral anodal stimulation with a rehabilitation program for the upper limb that spans over a two week period; whilst previous research has reported increased motor activity in chronic stroke patients after multiple sessions of stimulation, these lack an appropriate follow-up period that is necessary to analyse the true effects of repeated sessions.

Increased applications of brain stimulation over consecutive days has not been associated with any adverse effects (Fregni et al., 2006). Indeed, in a recent study that assessed the safety of repeated sessions of brain stimulation, no

serious intervention-related adverse events were experienced in stroke patients after 10 treatment sessions spaced over two weeks (Conforto et al., 2011). Previous research has indicated that if repetitive tDCS is performed with the intention of prolonging and stabilizing any after-effects, then stimulating subjects once daily is safe and suitable (Nitsche et al., 2008).

A secondary aim of this research is to investigate whether any variables can be used to predict how well chronic stroke patients will respond to rehabilitation. Variables of interest will include lesion characteristics, degree of presenting motor impairment, time since stroke, and measures derived from TMS and MRI techniques that assess the integrity of the CST. Previous research has led to conflicting reports regarding the impact and importance of these variables, so this thesis therefore aims to supplement such research and provide some direction as to how these variables can be clinically utilised within a chronic population. This has the potential advantage of directing the provision of post-stroke rehabilitation; if more research can show that, due to characteristics of their underling physiology or neural anatomy, chronic patients are still likely to benefit from physiotherapy even though they show a transient plateauing of function, then perhaps current physiotherapy provision might be increased.

Finally, this thesis aims to establish the effects of tDCS and motor rehabilitation across chronic stroke patients with a more diverse range of functional and motor impairments than has previously been tested. This will allow for a number of interesting questions to be answered, not only concerning the response to rehabilitation, but with regards to the underlying stroke anatomy and

physiology. For instance, it might be that models of abnormal IHI apply differently to patients with more severe deficits or larger lesions.

In the following chapters, I will discuss further the methods with which this research will be conducted, the results gathered from a cohort of chronic stroke patients, and what these mean in terms of chronic stroke rehabilitation.

Major Experimental Research Objectives:

1. *To establish behavioural changes occurring in response to repeated sessions of tDCS*
2. *To explore predictors of impairment and response to rehabilitation within a group of patients affected by chronic stroke*

2 METHODS

2.1 Study Design

In this double blind, randomised control trial I aimed to compare the effects of consecutive daily sessions of anodal or sham tDCS to the ipsilesional motor cortex (M1) as an adjunct to motor rehabilitation for the paretic arm and hand in chronic stroke patients. All patients participated in two baseline sessions that occurred within 6 weeks of starting a motor training intervention for the paretic limb that took place over nine consecutive working days, and four follow-up sessions distributed over the subsequent three months (figure 2.1).

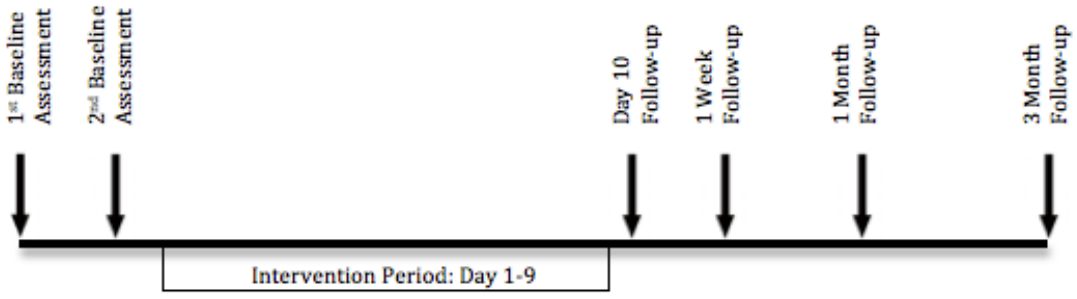


Fig 2.1. Study time plan.

2.2 Participants

Participants were 24 stroke patients (7 female, 17 male) who had previously suffered a single unilateral stroke (see appendix 1 for individual patient details). Mean age at intervention was 63 years (*SD* 11.54, range 37-79 years) and the

mean time post stroke was 54 months (*SD* 36.31, range 6-141 months). At baseline, the mean Upper Extremity Fugl Meyer (UE-FM) score in the paretic upper limb was 37.56 (*SD* 16.41, range 13-62) out of a possible 66 points.

All participants gave written informed consent to participate in accordance with the Declaration of Helsinki (World Medical Association, 1964) and Local Research Ethics Committee approval (Oxfordshire Research Ethics Committee A, 10/H0604/98). All patients were at least six months post a single unilateral (7 left hemisphere; 17 right hemisphere) ischemic (15 patients) or hemorrhagic (9 patients) stroke affecting motor function in the contralesional hand.

No participant had any history, signs or symptoms of any other neurological condition, nor did they have dysphasia significantly limiting communication. Exclusion criteria were: previous stroke or stroke affecting M1; inability to provide informed consent due to severe language or cognitive impairment; contraindications to tDCS, including a personal or family history of epilepsy, febrile convulsions or recurrent fainting fits; other neurological or psychiatric disease; drug abuse; use of prescription medication for depression or malaria; contraindications to MRI.

2.2.1 Baseline Sessions

During the initial assessment session, Baseline One, a trained physiotherapist specialising in stroke rehabilitation (CW) assessed each participant's movement range in the paretic hand and arm to ensure suitability for the study. In addition, baseline measures of gross and fine motor function (see 2.2.1.1, Functional

Assessments) were delivered to all patients by a trained researcher (CA). A single session of transcranial magnetic stimulation (TMS) was delivered to all patients to assess whether muscle movement could be observed in the paretic hand on stimulation of the stroke-affected hemisphere (see 2.2.1.2). During the second assessment, Baseline Two, patients underwent an MRI scan in addition to completing functional assessments for the second time. One patient (subject 8) did not undergo the scanning procedure due to claustrophobia, but did undertake all other measures. Data regarding this patient's stroke comes from clinical referral letters. The same researcher (CA) conducted all baseline and follow-up assessments for all participants.

2.2.1.1 Functional Assessments

All participants in the present study were assessed on three scales that are specifically designed to quantify a patient's gross and fine motor dexterity of the impaired arm. This battery of functional assessments was chosen in order to fully assess different spheres of motor and functional ability.

2.2.1.1.1 Primary Outcome Measures

The Upper Extremity Fugl-Meyer Assessment (UE-FM) was chosen as the primary outcome measure (for a detailed description of all outcome measures, see 1.2.3). The UE-FM assesses movement, coordination, and reflex action across a range of muscles (table 2.1) and is scored on a three-point ordinal scale for each point (0= cannot perform, 1= performs partially, 2= performs fully). The UE-FM ranges from 0-66, with higher scores indicating better performance.

Table 2.1. Motor Domain Items assessed by the UE-FM. Each point is scored on a three-point scale (0=cannot perform; 1=performs partially, 2=performs fully). Adapted from Gladstone et al., 2002.

Upper Extremity (66 points maximum)
Should retraction
Shoulder elevation
Shoulder abduction
Shoulder abduction to 90 degrees
Shoulder adduction/internal rotation
Shoulder external rotation
Shoulder flexion 0-90 degrees
Shoulder flexion 90-180 degrees
Elbow flexion
Elbow extension
Forearm supination
Forearm pronation
Forearm supination/pronation (elbow at 0 degrees)
Forearm supination/pronation (elbow at 90 degrees, shoulder at 0 degrees)
Hand to lumbar spine
Wrist flexion/extension (elbow at 0 degrees)
Wrist flexion/extension (elbow at 90 degrees)
Wrist extension against resistance (elbow at 0 degrees)
Wrist extension against resistance (elbow at 90 degrees)
Wrist circumduction
Finger flexion
Finger extension
Extension of metacarpophalangeal joints, flexion of interphalangeal joints
Thumb adduction
Thumb opposition
Grasp cylinder
Grasp tennis ball
Finger-nose speed
Finger-nose tremor
Finger-nose dysmetria
Finger flexion reflex
Biceps reflex
Triceps reflex

2.2.1.1.2 Secondary Outcome Measures

Secondary outcome measures used included the Action Research Arm Test (ARAT) and the Wolf Motor Function Test (WMFT).

