Novel assessment of functional adaptation and motor plasticity following stroke

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Publication Date:
2013

DOI:
https://doi.org/10.26190/unsworks/16506

NOVEL ASSESSMENT OF FUNCTIONAL ADAPTATION AND MOTOR PLASTICITY FOLLOWING STROKE

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Prince of Wales Clinical School, University of New South Wales

Neuroscience Research Australia

August 2013

THE UNIVERSITY OF NEW SOUTH WALES
Thesis/Dissertation Sheet

Surname or Family name: **Huynh**

First name: **William** Other name/s:

Abbreviation for degree as given in the University calendar: **PhD**

School: **Prince of Wales Clinical School** Faculty: **Medicine**

Title: **Novel assessment of functional adaptation and motor plasticity following stroke**

Abstract 350 words maximum: (PLEASE TYPE)

This thesis examined the physiological mechanisms of neuroplasticity in ischemic stroke, and its relation to functional and motor recovery. Novel electrophysiological techniques comprising of transcranial magnetic stimulation (TMS) and peripheral nerve excitability were utilized to assess the mechanisms of plasticity involving the central and peripheral nervous system in stroke patients, and whether biomarkers of such processes may be developed to assist in neurorehabilitative strategies for stroke recovery.

Initial peripheral nerve excitability studies demonstrated that peripheral nerve excitability results were reproducible over time in normal controls. The study also investigated changes in peripheral axonal properties in a patient with limb and demonstrated relative axonal depolarization in the ischemic limb that significantly improved following reperfusion.

Central and peripheral studies undertaken in acute stroke patients immediately after the event and assessed over the the first 3 months demonstrated significant reductions in intracortical inhibition in both lesioned and contralesional hemispheres that persisted at follow-up in association with clinical improvements, suggesting that bihemispheric intracortical excitability may be biomarkers of an adaptive plastic process after stroke. Furthermore, peripheral studies demonstrated complex changes in axonal biophysical parameters that suggest a down-stream (transynaptic) plastic process that may reflect changes occurring in the central nervous system.

To explore the changes in cortical excitability and potential maladaptive forms of neuroplasticity, chronic stroke patients with disabling spasticity were studied prior to and following the administration of peripheral intramuscular botulinum toxin. Results revealed significant intracortical disinhibition over the contralesional motor cortex that normalized after treatment with botulinum toxin. This suggests a maladaptive process in the contralesional motor cortex that may be contributing to the development of spasticity, and that this abnormality can be modulated with treatment.

Studies undertaken in acute cerebellar stroke patients demonstrated persistent motor cortex disinhibition bilaterally that correlated with the degree of impairment, suggesting an adaptive plastic process. This highlights the widespread reorganization on a neural network and provides insight into the complex pathophysiological mechanisms of recovery following stroke.

To further explore and clarify the cortical excitability changes that occur over the contralesional motor cortex, longitudinal studies were undertaken in stroke patients from the time of ictus and followed up to a period of 18 months. The study demonstrated that in well recovered stroke patients, contralesional intracortical hyperexcitability persisted in those patient groups with more severe baseline functional impairment and cortical location of stroke. The results suggest that ongoing cortical network recruitment in the contralesional hemisphere may be required in those patients with significant disruption to the integrity of ipsilesional motor pathways.

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ACKNOWLEDGMENTS

It has been a huge honour to have worked under the supervision of my primary supervisor, Professor Matthew Kiernan, who has offered not only guidance and support in regards to my research and PhD studies but has also continually assisted in different facets of my professional career. In addition, this body of work would not have been possible without the tremendous input from and mentorship of my co-supervisors, Dr Cindy Shin-Yi Lin, A/Prof Arun Krishnan and A/Prof Steve Vucic, who have all provided ongoing encouragement and motivation during my doctoral studies. I would like to also thank Dr Michael Hornberger for his contribution and supervision.

Thank you to all the members of Nerve Central (Kiernan group) who have all offered a nurturing and supportive environment over the past several years. I would like to thank Ria Arnold for being my fellow colleague on this tough and emotional PhD rollercoaster as well as enduring the countless debriefing sessions over coffee. Thank you to Dr Susanna Park who has been so patient and generous in sharing your time and knowledge. Thank you to Dr Cindy Lin and Hannah Pickering for being so thoughtful and supportive over the years and offering your social wisdom. Thank you to Ben Cheah for his statistical ingenuity and technical assistance with my studies. I would like to also thank Dr Michelle Farrar, Dr Neil Simon, Natalie Kwai, Jenna Murray, Dr Michael Lee, Dr James Burrell, Dr Eneida Mioshi and Dr Tim Eviston for their collegiality and moral support during my doctoral studies. Others in the group who deserve special mention are Margie Zoing, Eleanor Ramsay and Dianne Tyson who have also provided assistance and support over the years.

Thank you to my much loved family and friends (both human and canine) who have endured every single conceivable emotion that I have experienced during this critical time but have never failed to provide unconditional love, support and encouragement.

Last but not least, thank you to all the patients who have participated in studies, without whom, this thesis would not have been possible.

ABSTRACT

This thesis examined the physiological mechanisms of neuroplasticity in ischemic stroke, and specifically, its relationship to functional and motor recovery. Novel electrophysiological techniques comprising of threshold-tracking paired-pulse transcranial magnetic stimulation (TMS) and peripheral nerve excitability studies were utilized to assess the mechanisms of plasticity involving the central and peripheral nervous system in stroke patients, and whether electrophysiological biomarkers of such neuroplastic processes may be developed to assist in neurorehabilitative strategies to facilitate stroke recovery.

Initial peripheral nerve excitability studies were undertaken in a cohort of normal control participants to assess the reproducibility of study parameters. This study demonstrated that peripheral nerve excitability results showed minimal change over time in normal controls. The study also investigated changes in peripheral axonal properties in a patient with limb ischemia to enable later comparison to peripheral axonal changes in studies of patients with ischemic strokes. Results demonstrated relative axonal depolarization in the ischemic limb that significantly improved following reperfusion.

Central and peripheral studies were undertaken in acute stroke patients immediately after the event and assessed over the subacute period in the first 3 months. TMS studies demonstrated significant reductions in intracortical inhibition in both lesioned and contralesional hemispheres in the immediate phase of stroke

that persisted at follow-up in association with clinical improvements, suggesting that bihemispheric intracortical excitability may be biomarkers of an adaptive plastic process after stroke. Furthermore, peripheral studies demonstrated complex changes in axonal biophysical parameters that suggest a down-stream (transynaptic) plastic process that may reflect changes occurring in the central nervous system.

To explore the changes in cortical excitability and potential maladaptive forms of neuroplasticity, chronic stroke patients with disabling spasticity were studied prior to and following the administration of peripheral intramuscular botulinum toxin. Results revealed significant intracortical disinhibition over the contralesional motor cortex that normalized after treatment with botulinum toxin. This was associated with clinical improvements in spasticity indicating a maladaptive process in the contralesional motor cortex of chronic stroke patients that may be contributing to the development of spasticity, and that this abnormality can be modulated with treatment.

Studies undertaken in acute cerebellar stroke patients followed over a period of 12 months, also demonstrated persistent motor cortex disinhibition bilaterally that correlated with the degree of impairment, again suggesting an adaptive plastic process occurring in the motor cortices following cerebellar infarction. The study also illustrated that stroke recovery involved widespread reorganization on a neural

network level in the brain and provides insight into the complex pathophysiological mechanisms of recovery following stroke.

To further explore and clarify the cortical excitability changes that occur over the contralesional motor cortex, longitudinal studies were undertaken in stroke patients from the time of ictus and followed up to a period of 18 months. The study demonstrated that in well recovered stroke patients, intracortical disinhibition was maintained over the ipsilesional hemisphere over this period, whilst contralesional intracortical hyperexcitability remained a feature in those patient groups with more severe baseline functional impairment and cortical location of stroke. The results suggest that ongoing cortical network recruitment in the contralesional hemisphere may be required in those patients with significant disruption to the integrity of ipsilesional motor pathways.

In conclusion, threshold-tracking electrophysiological studies and in particular, paired-pulse transcranial magnetic stimulation, have enhanced our understanding of the physiological mechanisms associated with neuroplasticity in stroke recovery. These techniques may be used to further explore the mechanisms underlying novel neuromodulatory therapies as well as to improve the way in which these interventions are delivered. Furthermore, threshold-tracking transcranial magnetic stimulation may provide a means of developing neurophysiological biomarkers that can be incorporated into future clinical trials in stroke rehabilitation.

CONTENTS

PUBLICATIONS

Chapters 1 – 5 in this thesis are separate studies, which have been published or submitted for publication in international peer reviewed journals.

Methodology

Huynh W, Kiernan MC. Nerve conduction studies. *Australian Family Physician*. 2011. 40(9):693-697.

Chapter 1

Huynh W, Krishnan AV, Lin CS-Y, Vucic S, Kiernan MC. The effects of large artery ischemia and subsequent recanalization on nerve excitability. *Muscle & Nerve*. 2011. 44(5): 841.

Chapter 2

Huynh W, Vucic S, Krishnan AV, Lin CS-Y, Hornberger M, Kiernan MC. Longitudinal plasticity across the neural axis in acute stroke. *Neurorehabilitation & neural repair*. 2013. 27(3):219-29.

Huynh W, Krishnan AV, Vucic S, Lin CS-Y, Hornberger M, Kiernan MC. Unmasking the mechanisms of neuroplasticity in stroke: a study of the entire neural axis. Abstract in *Clinical Neurophysiology* 123 (7), e70-e70.

Chapter 3

Huynh W, Krishnan AV, Lin CS-Y, Vucic S, Katrak P, Hornberger M, Kiernan MC. Botulinum toxin modulates cortical maladaptation in post-stroke spasticity. *Muscle & Nerve*. 2012. 48(1):93-9.

Chapter 4

Huynh W, Krishnan AV, Vucic S, Lin CS-Y, Kiernan MC. Motor cortex excitability in acute cerebellar infarct. *Cerebellum*. June 2013. DOI: 10.1007/s12311-013-0493-8.

Chapter 5

Huynh W, Vucic S, Krishnan AV, Lin CS-Y, Kiernan MC. Exploring the long-term changes in cortical excitability after acute stroke. Submitted to *Brain* (August 2013).

Summary, conclusions and future strategies

Huynh W, Lee M, Kiernan MC. Neurorehabilitation approaches for disorders of the peripheral nervous system. In: Ward N and Dietz V (Eds). *Neurorehabiliation (The Oxford Textbook of Clinical Neurology).* UK: Oxford University Press (accepted, August 2013).

Other publications achieved during this candidature

Huynh W, Lam A, Cheah B, Vucic S, Clouston P, Kiernan MC. Corticospinal tract dysfunction and the development of Amyotrophic Lateral Sclerosis following electrical injury. *Muscle & Nerve*. 2010. 42(2):288-292.

Krishnan AV, Park SB, **Huynh W**, Lin CS-Y, Henderson R, Kiernan MC. Impaired energy-dependent processes underlie acute lead neuropathy. *Muscle & Nerve*. 2012. 46(6):957-61.

Huynh W, Lam A, Vucic S, Chia B, Kiernan MC. Corticospinal dysfunction and the development of Amyotrophic Lateral Sclerosis following Electrical Injury. Abstract in *Clinical Neurophysiology* 121 (4), e1-e1.

AWARDS

- National Health and Medical Research Council (NHMRC) of Australia Postgraduate Medical Scholarship (2010-2013).
- James Lance Young Investigator of the Year Award, Australian and New Zealand Association of Neurologists Annual Scientific Meeting, May 2011. "Defining mechanisms of neuroplasticity in stroke".

CONFERENCE PRESENTATIONS

- Combined Asian-Oceanic Congress of Neurology (AOCN) and Australian and New Zealand Association of Neurologists (ANZAN) Annual Scientific Meeting (ASM) 2012.
    - Platform presentation: “The role of the unaffected hemisphere in acute stroke”
    - Poster presentation: “Central effects of Botulinum toxin in stroke”

- Australian and New Zealand Association of Neurologists (ANZAN) Annual Scientific Meeting (ASM) 2011.
    - Platform presentation: "Defining mechanisms of neuroplasticity in stroke". Young investigator award division.

- 14th Biennial Clinical Neurophysiology Conference, Gold Coast, Australia 2011.
    - “Unmasking the mechanisms of neuroplasticity in stroke: a study of the entire neural axis”

- Coast Medical Association/Combined Prince of Wales Campus Hospitals TOW Awards 2011.
    - Open Junior Division platform presentation: "Unmasking the mechanisms of neuroplasticity in stroke".
    - Poster Division: "The effects of large artery ischemia and subsequent recanalization on nerve excitability".

- University of New South Wales Brain symposium 2011.
    - "Defining mechanisms of neuroplasticity in stroke"

- The Inaugural Sydney-Chiba Neuroscience Symposium 2011.
    - Platform presentation: "The Brain without limits: Unmasking mechanisms of neuroplasticity in stroke"

ABBREVIATIONS

ACh	acetylcholine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APB	abductor pollicis brevis
ATPase	Adenosine Triphosphatase
BI	Barthel index
BDNF	brain-derived neurotrophic factor
CMAP	compound muscle action potential
Contra-M1	contralateral primary motor cortex
CSP	cortical silent period
DCN	deep cerebellar nuclei
FM	Fugl-Meyer score
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
ICF	intracortical facilitation
Ipsi-M1	ipsilateral primary motor cortex
I/V	Current-Voltage (or Current-Threshold) relationship
K+	Potassium
M1	primary motor cortex
mAS	modified Ashworth scale
MEP	motor evoked potentials
mRS	modified Rankin scale
Na+	Sodium

NIBS	non-invasive brain stimulation
NIHSS	National Institute of Health Stroke Scale
NMDA	*N*-methyl-D-aspartate
PET	positron emission topography
PICA	posterior inferior cerebellar artery
RMT	resting motor threshold
rTMS	repetitive transcranial magnetic stimulation
S1	primary somatosensory cortex
SARA	scale for the assessment and rating of ataxia
SCA	superior cerebellar artery
SICI	short-interval intracortical inhibition
SM1	primary sensorimotor cortex
SMA	supplementary motor area
SR	stimulus response
tDCS	transcranial direct current stimulation
TMS	transcranial magnetic stimulation
TE	threshold electrotonus
TEd	depolarizing Threshold Electrotonus
TEh	hyperpolarizing Threshold Electrotonus
tPA	tissue-type plasminogen activator
τ_{SD}	strength-duration time constant

LITERATURE REVIEW

1 INTRODUCTION

Stroke is the rapid development of a focal neurologic deficit caused by a disruption of blood supply to the corresponding area of brain accompanied by characteristic abnormalities on brain imaging (Grysiewicz et al., 2008; Jovin et al., 2008). Strokes can either be ischemic (an occlusion of a blood vessel) or hemorrhagic (a rupture of a blood vessel), with the majority being ischemic (approximately 85%)(Grysiewicz et al., 2008). Ischemic strokes are categorized into subtypes according to injury mechanisms (Adams et al., 1993; Jovin et al., 2008): (1) cardioembolism (20-30%), where the thrombus originates from the heart; (2) large-artery atherosclerosis (15-20%), where emboli originate from proximal large vessels e.g. aorta, extracranial cervical and intracranial arteries; (3) small vessel disease (20-30%), where thrombotic occlusion occur in small penetrating arteries of the brain; (4) stroke of other determined cause, such as inherited thrombophilic states, sickle cell disease; and (5) Stroke of undetermined cause.

1.1 Epidemiology

Fifteen million people suffer a stroke each year worldwide (Onteniente and Polentes, 2011). Stroke is the third most frequent cause of death (10% of deaths worldwide) (Rothwell et al., 2011), and the most common cause of acquired adult disability in developed countries (Stinear, 2010), confining one-third of stroke survivors to nursing homes or institutional settings (Calautti et al., 2001b; Clarkson et al., 2010). In developed countries, stroke accounts for more than 4% of direct

health-care expenditure, with an absolute cost of more than US$40 billion in the USA (Rothwell et al., 2011).

Motor impairment is a frequent complication after stroke, and is an important contributory factor to a patient's ability to live independently (Stinear, 2010). Six months after the episode, up to two-thirds of stroke survivors have chronic upper limb impairment resulting in the inability to take part in activities of daily living with their paretic hand to the extent they were premorbidly (Boyd et al., 2010; Hummel and Cohen, 2006). In spite of this, there is a widely variable phase in stroke recovery, and only 30% to 50% of the variance is explained by the most commonly reported predictors, lesion volume and initial stroke severity (Marshall et al., 2009). The remainder is likely due to differences in plasticity of the central nervous system.

2 PATHOPHYSIOLOGY OF ISCHEMIC STROKE

The main factors determining tissue outcome are regional cerebral blood flow (CBF) and duration of vessel occlusion (Jovin et al., 2008). Normal CBF is greater than 50 ml/100mg/min and infarction occurs when CBF falls below 20 ml/100mg/min with irreversible neuronal death occurs below about 10 ml/100mg/min (Khaja, 2008). For any given blood flow level, low CBF values are tolerated for only a short period of time, while higher CBF values require longer a time for infarction to occur (Jovin et al., 2008). Brain tissue perfusions between CBF values of 8-22 ml/100mg/min with consequent cessation of function, have their structural

integrity maintained and can be salvaged with timely reperfusion (Symon et al., 1977). This region of brain tissue is known as the ischemic penumbra.

2.1 Ischemic penumbra

The term “ischemic penumbra” was originally coined by Astrup and colleagues in 1981 (Astrup et al., 1981), and describes the region of potentially viable ischemic tissue around an infarcted core that determines the final infarct volume following hypoperfusion (Mitsios et al., 2006) (Figure 1). It includes ischemic areas that recover spontaneously and areas that progress to irreversible change, unless prompt effective treatment is administered (Mitsios et al., 2006). Cells in this region are functionally silent with their electrical activity maintained, and blood flow is relatively sufficient for a degree of neuronal function and membrane integrity (Khaja, 2008; Mitsios et al., 2006). In middle cerebral artery (MCA) occlusions, the penumbra may comprise about 1/3 of the MCA territory, whilst infarct core varies between 20% to 70% of the MCA cortex. It is found that the extent of the core (Jovin et al., 2003), as well as the volume of the penumbra (Cramer, 2008), correlated with the final clinical outcome.

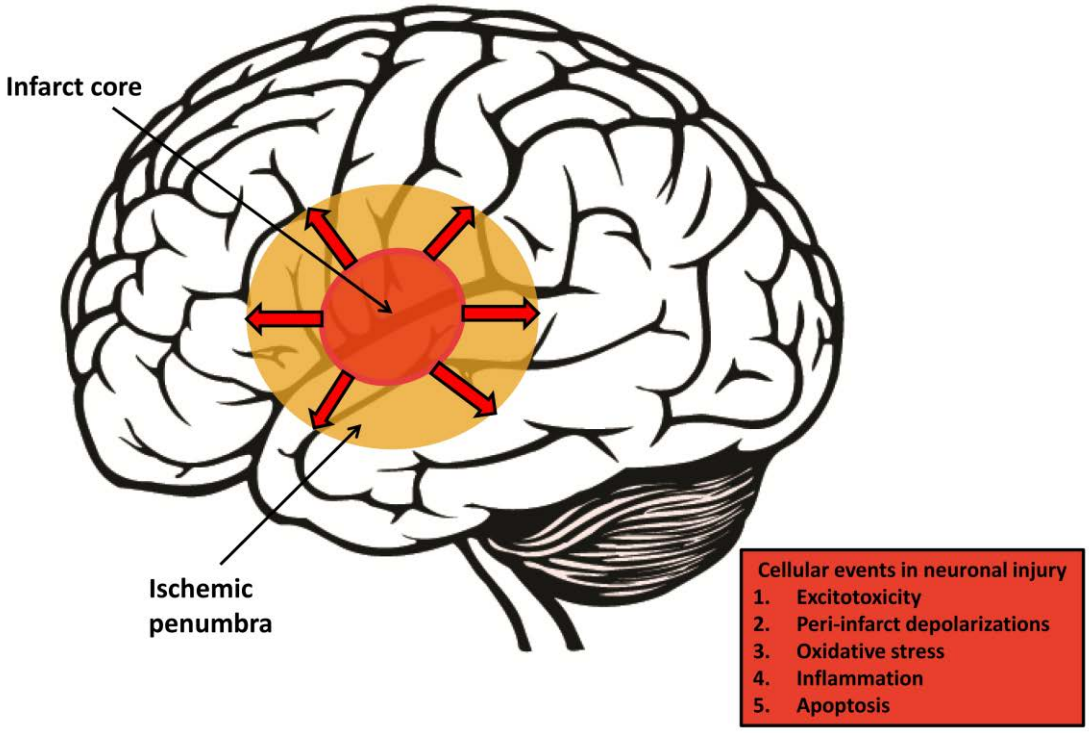


Figure 1. Cellular events occurring in the infarct core and surrounding penumbral region following ischemic injury to neurons in the brain. The penumbra comprises neurons that are functionally silent yet receive sufficient blood flow to maintain structural integrity. In the absence of timely reperfusion, the infarct core gradually expands, encroaching on and ultimately incorporating the ischemic penumbra, a process resulting in the final infarct volume.