The quality of movement for each item on the ARAT is rated on a four-point scale (0= no movement possible, 1= movement partially performed, 2=movement performed, but abnormally, 3= movement performed normally). Scores range from 0-57, with higher scores reflecting better function (table 2.2).

Table 2.2. ARAT Test Items. Adapted from Van der Lee et al.,2001.

Upper Extremity (57 points maximum)
Grasp subscale
Block, 10cm^3
Block, 2.5 cm^3
Block, 5 cm^3
Block, 7.5 cm^3
Cricket ball
Sharpening stone
Grip subscale
Pour water from one glass to another
Displace 2.25cm alloy tube from one side of the table to the other
Displace 1cm alloy tube from one side of the table to another
Put washer over bolt
Pinch subscale
Ball bearing, held between ring finger and thumb
Marble, held between index finger and thumb
Ball bearing, held between middle finger and thumb
Ball bearing, held between index finger and thumb
Marble, held between ring finger and thumb
Marble, held between middle finger and thumb
Gross movement scale
Hand to behind the head
Hand to top of head
Hand to mouth

The WMFT is performed as quickly and as accurately as possible, and test items are truncated at 120 seconds. Performance is rated on a five-point scale ranging from 0-75, with higher scores indicating better performance (see table 2.3).

Table 2.3. WMFT Task Items. Adapted from Morris at al., 2001.

Upper Extremity (75 points maximum)
Forearm to side table (side)
Forearm from table to 25cm box (side)
Extend elbow 28 cm on table top (side)
Extend elbow 28 cm on table top (1lb weight)
Hand to table (front)
Hand to box (front)
Retrieve .45kg weight from 28 cm line on table top by elbow flexion
Lift can to mouth
Lift pencil from table
Lift paper clip from table
Stack 3 checkers
Flip 3 cards
Turn key in lock: clockwise to 180 degrees and counter-clockwise to 180 degrees
Fold face towel
Lift basket with 1.35kg weight to fully raised bedside table

2.2.1.2 Transcranial Magnetic Stimulation (TMS)

For the purposes of this research, TMS was used during Baseline One to try and elicit muscle movement from the paretic hand (see appendix 2). The hand area of the primary motor cortex was initially estimated to be five centimetres lateral to the vertex (Cz) for each patient. This was measured bilaterally. The hand area was then more tightly localised by assessing visible elicited twitches in the fingers of the hand in response to TMS at different points around this mark. When the optimal point for eliciting a motor response in the contralateral hand was found, TMS pulses were then applied to that hemisphere at varying intensities until a minimum intensity was found which reliably elicited a visible finger twitch (this could be elicited in 18 patients). An 80mm wing diameter figure-of-eight coil was used for all stimulation sessions and was connected to a Magstim 200 Monophasic Stimulator (Magstim, Carmarthenshire, UK). The coil was oriented at 45° to the mid-sagittal axis.

2.2.1.3 MRI Data Acquisition

One aim of this thesis is to test whether information from standard clinical MRI scans could be used to predict response to treatment. A multi-modal battery of structural and functional MRI was performed before and after the intervention but only baseline T1-weighted scans are considered in this thesis. I have focused on the T1-weighted scans as these are routinely performed on clinical systems, whereas the other types of functional and structural data collected on participants in this study are rarely collected clinically.

Data were acquired on a 3-Tesla Siemens MRI scanner using a 32-channel head coil. T1-weighted high-resolution MRI scans (1 x 1 x 1 mm^3) were acquired for anatomical localisation and lesion volume calculation (magnetization prepared rapid gradient echo; MPRAGE; repetition time= 2040ms; echo time=4.7ms; inversion time= 900ms; field of view 192x192mm^2; flip angle= 8°). In addition, task based and resting functional MRI scans were performed, as well as diffusion tensor imaging. These imaging measures are being analyzed by other members of the research team and are outside the scope of this thesis.

In order to construct corticospinal tract (CST) maps to allow overlap between lesions and the CST to be calculated, I used data previously acquired in healthy controls (Stepens et al., 2010). Healthy controls (mean age 66 years) were recruited with approval from the Central Oxford Research Ethics Committee and gave their informed consent in accordance with the Declaration of Helsinki

(World Medical Association, 1964). Diffusion-weighted images were acquired on a Siemens Sonata 1.5 T scanner at the Oxford Centre for Magnetic Resonance, Oxford, with a maximum gradient strength of 40 mT/m. Three sets of echo-planar images of the whole head were acquired, each with nine volumes with no diffusion weighting (72x2-mm-thick axial slices, giving an isotropic resolution of 2x2x2 mm). Diffusion weighting was isotropically distributed along 60 directions using a b-value of 1000s/mm^2.

2.2.2 Intervention

Within six weeks of both baseline assessments being completed, participants attended for one hour per day on nine consecutive working days to complete a motor training intervention. On arrival, all participants completed a safety form before transcranial direct current stimulation (tDCS) electrodes were applied to the patient's scalp. Stimulation was commenced at the same time as the motor training protocol, and both ran simultaneously for 20 minutes. Once the stimulation had terminated the electrodes were removed, and motor training recommenced for another 40 minutes. As the trial was double blind, one researcher (UA or HJB) was in charge of delivering tDCS, and a different researcher (CA or CJS) was responsible for administration of all behavioural measures throughout the study, and for the delivery of the motor training intervention. This latter researcher remained unaware of the stimulation type being delivered. Due to the practical set-up of the study, it was straightforward for both patient and researcher to complete the motor training protocol without any knowledge of stimulation paradigms.

2.2.2.1 Motor Training Protocol

A Graded Repetitive Arm Supplementary Program (GRASP) was delivered to all patients by a researcher (CA) who had undertaken supervised training, both in the delivery of the manual itself and in stroke rehabilitative physiotherapy.

The GRASP protocol consists of different components of strengthening, range of motion, weight-bearing and trunk control (described in table 2.4). In addition, gross and fine motor skills are practiced. Both unilateral movements of the paretic hand and arm are practiced, as well as bilateral functional movements. The GRASP protocol consists of one hour's work, which for this study was administered daily for nine consecutive days, with a focus on building repetitions of exercises that patients continue to find challenging. Progression is achieved by increasing the number of repetitions performed, and by changing the weight or sizes of objects that were used in the program to make the tasks more difficult.

To ensure that all patients are able to work at a level that constantly challenges their ability, the GRASP protocol consists of three different levels based on UE-FM scores. Level 1 is appropriate for UE-FM scores of 10-25, level 2 for scores of 26-45, and level 3 for scores of 46 or greater. Level 1 is therefore the lowest and requires minimal (but some) hand function. Level 2 requires more graded control of hand grasp and release and fine motor skills, while level 3 has substantial fine motor tasks (Eng, 2009). In this study, patients were allocated to

GRASP levels depending on the average score of their baseline UE-FM assessments. Research has shown that therapy intensity is matched between patients across all three GRASP levels (Pang, Eng, Dawson, McKay, & Harris, 2005).

Table 2.4. Components of the GRASP intervention that were undertaken daily by the study participants

Activity
Total arm stretch
Shoulder shrug
Hand and wrist stretch
Table top push ups (both vs paretic arm only)
Arm flexion and abduction (with or without weights, progressing to heavier weights over time)
Elbow flexion (with or without weights, progressing to heavier weights over time)
Wrist extension (with or without weights, progressing to heavier weights over time)
Grip and finger power
Pick up/controlled drop of objects
Ball rolling (self or partner)
Dish cloth wringing
* Knife and fork practice
* Water pouring
* Ball catching
* Shirt buttons
* Clothes line pegs
* Lego block building
* Tower building
* Paper clip chains
* Removal of jar lids

* indicates an activity reserved for patients with greater finger dexterity, namely those patients assigned to levels 2 or 3 of the program. All patients were able to progress in their activities throughout the intervention, either by increasing the number of repetitions of activities or by increasing resistance and weights.

2.2.2.2 tDCS

The Principal Investigator (HJB) assigned participants to one of two groups - anodal tDCS with one hour daily motor training or sham tDCS with one hour daily motor training - using a block randomization with three strata of impairment based on baseline UE-FM score (10-25, GRASP Level 1; 26-45, GRASP Level 2, or 46 or more, GRASP Level 3) to achieve similar distributions of impairment between stimulation groups. Participants were first stratified by

GRASP level; then blocks of treatment allocation sequences were defined using a random number generator and participants were assigned to conditions accordingly (Altman & Bland, 1999). Participants were not aware of group assignment.

A direct current stimulator (Eldith GmbH; Germany) delivered a 1mA current to the brain via two electrodes measuring 5x7cm, one centred over ipsilesional M1 (positioned 5cm lateral to Cz: C3) and the other over the contralateral supraorbital ridge. Saline-soaked sponges were used as a conducting medium between the scalp and the electrode. For anodal stimulation the current was ramped up over ten seconds, held at a constant 1mA for 20 minutes and then ramped down over ten seconds. For sham stimulation, the current was ramped up over ten seconds and then immediately switched off. Subjects are not able to distinguish real from sham stimulation using this paradigm (Gandiga et al., 2006; Nitsche et al., 2008; Poreisz et al., 2007; Siebner, Lang, & Rizzo, 2004).

2.2.3 Follow-Up

Following the completion of the motor training intervention, participants were followed-up comprehensively on four separate occasions, including immediately following the intervention (day 10), one week post-intervention, one month post-intervention, and three months post-intervention. All follow-up assessments were completed accurately within specific time points; all day 10 follow-up appointments took place on Friday of the second intervention week, and all 1 week follow-up appointments took place on the Thursday following the end of the intervention. All 1-month follow-up appointments took place between

28 and 31 days following the end of the intervention, and all 3-month follow-up appointments took place between 85 and 90 days after the intervention. The same researcher who had assessed patients at baseline also conducted all follow-up assessments.

At all follow-up appointments, patients were assessed on the same functional assessments scales as at baseline in order for changes in performance to be assessed. In addition, at both day ten and one month post-intervention follow-ups, patients underwent an additional MRI scan following the same protocol as that used on the baseline scan.

2.3 Data Analysis

Behavioural data was analyzed from each visit (baseline one and two, day 10 follow-up, one week follow-up, one month follow-up and three month follow-up). Imaging data from the baseline scan was analyzed for the purpose of this thesis.

2.3.1 Behavioural Data Analysis

Data collected from baselines one and two were averaged for each participant to characterize arm function prior to beginning the intervention. In addition to analysing the raw scores provided at baseline and follow-up, the absolute change in score from baseline to day ten, baseline to one week follow-up, baseline to one month follow-up and baseline to three month follow-up was also calculated and analysed. Absolute change from baseline was calculated by subtracting the

baseline score from the follow-up score for each follow-up time point. Average baseline and follow-up data as well as absolute change for all behavioural measures were entered into PASW SPSS v 20.0. (IBM).

For each measure, differences between groups and over time were assessed using a repeated measure ANOVA with within-subject factor of time and between-subject factor of tDCS condition (anodal, sham). A multivariate regression analysis was performed to investigate the impact of lesion characteristics and patient variables such as age at intervention and time since stroke on both baseline impairment and response to intervention.

2.3.2 MRI Data Analysis

Analysis of MRI data was carried out using tools from the FMRIB Software Library (www.fmrib.ox.ac.uk/fsl) (Smith, Jenkinson, & Woolrich, 2004).

Stroke lesion volume was established for each patient by manually delineating regions of abnormally hypointense signal on the patient's high-resolution T1 images using fslview, and then calculating the volume of this region using fslstats, as implemented within FSL (see appendix 2 for full patient details). The traced lesion was automatically filled, and the resulting three-dimensional volume of interest, describing the area of tissue affected by the stroke, was saved as a lesion mask (see figure 2.2).

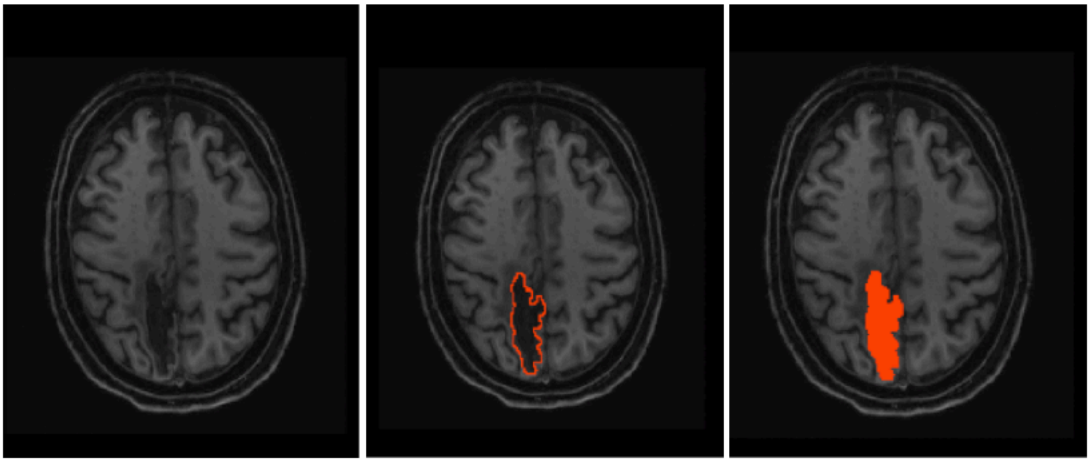


Fig 2.2. Lesion delineation procedure. Lesions were identified on each slice of T1 images. Lesions were traced manually and voxels were filled to create lesion masks.

Lesion-masks were subsequently registered to standard space using an initial linear registration (FMRIB's Linear Image Registration Tool, FLIRT) (Smith et al., 2004), and then optimized using a non-linear registration tool (FNIRT) (Andersson & Smith, 2008) (see Figure 2.3). Registrations were visually inspected to ensure accuracy. Lesion location was defined for each patient using the Harvard-Oxford atlas (available within FSLview).