2.2 Cellular and molecular mechanisms of neuronal injury

The obstruction of cerebral blood flow sets up a complex cascade of events beginning with adenosine triphosphate (ATP) depletion, resulting in failure of the Na^+/K^+ pump, and loss of ion homeostasis, release of glutamate, calcium accumulation in neurons and subsequent activation of a series of detrimental enzymatic processes (Khaja, 2008; Mitsios et al., 2006; Woodruff et al., 2011). All these occur within 1-2 hours (Mitsios et al., 2006) and lead to necrosis that occurs in the affected ischemic core (Woodruff et al., 2011). Neuronal cell death occurs as a result of 2 main mechanisms, necrosis and apoptosis (Jovin et al., 2008). Necrosis is not regulated or programmed, while apoptosis is a "programmed cell death". Evidence suggests that excitotoxicity and apoptosis comprise the major route of cell injury in the penumbra, whilst necrosis occurs in the core (Mitsios et al., 2006), with the latter morphologically characterized by initial cellular and organelle swelling, subsequent disruption of nuclear, organelle, and plasma membranes, disintegration of nuclear structure and cytoplasmic organelles with extrusion of cell contents into the extracellular space and resultant oedema (Woodruff et al., 2011). Moreover, areas that receive synaptic input from the primarily damaged area suffer a sudden withdrawal of excitation or inhibition. Such sudden changes in input lead to an additional loss of function and secondary cell death that combine with the primary cell death resulting directly from the injury itself (Kolb and Teskey, 2010).

2.2.1 Excitotoxicity

A significant portion of ischemia-induced neuronal damage is mediated by excessive accumulation of excitatory amino acids, namely glutamate, leading to toxic increases in intracellular calcium and other ions (Woodruff et al., 2011; Yenari, 2004). Soon after reduction in CBF, energy-dependent pump failure occurs and Ca^{2+} transport from the cell into the extracellular space is impaired resulting in intracellular accumulation of Ca^{2+} (Jovin et al., 2008). This then leads to the impairment of energy-dependent reuptake of glutamate resulting in its increased extracellular concentration and subsequent overstimulation of its receptors: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA), and kainate (KA), further contributing to increased influx of Ca^{2+} via these Ca^{2+} permeable glutamate receptors (Jovin et al., 2008; Yenari, 2004). Increased intracellular Ca^{2+} results in activation of a number of proteases, kinases, lipases, and endonucleases that lead to a series of events at both the cytoplasmic and nuclear level culminating in cell death by degrading key cytoskeletal and enzymatic proteins (Arai et al., 2011; Jovin et al., 2008; Mitsios et al., 2006; Woodruff et al., 2011; Yenari, 2004). Intracellular Ca^{2+} also participates in the formation of free radicals through activation of nitric oxide synthase (Samdani et al., 1997). Intracellular Na^{+} concentrations are also increased as a result of increased NMDA receptor activation via glutamate, leading to swelling of neuronal cell bodies and cytotoxic oedema (Jovin et al., 2008; Mitsios et al., 2006).

2.2.2 Peri-infarct depolarizations

Subsequent to energy loss as a result of hypoperfusion, depolarizations occur due to the inability to maintain membrane potentials. While these tend to be permanent within the infarct core, cells in the penumbra undergo repetitive depolarization that is an active energy-dependent process contributing to the increase in infarct size (Jovin et al., 2008). Glutamate acts as a mediator of this peri-infarct depolarization, or spreading depression, which originates from the marked disruption of ionic homeostasis, and causing acidosis and energy demand increases as well as neurotransmitter effluxes that commence at the core and propagate peripherally to involve the penumbral regions (Hossmann, 1996).

2.2.3 Oxidative stress

Following tissue ischemia, there is generation of reactive oxygen species (ROS) by mitochondria especially during reperfusion. In addition, reactive nitrogen species (RNS) such as nitric oxide (NO) and peroxynitrite (ONOO) are produced by NMDA receptor stimulation and inflammatory cells including microglia and monocytes. Together, these free radicals may be involved in activating several pathways of cell death such as apoptosis and inflammation, causing further tissue damage (Woodruff et al., 2011; Yenari, 2004).

2.2.4 Inflammation

Accumulation of inflammatory cells in the ischemic lesion occurs as a result of intracellular Ca^{2+} accumulation, increase in free radicals, hypoxia itself, as well as

during reperfusion (Jovin et al., 2008; Yenari, 2004). Pro-inflammatory cytokines are expressed de novo within 2 hours after ischemic onset and remain elevated for several days (Mitsios et al., 2006). Early inflammation further exacerbates ischemic injury for several days after onset through local activation of microglia (most important cellular component in post-stroke neuroinflammation (Thiel and Heiss, 2011)), astrocytes, endothelial cells and leukocytes, and subsequent release of various cytokines, chemokines, endothelial-leukocyte adhesion molecules and proteolytic enzymes (Jovin et al., 2008; Mitsios et al., 2006).

2.2.5 Apoptosis

Cell death following cerebral ischemia can be necrotic or apoptotic by histology – necrosis is the predominant mechanism that occurs in global ischemia as well as in the core of an ischemic lesion; while neurons at the periphery (penumbra) and in milder ischemia, show apoptotic features. (Mitsios et al., 2006; Yenari, 2004). Apoptosis is characterized by an ordered and tightly controlled set of changes in gene expression and protein activity resulting in neuronal cell death (Jovin et al., 2008), and is mediated by caspases. Caspases are a family of proteases that are proenzymes requiring cleavage to active forms leading to DNA cleaving and chromatin condensation which are hallmarks of apoptosis. (Mitsios et al., 2006; Yenari, 2004).

2.2.6 GABA signalling in peri-infarct cortex

Signalling in the brain occurs through 2 main γ-Aminobutyric acid (GABA)-ergic systems: phasic or synaptic GABA signalling is when an interneuron action potential depolarizes the presynaptic bouton, causing release of GABA and an effect on postsynaptic GABA receptors; whilst tonic or extrasynaptic GABA signalling respond to ambient GABA levels outside the synapse, and controls the overall membrane potential of the neuron and its propensity to fire (Carmichael, 2011; Glykys and Mody, 2007). Stroke causes an increase in tonic GABA currents for more than 1 month after the stroke. There is a substantial 50% increase in the tonic GABA current in peri-infarct motor neurons because of diminished GABA uptake by astrocytes in the peri-infarct cortex (Carmichael, 2011). Thus, on a physiological level, recovering peri-infarct motor cortex is hypoexcitable after stroke, and blocking tonic GABA currents may promote behavioural recovery after stroke (Clarkson et al., 2010).

The increase in tonic GABA inhibition after stroke occurs because of diminished GABA uptake and is not observed in studies of phasic or synaptic GABA signalling. Previous studies in brain inhibition after stroke have examined phasic or synaptic inhibition (Carmichael, 2011). For instance, synaptic inhibition as measured in brain slices by paired-pulse inhibition is decreased, particularly at 7 days after the stroke (Neumann-Haefelin et al., 1995), and may correspond to a reduced expression of GABA-A receptor subunits during this period (Sacco et al., 2009). Moreover, human studies using transcranial magnetic stimulation (TMS) looking at synaptic GABA

signalling demonstrate reduced paired-pulse inhibition in the early stages after stroke (Cicinelli et al., 2003) that may normalize (Wittenberg et al., 2007) with recovery.

Animal and human data indicate a possible contrast in the response to GABA systems after stroke: phasic GABA signalling is reduced in the first weeks after stroke, while tonic GABA signalling is potentiated.

3 NEUROPLASTICITY: DEFINITIONS AND HISTORICAL PERSPECTIVE

The concept of plasticity has been used for over a century. Santiago Ramon y Cajal, a Spanish neuroscientist, first speculated in 1894 that learning required formation of new neural connections (Stahnisch and Nitsch, 2002), followed later by Polish neuroscientist Jerzy Konorski (1948) and Canadian psychologist Donald Hebb (1949) who both proposed that the strength and effectiveness of neural synapses could change as a result of activity (Berlucchi and Buchtel, 2009). However, it was American psychologist William James who addressed the concept of neuroplasticity from a modern perspective suggesting that the human brain is capable of reorganization, in his *Principles of Psychology* (1890). Plasticity refers to a change in structure in response to an external force and the maintenance of that shape after removal of the force (as opposed to elasticity). In addition, the changes can reflect modifications of structure, operating principles or functions (Berlucchi and Buchtel, 2009). French neurophysiologist Jacques Paillard, in his 1976 paper published in the

first issue of the *Journal de Psychologie*, entitled *"Réflexions sur l'usage du concept de plasticité en neurobiologie*", furthered argued that the term plasticity is only appropriate in terms of the ability of a system to achieve novel functions, either by transforming its internal connectivity or by changing the elements of which it is made (Will et al., 2008).

When applied to the brain and nervous system, neuroplasticity is the ability to respond to intrinsic and extrinsic stimuli by reorganizing its structure, function and connections; and can be described at many levels, from molecular to cellular to systems to behaviour; and can occur during development, in response to the environment, in support of learning, in response to disease, or in relation to therapy (Cramer et al., 2011). This compensates for the limited ability of regeneration in the adult central nervous system (CNS), and includes axonal sprouting and dendritic arborization, increased synaptic efficiency (number and size), angiogenesis and neurogenesis (Onteniente and Polentes, 2011; Pekna et al., 2012). Neuroplasticity is the neurobiological basis for the ability to adapt and learn in an experience-dependent manner (Pekna et al., 2012). Plasticity can either be adaptive or maladaptive: adaptive plasticity refers to functional and structural changes that promote or improve function, whereas maladaptive forms of plasticity may result in pathogenesis of neurological disorders, such as those observed in dystonia and phantom-limb pain (Johnston, 2009; Quartarone et al., 2008).