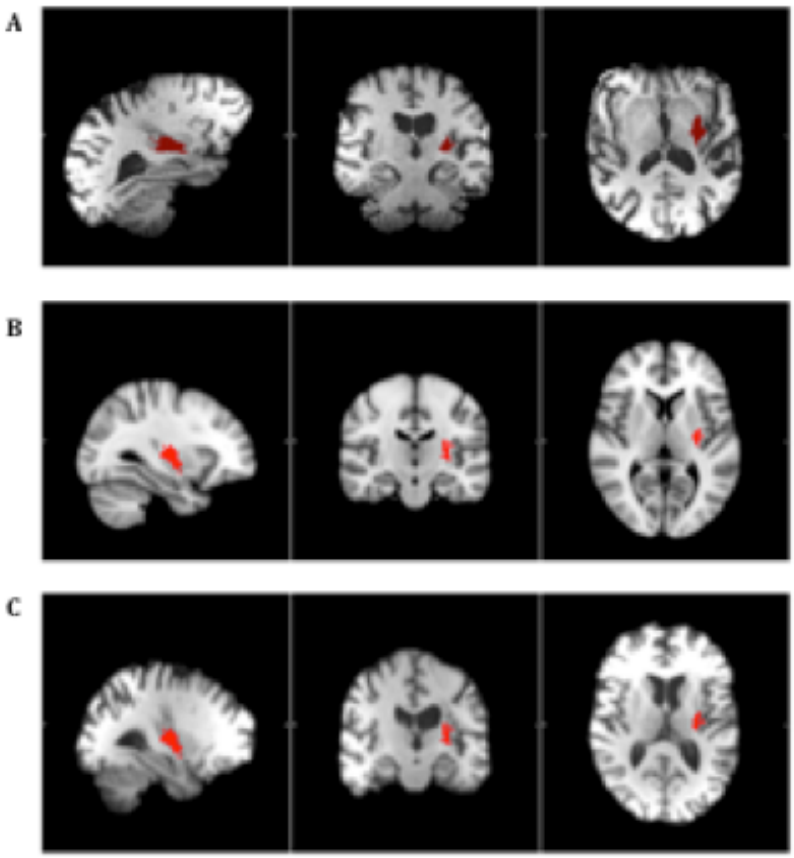


Fig 2.3. Lesions were manually drawn onto T1 weighted structural scans (**A**). Lesions masks were registered to standard space (**B**). Structural images (**C**) were also registered to standard space.

In order to study lesion overlap with the CST, two separate atlases for the left and right tracts were generated from pre-acquired Diffusion Tensor Imaging (DTI) data from ten healthy controls (see figure 2.4).

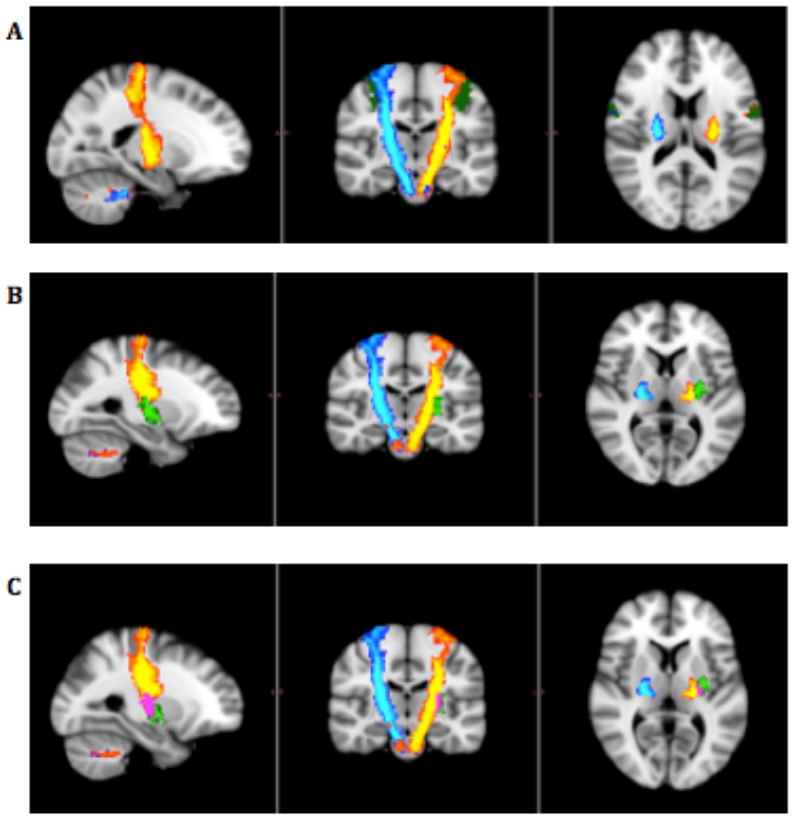


Fig 2.4. CST atlases were derived from DTI data of healthy controls (**A**; flipped images – blue shows right CST, yellow shows left CST, green areas show a seed mask in the M1s used to create the atlas, pink shows a way-point mask in the pons). Lesions (green) were overlapped onto CST maps for each subject (**B**), and common voxels (purple) were identified (**C**).

Two masks for left and right M1 and a single slice mask in the pons were separately drawn in standard space. Probtrackx software (FSL) was used to generate probabilistic tracts of left and right CST, using the M1 mask as a seed and the pons as a way-point. A midline exclusion mask was also used to prevent callosal fibre overlap with the CST. Individual left and right CST masks were thresholded to 0.01% to remove spurious tracts generated from the probabilistic approach. Masks were then combined across the 10 control subjects, binarised and overlapped. Left and right CST tracts were then further thresholded to 30% (so that for each voxel, at least seven subject had that voxel in common). The

total overlap and maximum cross-sectional area of overlap (peak overlap) between each individual lesion-mask and left and right CST atlases was calculated and correlated with behavioural measures. The derived CST maps were compared to those pre-existing CST maps available within the Juelich Histological atlas available with FSL. Both the left and right CST maps developed were similar to the left and right CST maps found in the Juelich Histological atlas, with 78.7% and 80.5% of voxels in common, respectively.

FMRIB's automated segmentation tool (FAST) was implemented for each subject, in order to segment the brain into different tissues types, including cerebrospinal fluid (CSF), grey matter and white matter. The aim of this step was to identify with greater sensitivity whole brain volume, whilst accounting for any brain atrophy or greater CSF volume occurring with enlarged ventricles (figure 2.5).

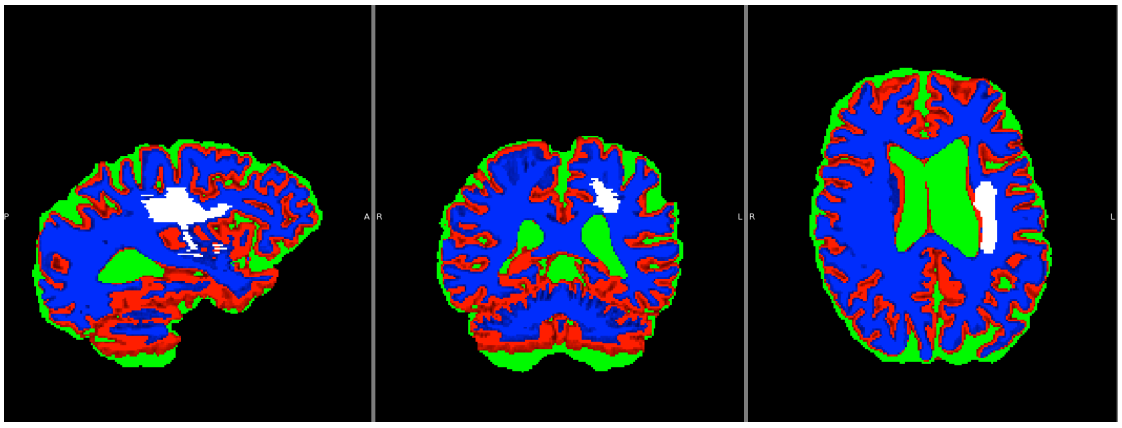


Fig 2.5. FAST segmentation of brain tissue. Green=CSF, blue=white matter, red=grey matter, white=lesion.

Within the sample, mean brain matter volume was 1,079,051 mm^3 (*SD* 110,162 mm^3, range 946,660 – 1,313,447 mm^3). Mean lesion volume was 44,235 mm^3

(*SD* 76,607 mm^3, range 431 – 366,389 mm^3), which meant that the mean volume of brain damage as a direct result of the lesion was 4.23% (*SD* 0.076%, range 0.05 – 36%). Mean lesion overlap onto the CST was 31,118 mm^3 (*SD* 26,545 mm^3, range 0 – 73,704 mm^3), whilst peak lesion-CST overlap was 1,562 mm^3 (*SD* 1077 mm^3, range 0 – 3,112 mm^3).

3 RESULTS

3.1 Introduction

The major objectives of this thesis were:

1. To establish behavioural changes occurring in response to repeated sessions of tDCS
2. To explore predictors of impairment and response to rehabilitation within a group of patients affected by chronic stroke

This section will explore each of these objectives in turn.