3.1 Neuroplasticity in stroke recovery

The majority of motor function recovery following stroke has been observed to be most rapid during the first month, slower during subsequent months, and then plateaus by 6 months post-stroke (Schaechter, 2004). Following infarction, part of the recovery process involves resolution of the initial reversible pathophysiological events resulting from the brain injury, that include oedema, necrotic tissue, reperfusion of the ischemic penumbra through collateral circulation, and diaschisis (Butefisch, 2004; Furlan et al., 1996). Diaschisis refers to the compromised function and reduced activity of remote uninjured brain regions (both ipsilateral and contralateral) that are connected to the area of damaged tissue as result of hypometabolism, neurovascular coupling and aberrant neurotransmission (Cramer, 2008; Wieloch and Nikolich, 2006). The ensuing phases of recovery encompass activation of cell genesis and repair, changing the properties of existing neuronal pathways with the alternative use of spared circuits, and structural plasticity leading formation of new neuronal connections (Mohajerani et al., 2011; Pekna et al., 2012).

The ultimate functional outcome in stroke patients will depend on the initial lesion characteristics that include size and topography, as well the degree and efficacy of plasticity, the latter which underlies the capacity of the individual patient's brain to recover function (Stinear, 2010). It is the variability in neuroplasticity between individuals that likely accounts for the differences observed in stroke recovery amongst those with similar baseline clinical characteristics (Figure 2). Current

standard and novel rehabilitative therapies aim to facilitate or stimulate neuroplasticity, in order to further enhance the natural process of stroke recovery.

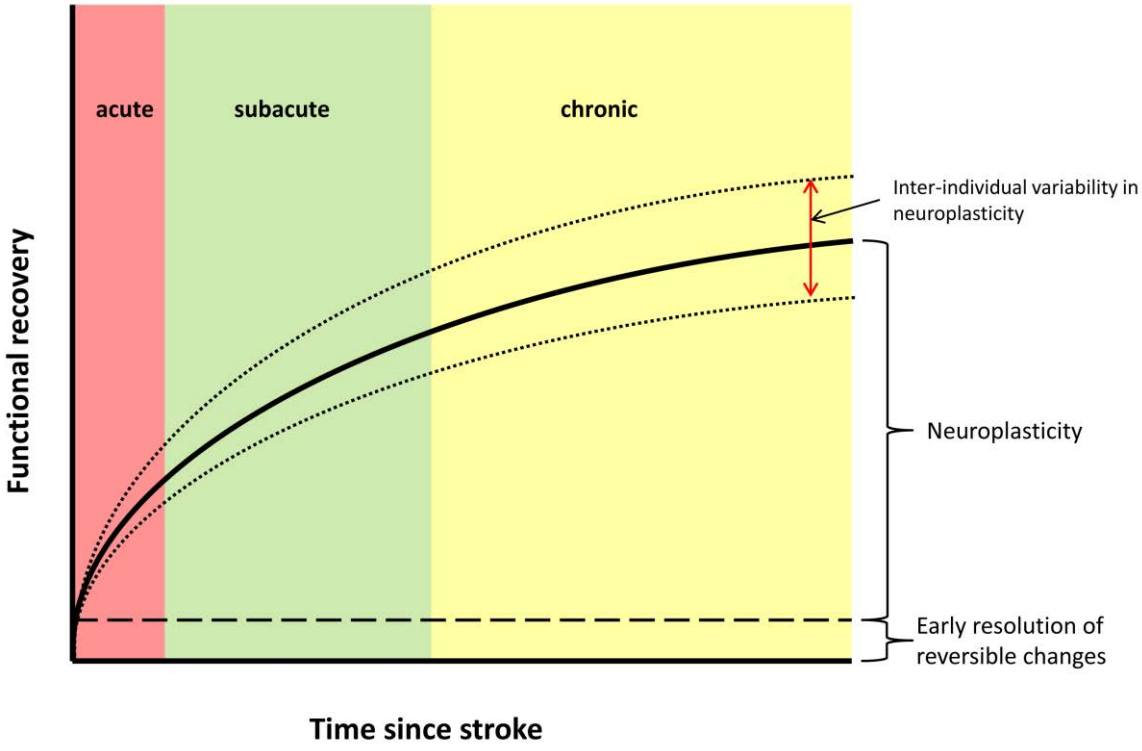


Figure 2. Functional recovery during the acute, subacute and chronic stages following stroke. The initial improvements observed immediately in the hyperacute period are secondary to resolution of reversible changes such as perilesional oedema, diaschisis, and reperfusion of the ischemic penumbra from collateral circulation. Following this, ensuing stroke recovery occurs with reorganization of neural networks in the brain (neuroplasticity), that varies between individual stroke patients and accounts for the heterogeneity in stroke outcomes observed even between patients with similar baseline clinical and lesion characteristics. Modulating the degree of neuroplasticity provides potential to further promote the functional recovery process, and is the basis of novel rehabilitative interventions.

4 MECHANISMS OF NEUROPLASTICITY AND STROKE RECOVERY

There are many parallels between the mechanisms of normal learning and memory and those of stroke recovery. The similarities between neuropsychological, brain imaging, cellular, and molecular aspects of learning and memory, and those of stroke recovery, suggest that the key elements in memory formation (the regulation and coding of brain excitability) may also play a role in stroke recovery (Carmichael, 2011). In addition to the similar principles underlying motor learning and stroke recovery on a neuropsychological level, similar events are also observed on a brain imaging level (Ward and Cohen, 2004). On a cellular level, learning and memory processes and recovery after stroke are both identified with changes in network excitability such as long-term potentiation (LTP) (Carmichael et al., 2005). The functional recovery following CNS lesions and the encoding of information during perceptual learning may invoke the same underlying mechanism mediated by long-range horizontal connections (Gilbert and Li, 2012). However, there are likely differences between de novo motor learning and relearning that follows from stroke recovery. Given that the primary motor cortex (M1) is a key structure for the storage of motor engrams, the mechanisms whereby novel motor memory is stored in the lesioned brain especially those involving M1 remains to be elucidated (Hosp and Luft, 2011).

4.1 Activity-dependent mechanisms in normal brains

A change in neural networks occurs in response to the normal process of learning. These changes are observed on different levels including gene and protein expression, synaptic modification, electrophysiological alterations and changes in cortical mapping (Kolb and Teskey, 2010). In humans and animal models, motor skill learning is accompanied by changes in the strength of connections within the primary motor cortex (Butefisch et al., 2000; Rioult-Pedotti et al., 1998; Ziemann et al., 2004). Motor cortical representations can reorganize rapidly in response to different stimuli with resultant improvement in behavioural responses. For example, a study demonstrated that taxi drivers had larger posterior hippocampi compared to controls that was proportional to the duration of their driving experience (Maguire et al., 2000), whilst the contralateral digit representation in M1 was significantly enlarged after learning finger sequences on the piano (Pascual-Leone et al., 1995).

The primary mechanisms mediating reorganization in the cerebral cortex involve the unmasking of existing, but latent, horizontal connections and modulation of synaptic efficacy such as long-term potentiation (LTP) or long-term depression (LTD) (Butefisch, 2004). LTP is a measure of the ability of an excitatory synapse to undergo long-term modification and is the key event underlying learning, memory, and the enhanced excitability that supports neuronal network modification by experience (Lynch, 2004). LTP is associated with an increase in dendritic length and spine density, whilst LTD is associated with a decrease in dendritic length and spine

density (Kolb and Teskey, 2010). LTP is enhanced in the immature brain compared to the adult brain (Crair and Malenka, 1995).

Although unmasking of horizontal connections provide means for rapid dynamic modulation of motor output zones in the primary motor cortex, modification of synaptic strength such as LTP and LTD provides more stable changes of these horizontal cortical connections. The neurotransmitter systems involved in mediating these effects include the inhibitory GABA-ergic system, as well as the excitatory glutamatergic system with activation of NMDA receptors (Butefisch, 2004). LTP in the motor cortex requires activation of NMDA receptors and down-regulation of GABA-ergic systems (Butefisch, 2004). Thus, reductions in GABA alone are necessary but may not be sufficient for plasticity. It has been identified that motor learning is accompanied in humans by a decrease in cortical GABA, and drugs blocking tonic GABA signalling promote neuronal excitability, enhance LTP formation, and lead to long-lasting enhancements in learning paradigms (Carmichael, 2011; Floyer-Lea et al., 2006). Furthermore, inhibitory GABA-ergic neurons show experience-dependent change, both in their dendrites and their axons (Gilbert and Li, 2012). The release of neuronal growth factor, brain-derived neurotrophic factor (BDNF) from electrically active neurons enhances LTP formation (Johnston, 2009).

4.2 Mechanisms in stroke recovery

4.2.1 The concept of functional connectivity

The brain consists of a complex network of cortical and subcortical areas in which neuronal populations interact with each other by both excitatory and inhibitory mechanisms, in a temporally and spatially specific fashion (Grefkes and Fink, 2011). In particular, activity in the primary motor cortex might be driven by facilitatory or inhibitory influences from premotor areas that themselves interact with activity in prefrontal, posterior-parietal or sensory regions. For this reason, a structural lesion resulting from stroke with its associated impairment in function, may disturb the complex balance of excitatory and inhibitory influences within the brain network, and affect the functional architecture of distributed nodes both intra- and interhemispherically (Grefkes and Fink, 2011). Furthermore, neurological deficits following a focal stroke lesion may reflect not only local dysfunction at the site of injury, but are also influenced by the distributed impairment of the neural network. For example, other than lesions affecting M1, lesions of the supplemental motor area, the premotor cortex, the thalamus and the parietal cortex, can also result in contralateral hemiparesis, suggesting that de-afferentation of M1 from these regions and the interruption of intracortical circuits, may induce functional inhibition of pyramidal neurons (Trompetto et al., 2000). Moreover, the functional architecture of brain reorganization after stroke is in part related to the nature of the organization of that function in the normal premorbid state, such as swallowing, facial movements, gait and proximal arm movements that are often bilaterally organized and thus functional changes may involve contralateral networks following

disturbances of these functions (Cramer, 2008). It is therefore crucial to move beyond segregated perspectives of brain function and characterize the lesioned brain as an integrated and reorganized functional network, which plays a key role in recovery of function after stroke (Westlake and Nagarajan, 2011).

4.2.2 Functional reorganization at a systems level

Following an acute stroke, there are functional changes that occur throughout the integrated neural network that may represent mechanisms of neuroplasticity and recovery (Takeuchi et al., 2010). These regions include the ipsi- and contralesional motor cortices, secondary motor areas and other cortical and subcortical structures of hemispheres, the crossed and uncrossed motor pathways, as well as peripheral motor axons. It may be that following stroke, diffuse connectivity together with the inherent redundancy in neuronal processing, allows for the recruitment of circuits over a wide network to mediate recovery. This wide-scale bihemispheric circuit level rearrangement could be the result of unmasking of latent neural networks and occurs in the absence of new structural connectivity (Mohajerani et al., 2011). Moreover, results of functional connectivity studies in stroke imply that recovery of motor function depends on reorganization processes within both hemispheres leading to enhanced inter-hemispheric connectivity (Grefkes and Fink, 2011).

4.2.2.1 Bihemispheric reorganization

4.2.2.1.1 Evidence from neuroimaging studies

Functional imaging permits the investigation of brain regions that may be active during the performance of a motor or "activation" task. Studies using positron emission topography (PET) or blood oxygenation level-dependent (BOLD) functional MRI (fMRI) evaluate the brain's hemodynamic response to such tasks, when brain areas with neurons actively involved in the activation task receive an increased supply of blood, thereby resulting in an increase in signal (Schaechter, 2004).

Results from such functional imaging studies in hemiparetic stroke patients have demonstrated task-related brain over-activation over and above normal controls during recovery, that were often bilateral, in sensorimotor, premotor, prefrontal, parietal and insular cortices, cerebellum, supplementary motor (SMA) and cingulate motor areas, (Calautti and Baron, 2003; Chollet et al., 1991; Cramer et al., 1997; Foltys et al., 2003; Marshall et al., 2009; Rehme et al., 2011b; Ward et al., 2003a; Ward et al., 2003b; Weiller et al., 1993) and that the recruitment of these brain regions may facilitate the stroke recovery process (Ward, 2005a), at least in the early stages of recovery (Rehme et al., 2011b). However, there continues to be contention regarding the role of these widespread changes in the more chronic phases of the recovery process, with a number of studies demonstrating their association with incomplete or poorer motor recovery (Chollet et al., 1991; Grefkes et al., 2008; Grefkes and Fink, 2012; Ward et al., 2003b). Moreover, there appears to be a negative correlation between the size of the brain activation in these

regions and stroke outcome (Ward et al., 2003a). The distribution and size of regional overactivations may also depend on the location of stroke (Luft et al., 2004).

In the ipsilesional hemisphere, there is recruitment of motor areas that were not making significant contributions to the lost function prior to the injury (Pekna et al., 2012). It has been suggested that stroke recovery is influenced by the integrity of the fast direct motor output pathways from M1 to motor neurons of the spinal cord and that patients who have the greatest disruption to this pathway, tend to have a poorer outcome (Ward, 2005a). In fact, task-related activation shifts from primary to secondary motor networks were observed in patients with the greatest functional disruption to the corticospinal tract (Ward et al., 2006). The non-primary motor system involving the premotor, supplementary motor, parietal and subcortical regions, has been suggested to be organized into a number of neural networks or loops, with its own projection from each region to the spinal cord motor neurons, as well as interactions with M1 (Strick, 1988; Ward, 2006). A recent study using tractography demonstrated that M1, premotor and supplemental motor had connections to the cerebral peduncle via the posterior limb of the internal capsule (Newton et al., 2006). Furthermore, projections from the premotor cortex to the reticular formation give rise to bilateral reticulospinal pathways to propriospinal premotoneurons (Benecke et al., 1991), and there is evidence suggesting that in stroke patients with greater damage to direct motor pathways, a greater degree of descending motor command is mediated via these propriospinal

projections (Mazevet et al., 2003; Stinear and Byblow, 2004). It is proposed that generation of a motor output following disruption to the primary pathway, will require an increase in signals via alternate pathways (monosynaptic and oligosynaptic), and that damage in one of these networks could at least, be partially compensated for by activity in another (Liu and Rouiller, 1999; Newton et al., 2006; Ward, 2005a). However, this compensation or substitution is unlikely to be complete as the alternate projections to the spinal cord neurons are less in number and less efficient (Maier et al., 2002).

In addition, cortical remapping frequently involve dorsal, ventral and posterior shifts of the ipsilesional M1 hand representation (Cramer, 2008; Pineiro et al., 2001; Weiller et al., 1993) most commonly in the face motor area (Cramer, 2008), as well as expansion in the ipsilesional primary somatosensory cortex (Calautti and Baron, 2003; Cramer, 2008; Roiha et al., 2011), that may be related to unmasking or disinhibition of silent connections and facilitate access to stronger connections with undamaged portions of the corticomotoneuronal pathway – representing an intrinsic redundancy of the neural network (Calautti and Baron, 2003; Ward, 2005a). It may also represent recruitment of neurons and connections that are not normally devoted to the task, likely a result of axonal sprouting and synaptogenesis (Calautti and Baron, 2003). The enlargements or size of the shift however, did not appear to correlate with the size of the infarct (Roiha et al., 2011) and may (Zemke et al., 2003) or may not correlate with ultimate outcome (Calautti et al., 2003).

It is postulated that these ipsilesional cortical map rearrangements represent functionally relevant changes for stroke recovery. Increased activity in ipsilesional secondary motor areas have been associated with improvements in upper limb function and gait during therapy (Johansen-Berg et al., 2002a), and that disruption of these regions using non-invasive brain stimulation impairs simple motor task performance in stroke patients but not controls (Fridman et al., 2004). Moreover, task-related activations in ipsilesional premotor regions, were shown to increase linearly as a function of increasing force of hand grip in those with incomplete recovery but not in control subjects (Ward et al., 2003b).

The contralesional primary and secondary motor, as well as other cortical areas, may represent potentially relevant substrate for stroke recovery (Westlake and Nagarajan, 2011), although the direct role of the contralesional hemisphere and its regional activations in recovery, remains controversial. Neuroimaging studies demonstrate increased activity of the contralesional hemisphere with movements of the paretic hand in stroke patients and is common regardless of lesion location (Boyd et al., 2010), but it is likely that the role of these changes in recovery and motor control will vary depending on time from stroke, severity of impairment, and lesion site (Hummel and Cohen, 2006).

The contralesional M1 can be viewed as another “secondary” node in the motor network that contributes to motor performance as required. In healthy individuals, ipsilateral motor cortex activations are observed during performance of unilateral finger-hand movements with increasing task complexity and/or difficulty (Krakauer

et al., 2004) potentially by modulation of transcallosal projections as well as increased utilization of ipsilateral pathways (Bradnam et al., 2013). These ipsilateral M1 activations are not observed with simple hand squeeze or grip tasks (Grefkes and Fink, 2011). In this context, it may be conceivable that execution of simple hand grip tasks in the paretic hand of stroke patients, are associated with enhanced neural activity observed on neuroimaging in the contralesional hemisphere in response to the perceived task difficult by virtue of the motor deficit caused by the stroke – representing a kind of procedural adaptation making use of available resources (Boyd et al., 2010; Calautti and Baron, 2003). In normal subjects, functional disruption to ipsilateral M1 resulted in impaired motor tasks involving the hand, suggesting a role of ipsilateral M1 in planning and execution of hand movement (Chen et al., 1997). Furthermore, ipsilateral M1 function has been shown to compensate for the acute dysfunction induced by repetitive TMS (rTMS) over the contralateral M1 (Strens et al., 2003), and disruptions to contralesional secondary motor areas were found to impair motor tasks in chronic stroke patients with greater deficits, but not controls (Johansen-Berg et al., 2002b), although contralesional M1 activity disruption by rTMS did not affect performance of the paretic hand in stroke patients in another study (Werhahn et al., 2003). However, rTMS may not be able to disrupt the deep posterior part of the contralesional M1 that may be contributing to recovery (Ward, 2004). Taken together, these suggest that functionally relevant recruitment does occur in certain contralesional regions in those patients with the greatest level of impairment (Ward and Cohen, 2004).

There continues to be debate regarding the temporal evolution of these overactivations observed in regions of both hemispheres, and how they correlate with recovery. Recent reviews and studies have suggested that restoration of neural activity in the ipsilesional M1 was the most important predictor of hand function recovery after stroke (Calautti and Baron, 2003; Carmichael, 2011; Cramer et al., 2002; Grefkes and Fink, 2011; Nair et al., 2007; Rehme et al., 2012; Ward et al., 2006; Werhahn et al., 2003). In particular, the initial task-related overactivations in motor and non-motor related areas in the brain are followed by reductions over time in patients with good recovery regardless of stroke location (Cramer, 2008; Jang et al., 2003; Jang et al., 2004), whilst persistent activation of contralesional networks was associated with poorer recovery and may indicate less efficient compensation (Calautti et al., 2001a; Calautti et al., 2001b; Feydy et al., 2002; Marshall et al., 2000; Ward et al., 2003b). The lesser recruitment over time in some of these regions may suggest that compensatory strategies and reliance upon redundant nodes in the neural network are required less as recovery proceeds (Calautti and Baron, 2003).

Other longitudinal studies in subcortical strokes have established early widespread activation to be most prominent in those with the greatest functional deficit, followed by a negative correlation between the size of activation and recovery over time in regions involving M1, secondary motor and other cortical areas in both hemispheres (Ward et al., 2003b). The increased recruitment of non-motor regions, such as the occipital cortex, may suggest that other modalities such as vision are

increasingly utilized in patients with the greatest deficit in an attempt to optimize task performance (Ward et al., 2003b; Ward et al., 2006).

In contrast, there are other studies that have shown persistent overactivation in the contralesional primary and secondary sensorimotor cortices in well recovered patients (Butefisch, 2004; Butefisch et al., 2005; Cramer et al., 1997; Gerloff et al., 2006; Marshall et al., 2009). The balance of brain activation after stroke may be governed by the integrity of ipsilesional sensorimotor cortex and its corticospinal tract (Newton et al., 2006; Ward et al., 2006). Those in whom the cortex is spared and the ipsilesional motor pathway integrity is sufficiently intact, good recovery may be mediated by normalization of activity back to the ipsilesional hemisphere. Of interest, studies have shown that approximately 20% of pyramidal fibres are sufficient to ensure restitution of fractionated hand-finger movements (Rossini and Dal Forno, 2004).

On the contrary, in patients with lesions involving the primary motor cortex and/or damaged ipsilesional corticospinal tract, persistent over-recruitment of contralesional networks may be required to achieve functional recovery (Cao et al., 1998; Chollet et al., 1991; Cramer et al., 1997; Feydy et al., 2002; Foltys et al., 2003; Murase et al., 2004; Schaechter, 2004; Ward et al., 2003b; Ward, 2006; Ward et al., 2006; Weiller et al., 1993). Functional relevance of the contralesional hemisphere involvement in stroke recovery is further supported by cases that report

impairment of the well-recovered limb following a second stroke affecting the previously intact hemisphere (Ago et al., 2003).