Regarding the first objective, this thesis aims to investigate whether multiple, consecutive sessions of transcranial direct current stimulation (tDCS), when delivered in conjunction with a two-week motor training program, will result in long-lasting enhancement of motor and functional abilities in chronic stroke patients, and will be the first to do so in a patient population with an extensive follow up.

The second objective aims to explore a clinician's ability to make an accurate prediction of a patient's potential for recovery following stroke. This is very difficult, as the time period for prediction may span several decades; patients may continue to recover from a stroke for many years, so a predictive framework that is useful and accurate for both the patient and those involved in their care is empowering and supportive.

As well as being useful at an individual patient level, an accurate predictive framework would also serve as a practical tool for the correct stratification of patients into clinical trials; currently, stroke patients are frequently enrolled in clinical trials without knowing how they are likely to respond to the proposed intervention. Consequently, one of the aims of this thesis is to consider which patients with which constellation of impairments may respond to a motor rehabilitation intervention.

The impact of the following variables will be considered, both on predicting impairment, and on predicting response to an intervention: *time since stroke, age at intervention, degree of initial motor impairment, lesion volume and corticospinal tract (CST) damage, lesion location, presence of a muscle twitch in the paretic hand in response to transcranial magnetic stimulation (TMS), and tDCS condition* (for response measures only).

3.2 Methods

24 chronic stroke patients were randomly allocated to receive 20 minutes of anodal (*n*=11) or sham (*n*=13) tDCS daily for 9 consecutive working days, whilst simultaneously completing a motor rehabilitation package for the paretic arm and hand. The motor rehabilitation package, or GRASP, took one hour to complete each day for all patients, and focused on strengthening, range of motion and gross and fine motor skills. Prior to completing the motor training intervention, at baseline all patients completed a battery of functional assessments [The Upper Extremity subscale of the Fugl-Meyer Assessment, (UE-

FM); the Action Research Arm Test, (ARAT); and the Wolf Motor Function Test, (WMFT)]. This battery was then repeated following the intervention at the day 10, 1 week, 1 month and 3 months follow-up sessions.

T1 weighted structural scans were collected at baseline sessions (see 2.2.1.3) for all patients (excluding patient no. 8). Stroke lesion volume was calculated using tools from the FMRIB Software Library, as was overlap of lesions onto the CST (see section 2.4.2). Lesions were identified as affecting either the left or right hemisphere, and as being either cortical or subcortical. Presence of a visible muscle twitch in the paretic hand was determined by a single TMS session at baseline. Individual patient values for all variables of interest are given in appendix 4.

3.3 Objective 1 - Behavioural changes occurring in response to repeated sessions of tDCS

3.3.1 The Effect of Time and tDCS

Three functional assessments were investigated (UE-FM, ARAT, WMFT). Descriptive statistics for all three scales are available in appendix 3.

Individual patient results as assessed on the UE-FM, ARAT and WMFT are shown in figures 3.1, 3.2 and 3.3 respectively.

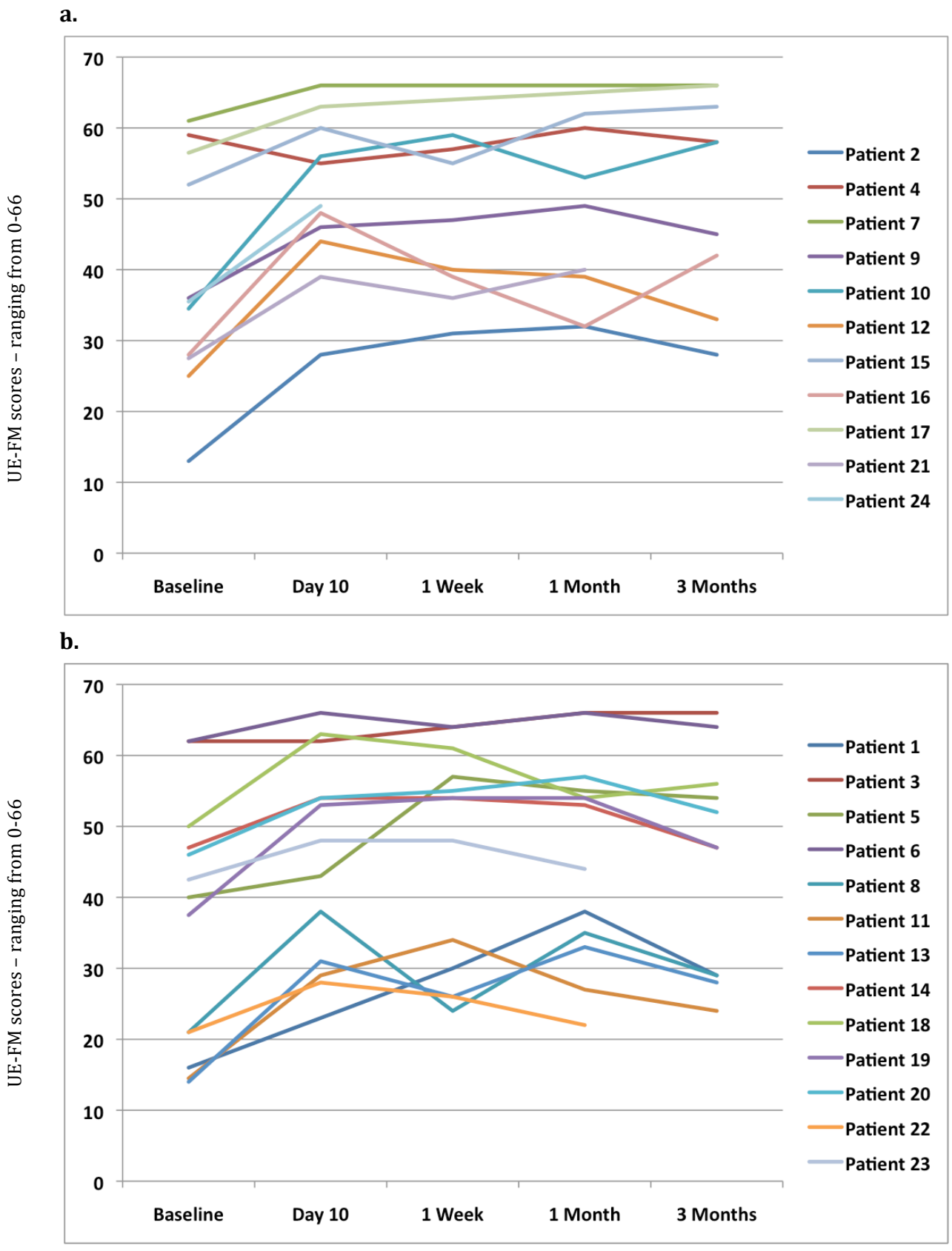


Fig 3.1. Individual results for the UE-FM. **a.** Patients who received anodal tDCS. **b.** Patients who received sham tDCS.

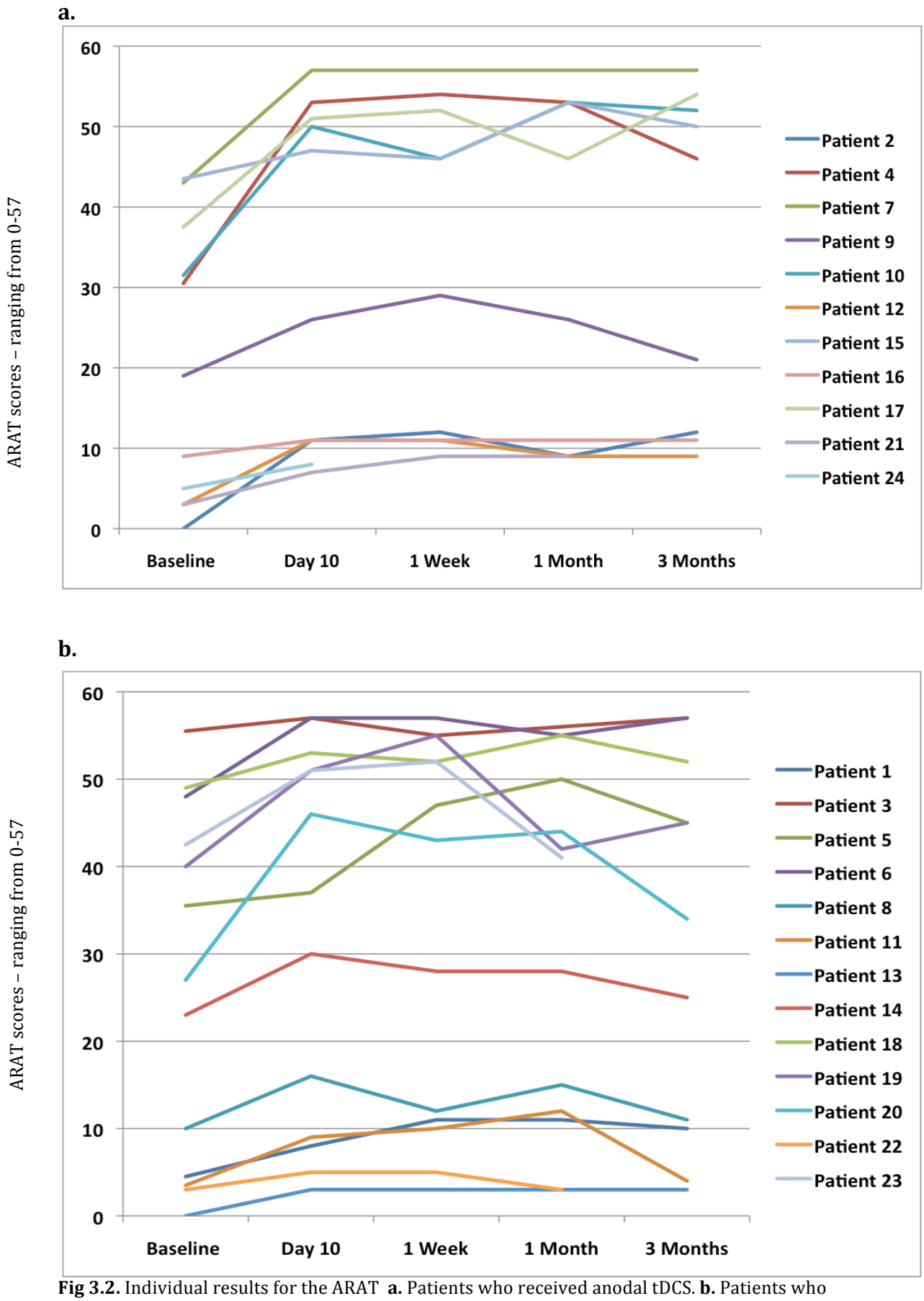
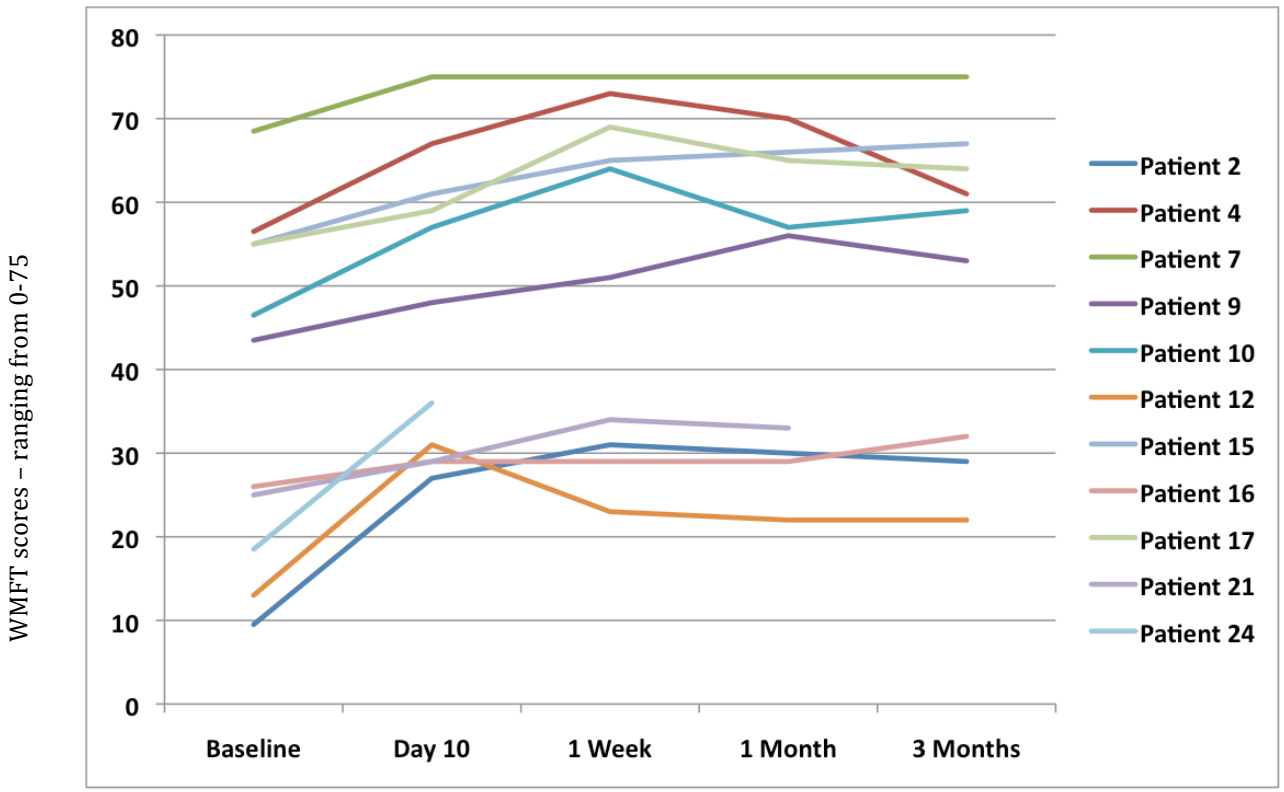


Fig 3.2. Individual results for the ARAT **a.** Patients who received anodal tDCS. **b.** Patients who received sham tDCS.

a.



b.