4.2.2.1.2 Evidence from electrophysiological studies

Transcranial magnetic stimulation (TMS) is a safe, painless and non-invasive technique used increasingly in investigations of brain plasticity and is a powerful tool for studying mechanisms of intracortical inhibition and excitation (Rossini and Dal Forno, 2004) as well as corticospinal physiology. The technique of paired-pulse TMS is particularly sensitive in activating cortico-cortical circuits, and provides information on cortical reorganization following brain injury (Ziemann et al., 1996). Widespread areas of intracortical hyperexcitability appear days after focal brain infarct in animal models that subsequently reduce over the ensuing months (Buchkremer-Ratzmann et al., 1996). Human studies utilizing TMS in stroke patients have demonstrated changes in cortical excitability in both hemispheres (Butefisch et al., 2003; Butefisch et al., 2008; Cicinelli et al., 2003; Liepert et al., 2000d; Liepert et al., 2000e; Manganotti et al., 2008; Shimizu et al., 2002; Swayne et al., 2008), that reflect changes in the intrinsic circuits of the cortex (Swayne et al., 2008), but there continues to be conflicting data regarding the relationship of these changes to the timing and location of stroke, and more importantly the evolution of these changes as a function of stroke recovery. Inconsistencies across studies may in part be a reflection of heterogeneity in patient and stroke characteristics (lesion location, chronicity of stroke and degree of impairment) as well as the cross-sectional design of most studies. The hyperexcitability may in fact represent the

electrophysiological correlate of the diffuse overactivations observed over both hemispheres in functional imaging studies of stroke patients. The BOLD signal as measured by fMRI primarily reflects input and processing within an area, such that a hyperexcitable cortex will result in an increase in and more diffuse BOLD signal (Logothetis et al., 2001; Ward and Frackowiak, 2006).

Immediately after stroke, there is reduced intracortical inhibition in the lesioned hemisphere (Butefisch et al., 2008; Cicinelli et al., 2003; Liepert et al., 2000e; Manganotti et al., 2008; Nardone and Tezzon, 2002; Swayne et al., 2008; Takeuchi et al., 2010) regardless of whether the stroke is cortical or subcortical in location (Butefisch et al., 2008; Takeuchi et al., 2010), that appears to persist or be associated with the recovery process (Butefisch et al., 2008; Liepert et al., 2000e; Manganotti et al., 2008; Wittenberg et al., 2007). In addition, short-interval intracortical inhibition (SICI) of the affected hemisphere was more reduced in chronic patients with better function, but was observed in the subcortical and not the cortical stroke groups (Takeuchi et al., 2010). Furthermore, in a longitudinal 6-month follow-up study, ipsilesional intracortical disinhibition persisted in association with clinical improvement over the duration of the study, with correlations between reduced SICI and worse clinical scores only present at 3 months, but not acutely or 6 months after stroke (Swayne et al., 2008).

Intracortical facilitation (ICF), also a measure of intracortical excitability that is likely mediated by glutamatergic processes, was found to be no different compared to

controls at any stage during the stroke recovery process in the affected hemisphere (Butefisch et al., 2003; Carmichael, 2011; Liepert et al., 2000e; Swayne et al., 2008). This is consistent with studies in rats that have demonstrated only transient alterations in NMDA neurotransmission following vascular lesions (Mittmann et al., 1994).

Intracortical inhibition is also reduced in the contralesional hemisphere following stroke (Butefisch et al., 2003; Butefisch et al., 2008; Liepert et al., 2000d; Liepert et al., 2000e; Manganotti et al., 2008; Shimizu et al., 2002) with some studies only observing such changes in those patients with cortical (Liepert et al., 2000d) but not subcortical lesions (Liepert et al., 2000e; Liepert et al., 2005; Shimizu et al., 2002), whilst others demonstrated changes in both location groups (Butefisch et al., 2003; Butefisch et al., 2008; Cicinelli et al., 2003; Manganotti et al., 2002; Manganotti et al., 2008). On the other hand, other studies have failed to demonstrate changes in intracortical inhibition in the contralesional hemisphere with group comparisons, but showed correlations with clinical parameters suggesting hyperexcitability in those with greater impairment (Swayne et al., 2008). Like the ipsilesional hemisphere, there were no observed alterations in contralesional ICF at any stage of the stroke recovery process (Carmichael, 2011; Liepert et al., 2000d; Swayne et al., 2008).

There continues to be much controversy and debate surrounding the functional relevance of hyperexcitability in the intact hemisphere pertaining to stroke

recovery. There are cross-sectional studies that have shown increased cortical excitability in patients with good recovery (Butefisch et al., 2008), whilst those without such changes in the contralesional hemisphere have poorer recovery (Butefisch et al., 2003; Shimizu et al., 2002). Findings from longitudinal studies however, have suggested that patients with good motor recovery tend to demonstrate normalization of these parameters with time, whilst persistently reduced intracortical inhibition in the contralesional motor cortex was observed in those with poorer recovery (Manganotti et al., 2008). There are suggestions that the cortical hyperexcitability in the intact hemisphere are an epiphenomenon secondary to release of interhemispheric inhibition (IHI) from the damaged hemisphere, and furthermore, that this contralesional excitability may be exerting an inhibitory influence via the transcallosal inhibition onto the lesioned hemisphere to impede recovery (Murase et al., 2004). Studies however, have demonstrated contralesional reductions in intracortical inhibition occurred independent of changes in IHI from the ipsilesional cortex (Butefisch et al., 2008) and that this IHI from lesioned M1 to the intact hemisphere did not differ from healthy controls (Murase et al., 2004). In addition, the contralesional cortical hyperexcitability did not appear to result in excessive IHI onto the lesioned hemisphere, at least in the acute period (1-6 weeks) after stroke (Butefisch et al., 2008). These results suggest that the contralesional changes are unlikely to be an epiphenomenon, but may in fact be an adaptive process that promotes functional recovery.

Intracortical hyperexcitability may serve as a functional substrate facilitating activity-dependent plastic changes after stroke that may be modulated by therapeutic intervention (Benali et al., 2008; Ward, 2004). A reduction in intracortical inhibition contributes to cortical reorganization by unmasking latent or silent networks and recruitment of ipsilesional and contralesional primary and secondary sensorimotor areas necessary for functional adaptation and recovery. Early hyperexcitability may allow for the increase in the amount of ipsilesional M1 that is activated to facilitate subsequent shifting of sensorimotor representation (Pineiro et al., 2001; Weiller et al., 1993) and access to undamaged fast corticospinal tracts (Ward and Frackowiak, 2006). Later on, the disinhibition may represent an adaptive response promoting access to residual motor output system and the recruitment of non-motor regions of the brain, thereby facilitating structural change and promoting recovery. However, in the more chronic stages of stroke when alternative motor networks become better established, this disinhibition may be less required (Marconi et al., 2011). Evaluation of excitatory and inhibitory circuits has revealed that the reduction in SICI is secondary to loss of inhibitory function rather than an epiphenomenon of shift towards excitation (Takeuchi et al., 2010). The reduction in inhibition and subsequent hyperexcitability is a result of down-regulations in GABA as well as decreases in GABA-ergic mechanisms (Neumann-Haefelin et al., 1995), thereby facilitating LTP which is likely fundamental in the process of cerebral reorganization and stroke recovery (Castro-Alamancos et al., 1995; Hagemann et al., 1998).

Whilst changes in intracortical hyperexcitability immediately after acute stroke are somewhat consistent across the majority of studies, measures of corticospinal excitability have demonstrated considerable variability. Acutely, the resting motor thresholds (RMT) in the lesioned hemisphere were raised in some studies (Caramia et al., 1996; Cicinelli et al., 2003; Liepert et al., 2000e; Manganotti et al., 2002; Wittenberg et al., 2007) but not in all (Delvaux et al., 2003), with a lower RMT associated with better outcomes (Cicinelli et al., 1997; D'Olhaberriague et al., 1997; Heald et al., 1993; Trompetto et al., 2000) and correlated with grip strength (Thickbroom et al., 2002). The presence of motor evoked potentials (MEPs) in the ipsilesional hemisphere was also associated with better recovery compared to those with absent responses (Cruz Martinez et al., 1999; Delvaux et al., 2003; Escudero et al., 1998; Heald et al., 1993; Pennisi et al., 1999; Rapisarda et al., 1996; Trompetto et al., 2000), with some further describing most improvements seen in those with greater MEPs (Cicinelli et al., 1997; D'Olhaberriague et al., 1997; Heald et al., 1993) with correlations between size and grip strength (Thickbroom et al., 2002). However, there are patients with good outcomes that initially had no demonstrable MEPs (Butefisch, 2004; Escudero et al., 1998; Trompetto et al., 2000). Overall, MEP and RMT are measurements of the functional integrity and excitability of corticospinal pathways (Stinear, 2010; Swayne et al., 2008), with the ipsilesional excitability typically reduced following stroke and normalizing over time (Murase et al., 2004).

In addition, ipsilesional measures of corticospinal tract integrity were only correlated with clinical scores acutely after stroke, likely a reflection of motor impairment at time of testing (Talelli et al., 2006), and this association diminished at the more chronic stages, whilst intracortical inhibition was correlated with clinical parameters more so in the chronic phases of stroke compared to the immediate stages (Swayne et al., 2008). This suggests that acutely, the degree of functional impairment following stroke is dependent on the amount of damage to the primary motor pathways, whereas in the later stages of recovery, the impairment will be determined by the degree of cortical reorganization that may involve the recruitment and utilization of alternate pathways for motor output generation.

On the contralesional hemisphere, RMT and MEP parameters are generally unchanged (Butefisch et al., 2003; Cicinelli et al., 2003; Liepert et al., 2000d; Manganotti et al., 2002; Shimizu et al., 2002).

4.2.2.2 Ipsilateral motor pathways

Ipsilateral descending motor control is mediated by both direct and indirect pathways (Bradnam et al., 2013). Approximately 10% of corticospinal pathways originating from the ipsilateral M1 are uncrossed at the medullary decussation and project directly onto spinal motoneurons (Davidoff, 1990; Jang, 2009), whilst the indirect pathways descend from the ipsilateral M1 to reticular neurons in the brainstem and then onto cervical spinal cord motoneurons, constituting the cortico-reticulo-propriospinal pathway (CRPP) (Bradnam et al., 2013). Although such

ipsilateral M1 projections are thought to usually control axial and proximal muscles with little contribution to distal motor function in normal subjects (Brinkman and Kuypers, 1973), these properties may change under certain circumstances such as stroke and provide a means for functional recovery (Mohajerani et al., 2011; Ward, 2006).