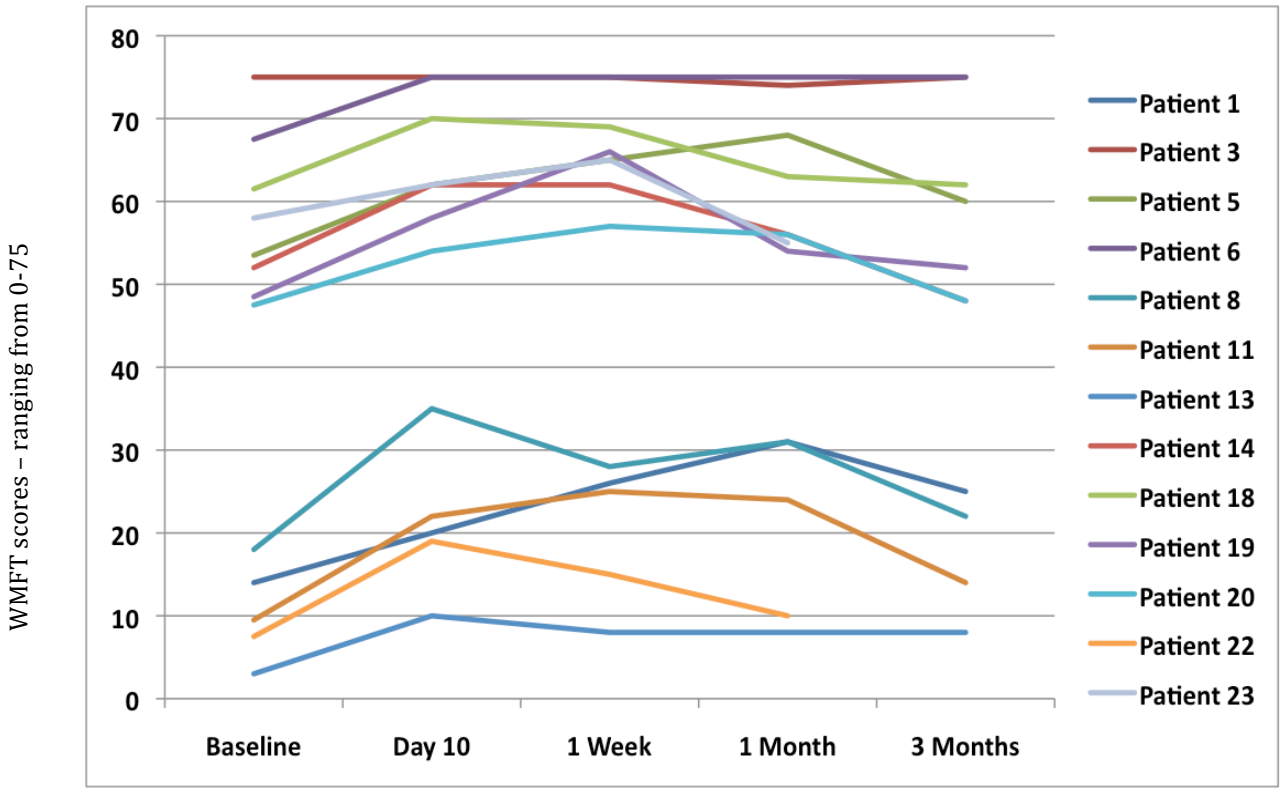


Fig 3.3. Individual results for the WMFT **a.** Patients who received anodal tDCS. **b.** Patients who received sham tDCS.

Repeated measures ANOVAs (RM-ANOVAs) were used to investigate the effects of time and tDCS on functional assessment measures as detailed below. Importantly, there were no significant differences between the sham and anodal tDCS groups in behavioural performance at baseline.

3.3.2 UE-FM results

UE-FM absolute changes (the change in score between baseline and each follow-up time point) are displayed in figure 3.4. A RM-ANOVA showed no main effect of time (p=.486) or tDCS (p=.777). There was no significant interaction between time and tDCS (p=.396).

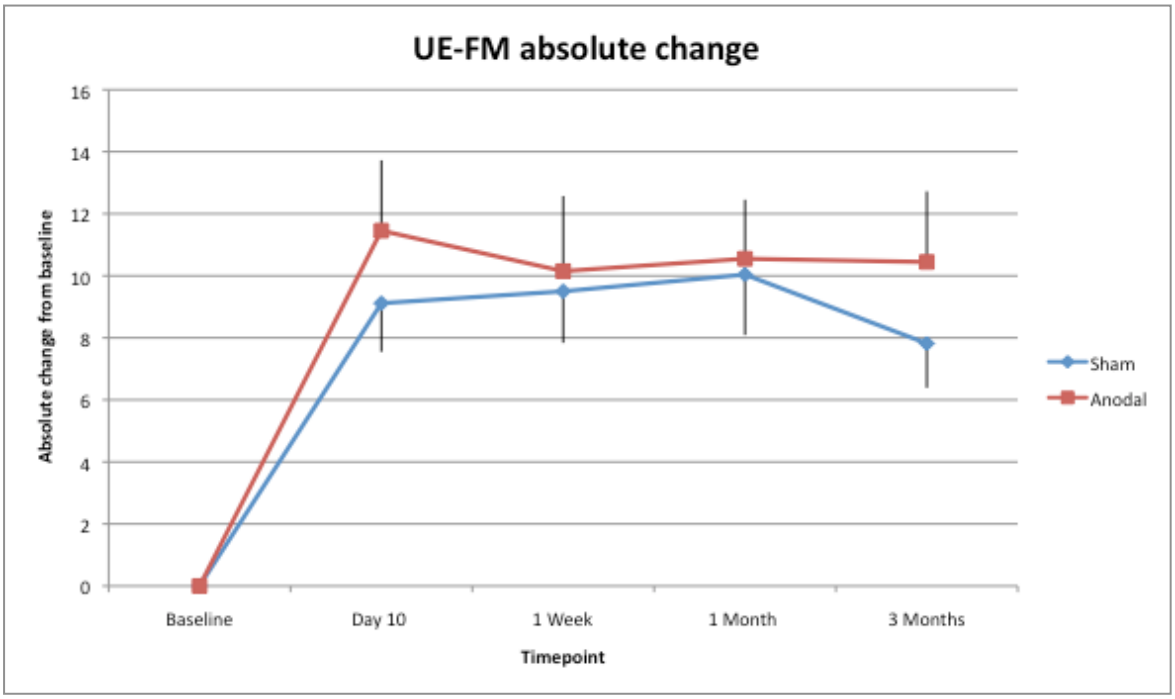


Fig 3.4. Absolute change in scores from baseline to follow-up for anodal and sham tDCS groups. Bars indicate standard error mean.

3.3.3 ARAT results

Change in ARAT scores from baseline are plotted in Figure 3.5. A RM-ANOVA on the change measures at all 4 follow up time points revealed a significant main effect of tDCS ($F(1, 18)=4.49$, $p=.048$) and no main effect of time (p=.270) or time by tDCS interaction ($p=.675$). These results reflect greater change scores in the anodal group consistently across time (figure 3.5). Independent samples t-test showed a significant difference between anodal and sham groups at 3 month follow-up, $t(18)=-2.59$, $p<.05$. There were no significant differences between anodal and sham tDCS groups at any other time points (day 10, $p=.192$, 1 week, $p=.138$, 1 month, $p=.075$).