In a study on cats, unmasking of ipsilateral M1 pathways was evident after treatment with 4-aminopyridine (a potassium channel blocker) (Jankowska et al., 2005). Other animal studies have shown corticospinal tracts from each motor cortex initially projecting bilaterally onto spinal cord anterior horn cells that subsequently become pruned according to neuronal activity and maturity, resulting in final contralateral predominance. And with experimental hemispheric silencing, axons are retracted on the contralateral side of the spinal cord ventral horn and ipsilateral fibres from unaffected hemisphere predominate (Eyre, 2007). In human studies, structural imaging has documented hypertrophy of ipsilateral corticospinal tract from contralesional hemisphere in cerebral infarcts in infants (Johnston, 2009). This hypothesis is further supported by electrophysiological studies demonstrating the presence of ispilateral MEPs from the contralesional M1 to distal hand muscles in patients with good stroke recovery, that are not normally present in control subjects (Caramia et al., 1996; Trompetto et al., 2000; Wassermann et al., 1994). Other studies have further demonstrated up-regulations of the indirect cortico-reticulo-propriospinal pathways (CRPP) after stroke with compromise to the corticospinal tract (Stinear and Byblow, 2004), and hypothesized that whilst

contralesional CRPP may interfere with residual descending inputs from the ipsilesional cortex in patients with minimal damage to the corticospinal tract and hence negatively impact recovery, contralesional CRPP pathways may provide the only descending cortical drive to the spinal cord in the presence of extensive damage to the corticospinal tract (Bradnam et al., 2013). Activation of such ipsilateral pathways may have come about through the unmasking of existing connections via synaptic reorganization or the creation of new ones through sprouting and synaptogenesis (Caramia et al., 1996). On the other hand, there are also studies that have shown the presence of ipsilateral MEPs from the contralesional M1 in patients with poor outcome (Netz et al., 1997; Turton et al., 1996). Furthermore, a study using fMRI and TMS could not demonstrate the presence of an ipsilateral corticomotoneuronal pathway from the unaffected activated hemisphere to the affected hand in stroke patients (Foltys et al., 2003). This study, however, was in the relatively early phase after stroke (14 days), thus it may be possible that recruitment and development of ipsilateral motor pathways occur at a later stage.

4.2.3 Cellular and molecular mechanisms of plasticity in stroke

4.2.3.1 Neurogenesis and growth factors

Evidence suggests that neurogenesis persists in regions of the adult brain beyond the fetal period, including the subventricular zone of the lateral ventricles and subgranular zone of the dentate gyrus in the hippocampus, and these areas may be substrates for neuronal repopulation following stroke (Pekna et al., 2012; Toni et

al., 2008). Adult neurogenesis is regulated by extracellular signals that include growth factors, neurotransmitters, hormones, extracellular matrix components and cytokines, with the expression and release of these agents being modified following stroke to promote neurogenesis (Onteniente and Polentes, 2011).

Brain-derived neurotrophic factor (BDNF) is the most abundant factor in the brain and affects neuroplasticity both directly, through modulation of cellular processes, and indirectly, through its modulation of other factors involved in the process of neuroplasticity (Pearson-Fuhrhop and Cramer, 2010). BDNF can induce lasting changes in synaptic plasticity, neurotransmitter and neuropeptide production, and excitability, as well as facilitating LTP and mediating use-dependent plasticity (Pearson-Fuhrhop and Cramer, 2010). In rodent stroke models, BDNF levels have been associated with CNS repair (Comelli et al., 1992) and angiogenesis (Qin et al., 2011), and that treatment with exogenous BDNF was associated with better motor recovery (Schabitz et al., 2007).

Growth factors such as transforming growth factor-β or fibroblast growth factor-2 and chemokine stromal cell-derived factor-1 are overexpressed in the penumbra, and are involved in the recruitment bone-marrow derived cells and neural stem cells to sites of ischemic injury (Issa et al., 2005). It is thought that stromal cell-derived factor-1 may play a role in promoting plasticity following brain ischemia (Hill et al., 2004).

4.2.3.2 Synaptic plasticity and changes in neurotransmission

Widespread cortical hyperexcitability following stroke is mediated by alterations in the balance between inhibitory (GABA-ergic) and excitatory (glutamatergic) neurotransmission that result in functional reorganization of cerebral networks, ultimately leading to structural reorganization that is conducive to motor and functional recovery. Greater plasticity is associated with greater degrees of excitability, whilst enhanced inhibition is associated with impaired plasticity (Benali et al., 2008). These changes occur in regions structurally connected to the lesion in both hemispheres and are due to down-regulation of GABA and GABA-ergic inhibition (Neumann-Haefelin et al., 1998).

Magnetic resonance spectroscopic (MRS) studies in stroke patients have shown reductions in GABA levels in both the ipsi- and contralesional hemispheres at the acute and subacute periods (Glodzik-Sobanska et al., 2004). Furthermore, immunohistochemistry studies have identified reduced GABA-A receptors in both hemispheres of patients in the acute and chronic stages of stroke (Neumann-Haefelin et al., 1998; Sacco et al., 2009). Animal models have demonstrated widespread decreases in the levels of GABA-ergic intracortical inhibition (Hagemann et al., 1998) and associated reductions in in cortical inhibitory interneurons mediating this GABA-ergic neurotransmission in ipsi- and contralesional regions (Buchkremer-Ratzmann et al., 1996; Imbrosci and Mittmann, 2011; Neumann-Haefelin and Witte, 2000; Zeiler et al., 2013) following focal cortical lesions that were a result of recovery rather than the infarct itself (Zeiler et al., 2013).

Studies have demonstrated that reductions in GABA-mediated inhibition below certain thresholds, parallel augmentation in plastic properties of cortical networks (Imbrosci and Mittmann, 2011). Reduced GABA-ergic transmission may act as a permissive substrate allowing sensory experience to remodel structure and functions of cortical networks (Imbrosci and Mittmann, 2011). Moreover, GABA blockade and the resultant reduced GABA inhibition facilitate LTP (Castro-Alamancos et al., 1995; Hagemann et al., 1998), a mechanism fundamental to synaptic plasticity and hence cortical reorganization governing recovery after stroke. Other studies that have supported the role GABA-ergic down-regulation in stroke recovery, have shown that introducing a selective GABA receptor antagonist in the chronic stages of stroke in rats resulted in motor recovery (Clarkson et al., 2010), whilst another study that involved administration of a GABA-ergic drug (midazolam) in chronic stroke patients led to the re-emergence of stroke deficits (Lazar et al., 2002).

Up-regulations in glutamatergic neurotransmission and their associated NMDA and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors may also contribute to the observed cortical hyperexcitability following stroke, and have positive influences on recovery through LTP mechanisms (Arai and Kessler, 2007). These were also observed in both the ipsi- and contralesional hemispheres (Hagemann et al., 1998; Witte, 1998).

The lesion-induced reductions in inhibition that occur in the peri-infarct and more remote regions including the opposite hemisphere, subsequently allows for unmasking of previously silent networks and by converting these to functional networks is per se a mechanism of functional reorganization and cortical map plasticity (Imbrosci and Mittmann, 2011). This process permits surviving neurons remote but connected to the damaged area to acquire information previously processed by the damaged tissue. Following this, LTP and LTD processes are required for further reinforcement of relevant inputs (Hagemann et al., 1998) and masking of improper inputs (Allen et al., 2003) respectively, leading to final structural modification to stabilize the new connectivity patterns (Imbrosci and Mittmann, 2011) by way of enhanced synaptic efficiency and synaptogenesis.

Terminal axonal sprouting, dendritic branching and synaptic numbers are enhanced in both the ipsi- and contralesional hemispheres in animal models of stroke (Hermann and Chopp, 2012; Jones and Schallert, 1992; Jones and Schallert, 1994; Jones et al., 1996; Stroemer et al., 1995). This occurs within days after a lesion and then followed by the pruning back of connections over time (Brown et al., 2010; Ward and Frackowiak, 2006). In addition, remodelling of transcallosal projections connecting the two motor cortices occur with homologous fibres originating from the contralesional cortex growing out across the midline to reach denervated neurons in the target structures of the lesioned hemisphere (Hermann and Chopp, 2012). Projections from the ipsilesional supplemental motor area to the

contralateral spinal cord were also observed to increase following stroke involving M1 (McNeal et al., 2010).

Up-regulation of growth-promoting genes and down-regulation of growth-inhibiting genes in the perilesional regions (Carmichael et al., 2005; Urban et al., 2012) also contribute to the formation of axonal projections that are specific to reorganization after stroke and differs from axonal growth during development or recovery from peripheral nerve injury (Hosp and Luft, 2011).

4.2.4 Genetic influences

Genetic factors can strongly influence neuroplasticity and subsequent stroke recovery (Pearson-Fuhrhop et al., 2012). For instance, common polymorphisms of the gene for brain-derived neurotrophic factor (BDNF) are associated with a reduction in neuroplasticity and motor learning in healthy adults. Variations in genotype could, therefore, have important roles in recovery (Stinear, 2010) and partly account for the variability in functional recovery between individual stroke patients or their response to pharmacological or training-based interventions (Sharma et al., 2013). In particular, individuals with the val66-met polymorphism, have impaired electrophysiological responses to motor training (Kleim et al., 2006) and non-invasive brain stimulation (Cheeran et al., 2008) following stroke. This highly studied genetic variant occurs when a valine is replaced with methionine at position 66, resulting in 18%-39% less activity-dependent secretion of BDNF protein (Chen et al., 2004; Egan et al., 2003), with approximately 30%-50% of the

population either homozygous or heterozygous for this polymorphism (Pearson-Fuhrhop and Cramer, 2010). Furthermore, patients with the ApoE4 allele and BDNF Met allele were associated with poorer recovery and greater disability after stroke (Cramer and Procaccio, 2012).

Other candidate polymorphisms that may be associated with variations in neuroplasticity include the genes coding for catechol-O-methyltransferase (COMT), serotonin transporter and other proteins involved in the modulation and regulation of neurotransmission (Pearson-Fuhrhop et al., 2009; Sharma et al., 2013). In particular, a valine-to-methionine change at position 108/158 of the COMT enzyme results in a protein with 3-4 times less enzymatic activity and differences in recovery following neural injury (Lotta et al., 1995). Polymorphism in the KIBRA gene, which interacts with proteins involved with synaptic plasticity and LTP consolidation, may also influence variations in neuroplasticity (Floel and Cohen, 2010).

Recent evidence has also suggested the role of inflammation-related genes in stroke recovery including single nucleotide polymorphisms in interleukin-4, interleukin-10, and cyclo-oxygenase-2 (COX-2) gene (Maguire et al., 2011; Marousi et al., 2011). A novel polymorphism involving the gene for tissue-type plasminogen activator (tPA) may also be highly involved with neurotransmission and post-stroke plasticity (Samson and Medcalf, 2006).