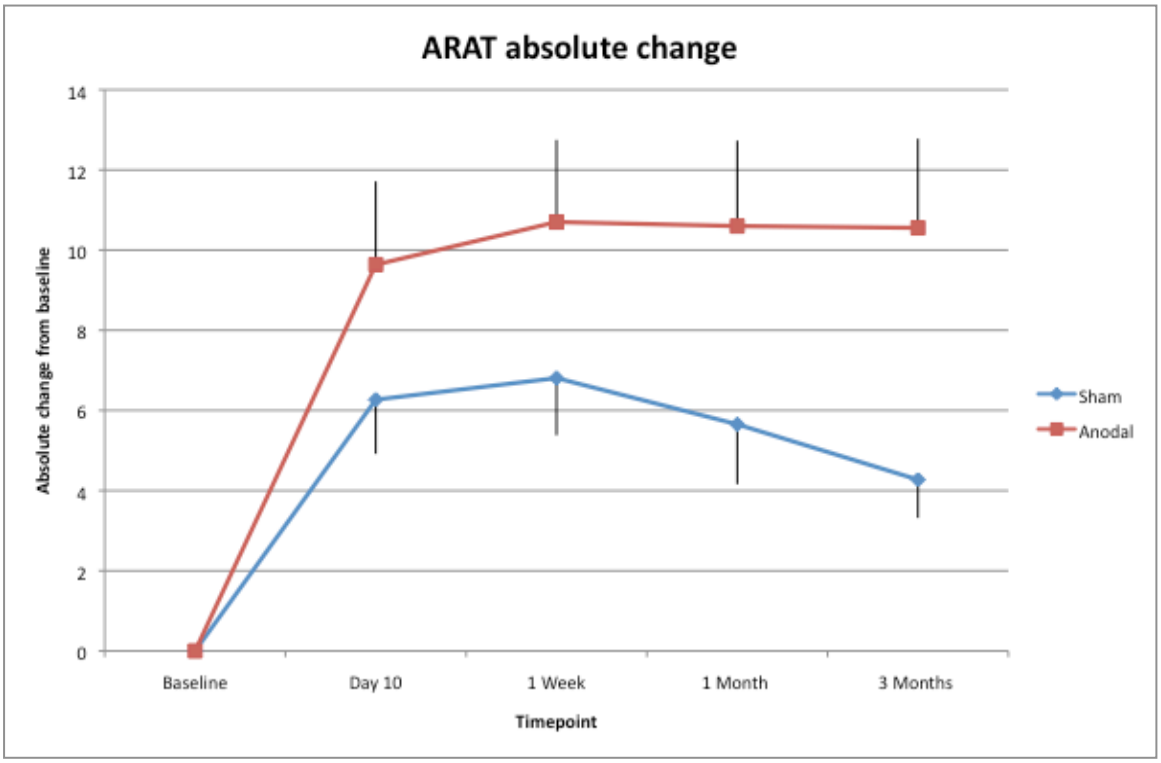


Fig 3.5. Absolute change in scores from baseline to follow up for anodal and sham tDCS groups. Bars indicate standard error mean.

3.3.4 WMFT results

WMFT absolute changes are displayed in figure 3.6. A RM-ANOVA on WMFT change showed a main effect of time, $F(3, 54)=4.92$, $p<.010$, but not of tDCS ($p=.132$). There was a trend for a time by tDCS interaction ($F(3, 54)=2.56$, $p=.064$). Independent samples t-test showed a significant difference between anodal and sham groups at 3 month follow-up, $t(18)=-3.33$, $p<.010$. There were no significant differences between anodal and sham tDCS groups at any other time points (day 10, $p=.668$, 1 week, $p=.311$, 1 month, $p=.134$)

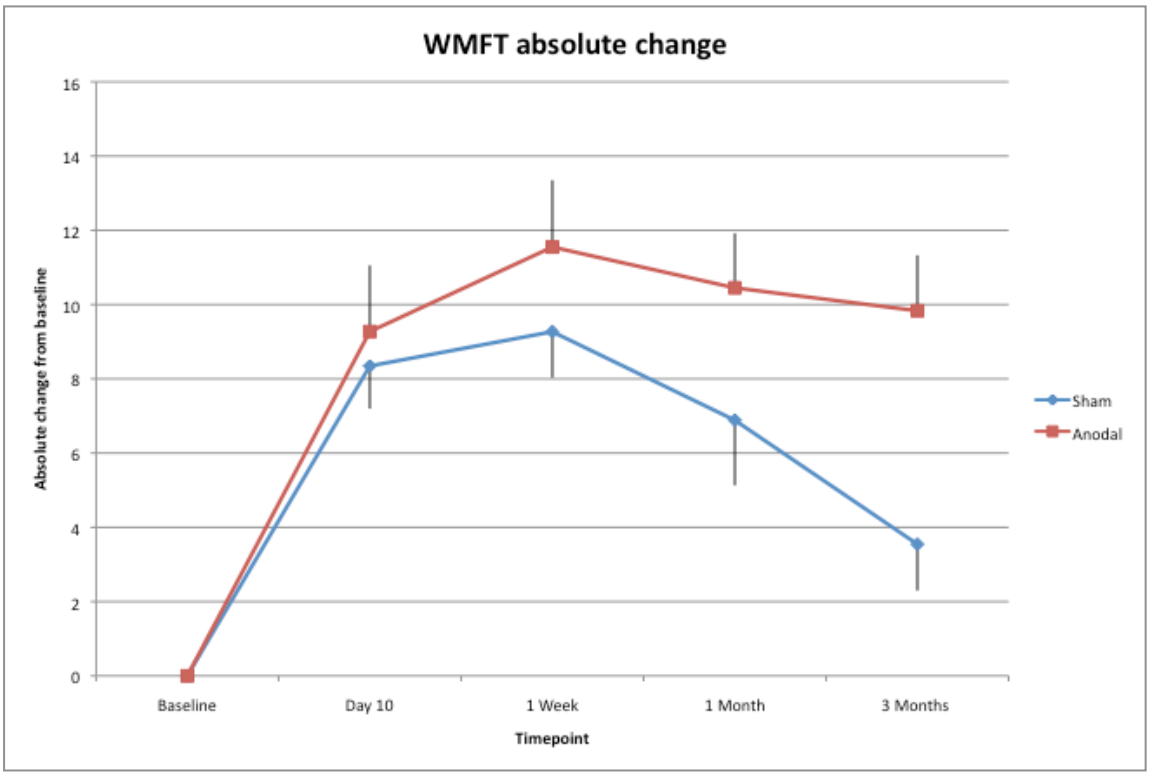


Fig 3.6. Absolute change in scores from baseline to follow up for anodal and sham tDCS groups. Bars indicate standard error mean.

3.3.5 Summary of results for research objective 1

All patients were found to improve over time, regardless of tDCS condition, with follow up scores higher than original baseline scores. However, as is evident

from the individual patients scores plotted in figures 3.1-3.3, there is substantial variability. Despite this, there is evidence to suggest that changes over time differed between the anodal and sham tDCS groups, with the anodal tDCS group achieving greater changes on the behavioural measures that were more enduring at follow up.

3.4 Objective 2 – predicting impairment and response to rehabilitation

3.4.1 The effect of patient variables and lesion characteristics on impairment

Individual patient values for all variables of interest are given in appendix 4. Stepwise multivariate regression was used to determine the relative impact of the predictor variables (*time since stroke, age at intervention, degree of initial motor impairment, lesion volume and CST damage, lesion location, presence of a muscle twitch in the paretic hand in response to TMS, and tDCS condition for response measures*) on baseline functional assessments as a measurement of impairment following stroke.

3.4.2 Baseline functional assessments

Three scales were assessed at baseline – the upper extremity subscale of the Fugl-Meyer (UE-FM), Action Research Arm Test (ARAT), and Wolf Motor Function Test (WMFT), and were run in separate regression analyses. Similar predictive models emerged for each scale, with the presence of a muscle twitch in the paretic hand in response to TMS and lesion volume being the most consistent significant predictors. For UE-FM, a significant predictive model emerged ($F(3, 21)=9.06$, $p<.010$) in which 60.2% of the variance in the baseline

UE-FM was explained. This model included the significant predictors of muscle twitch presence in the paretic hand (24.7%), age at intervention (22.4%), and lesion volume (13.1%) (for full model and Beta values see table 3.1). For the ARAT, a significant predictive model emerged ($F(1,21)=12.61$, $p<.010$) in which 38.7% of the variance for the baseline ARAT was explained. This model included the significant predictor of muscle twitch presence in the paretic hand (for full model and Beta values see table 3.2). For WMFT, a significant predictive model emerged ($F(3, 21)=8.69$, $p<.010$) in which 59.1% of the variance for the baseline WMFT was explained. This model included the significant predictors of muscle twitch presence in the paretic hand (35.3%), lesion volume (13.5%), and age at intervention (10.3%) (for full model and Beta values see table 3.3).

Beta values for all predictor variables, including those that were not found to be significant, are displayed below for each functional assessment (table 3.1-3.3). Beta values range from -1 to 1, and large beta values (either positive or negative) indicate a greater relative impact of that variable on the assessment measure in question.

Table 3.1. Beta values for predictor variables for baseline UE-FM

Prediction Variable	Beta Value	*P* Value
Muscle twitch presence in the paretic hand following TMS	.813	<.001*
Age at intervention	-.526	.006*
Lesion volume	-.368	.026*
Time since stroke	.260	.144
CST-lesion overlap	.389	.312
Subcortical stroke	.094	.574

Note: significant predictors included in this model *