5 MODULATION OF NEUROPLASTICITY IN STROKE

Studies in animal models have demonstrated that environmental, behavioural and pharmacological manipulation can influence cerebral reorganization and the process of stroke recovery (Ward and Cohen, 2004), through their effect on specific therapies rather than a direct effect on recovery alone. Specifically, these approaches are capable of conditioning the brain and temporarily enhance its responsiveness to afferent stimulation, an optimal state for driving cerebral reorganization (Ward, 2005b). Such environmental enrichment is associated with improved outcome, greater brain weight, altered growth factor expression, increased neurogenesis and increased dendritic branching and synapses (Cramer, 2008; Ohlsson and Johansson, 1995).

5.1 Neurorehabilitation

The ability of training and physical activity to restore motor function after neuronal injury has been long appreciated and has formed the basis for neurorehabilitation in stroke patients (Dimyan and Cohen, 2011), and is focussed on the concept of brain reorganization and neuroplasticity (Roiha et al., 2011). The current gold standard therapies for post-stroke rehabilitation comprise a combination of task-specific and general aerobic exercises (Dimyan and Cohen, 2011).

On a systems level, the shrinkage of hand muscle representation in M1 following stroke reversed following 8-10 weeks of rehabilitation training and correlated with improved motor function (Nudo et al., 1996; Traversa et al., 1997), but cortical map

shrinkage occurred despite training if therapy was commenced after a month of the acute event, indicating a critical time window in which cortical reorganization in M1 networks can be significantly influenced (Barbay et al., 2006). Furthermore, an increase in ipsilesional MEP amplitudes and expansion of the representation of the paretic hand were observed in response to rehabilitation and other therapeutic interventions in subacute and chronic stroke patients (Liepert et al., 2000b; Marconi et al., 2011), even after only a single therapy session that correlated with clinical improvement (Liepert et al., 2000b). In addition, task-specific or task-oriented training produced motor gains that were associated with normalization of sensorimotor cortex lateralization (Carey et al., 2002) and an increase in ipsilesional activation (Nelles et al., 2001) observed on functional imaging. Of interest, functional and connectivity studies demonstrated increased task-related brain activity in cortical and subcortical regions bilaterally following long term motor practice in chronic stroke patients, whilst these regions showed reductions following the same practice in controls (Bosnell et al., 2011). Moreover, these overactivations occurred in regions with reduced structural connectivity (Bosnell et al., 2011), and are consistent with other studies showing increased regional brain activation relevant to the task in patients undertaking rehabilitative interventions associated with good outcomes (Johansen-Berg et al., 2002a; Liepert et al., 2000a). This highlights the difference between motor practice in stroke patients compared to healthy control, in that a regional increase in functional efficiency occurs with controls whilst stroke patients show an increase in functional recruitment of regions that are structurally compromised (Bosnell et al., 2011).

On a cellular level, animal models of ischemia have revealed an increased synthesis of proteins in the somatosensory cortex that are implicated in neuroplasticity in those involved in skilled versus unskilled training (Pagnussat et al., 2012). These comprise neurotrophic factors such as nerve growth factor (NGF), fibroblast growth factor (FGF-2), and brain-derived neurotrophic factor (BDNF) (Carmichael, 2006; Kolb and Teskey, 2010) that stimulate neural sprouting, synaptogenesis and dendritic branching (Jones et al., 1999; Stroemer et al., 1995) and synaptic potentiation (Adkins et al., 2006).

Despite this, approximately 50%-60% of stroke patients continue to have some degree of motor impairment following standard rehabilitation (Schaechter, 2004), and even with intensive task-specific training, 15%-30% are permanently disabled (Dimyan and Cohen, 2011). This therefore calls for the development of more novel rehabilitative strategies to enhance the neuroplastic process and promote further stroke recovery.

5.2 Novel strategies for neurorehabilitation

5.2.1 Non-invasive brain stimulation

The two cerebral hemispheres are functionally coupled and balanced. The balance is maintained and controlled by mutual interhemispheric inhibition. Following stroke, the interhemispheric balance may be altered such that activity in contralesional M1 influences negatively on the recovery in some patients by contributing to abnormal interhemispheric interactions during movement of the

paretic hand (Grefkes et al., 2008; Murase et al., 2004; Rehme et al., 2011a; Westlake and Nagarajan, 2011). Based on this interhemispheric model, motor deficits are thought to be due to reduced output from the lesioned hemisphere or excessive interhemispheric inhibition from the contralesional to the lesioned hemisphere (Murase et al., 2004; Takeuchi and Izumi, 2012) (Figure 3). Further to this, a suggested model to improve function is the up-regulation of cortical excitability in M1 of the lesioned hemisphere and/or down-regulation of excitability in M1 of the intact hemisphere (Hummel and Cohen, 2006).

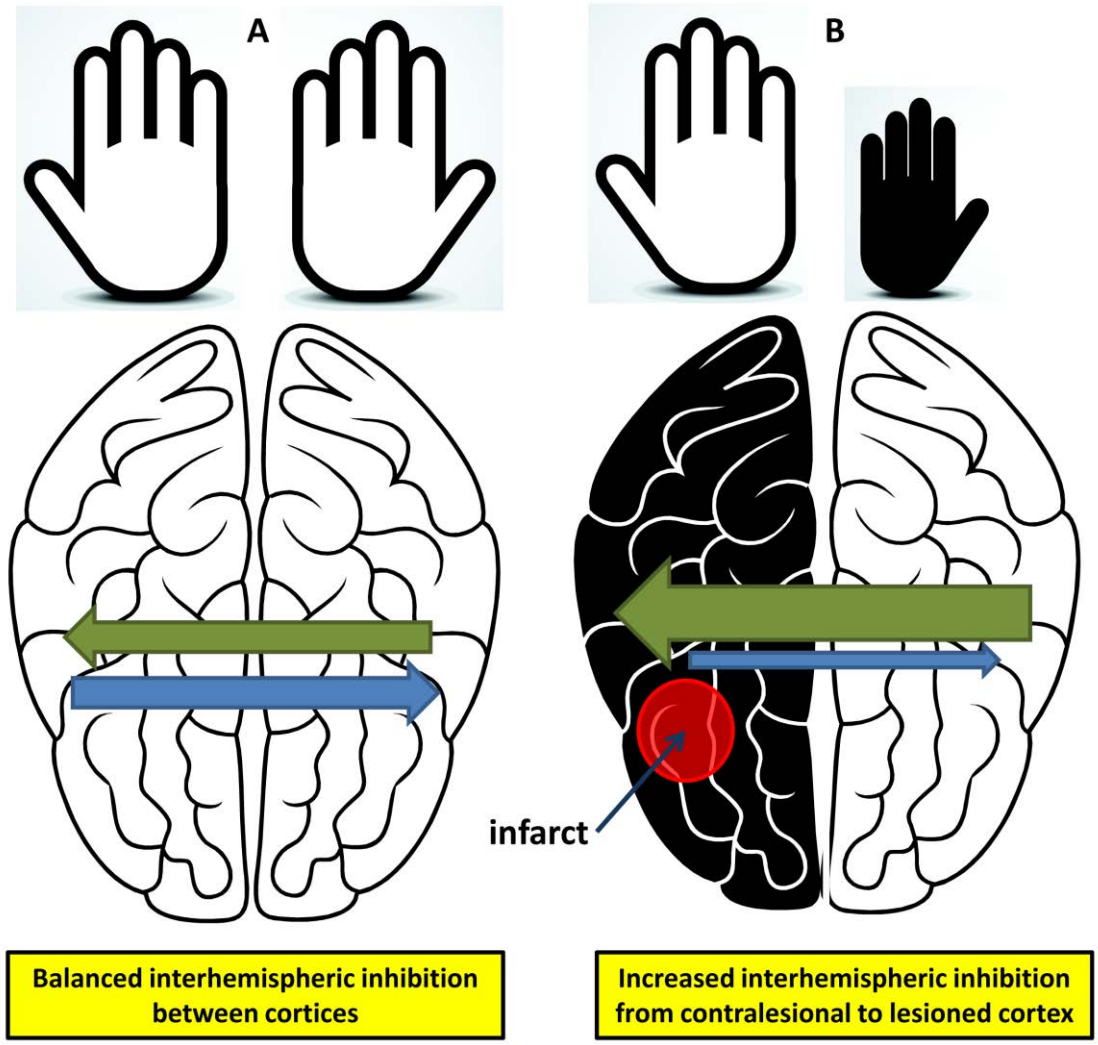


Figure 3. Interhemispheric inhibition model. (A) In normal subjects, the 2 hemispheres are functionally coupled and balanced by an approximately equal amount of interhemispheric inhibition exerted by one motor cortex onto the other; (B) In stroke subjects, the interhemispheric interactions are altered such that the lesioned hemisphere now exerts less inhibition onto the opposite side, resulting in abnormally enhanced inhibition from the contralesional to the ipsilesional motor cortex, consequently leading to reduced output to the paretic limb and impairing recovery.

This has led to the advent of non-invasive brain stimulation (NIBS) using either repetitive TMS (rTMS) or transcranial direct current stimulation (tDCS), which are powerful methods with the potential of modulating neuroplasticity and thereby human brain function in stroke patients. TMS acting as both a neurostimulator and neuromodulator is a painless procedure that can up- or down-regulate cortical excitability of neural structures under the stimulating coil depending on stimulation parameters (Hummel and Cohen, 2006; Pekna et al., 2012). On the hand, tDCS acting as a neuromodulator, polarizes brain regions through non-invasive application of weak direct currents that elicit focal reversible shifts in cortical excitability depending on the polarity, strength and duration of stimulation (Hummel and Cohen, 2006) and can induce sustained changes in neural membrane potential (Pekna et al., 2012).

Different neurotransmitters and neuromodulators such as GABA, glutamate, dopamine and serotonin are altered in defined regions of the brain after stimulation with both repetitive transcranial magnetic stimulation and tDCS (Pekna et al., 2012). Further to this, early genes like c-fos and genes coding for neurotrophic factors like BDNF have shown to be expressed in the rat brain after repetitive transcranial magnetic stimulation (Bolognini et al., 2009; Dimyan and Cohen, 2011). There is a growing body of evidence that has demonstrated that active stimulation of either the ipsilesional or contralesional motor cortex in combination with physical and occupational therapy improves motor outcomes after stroke (Figure 4).

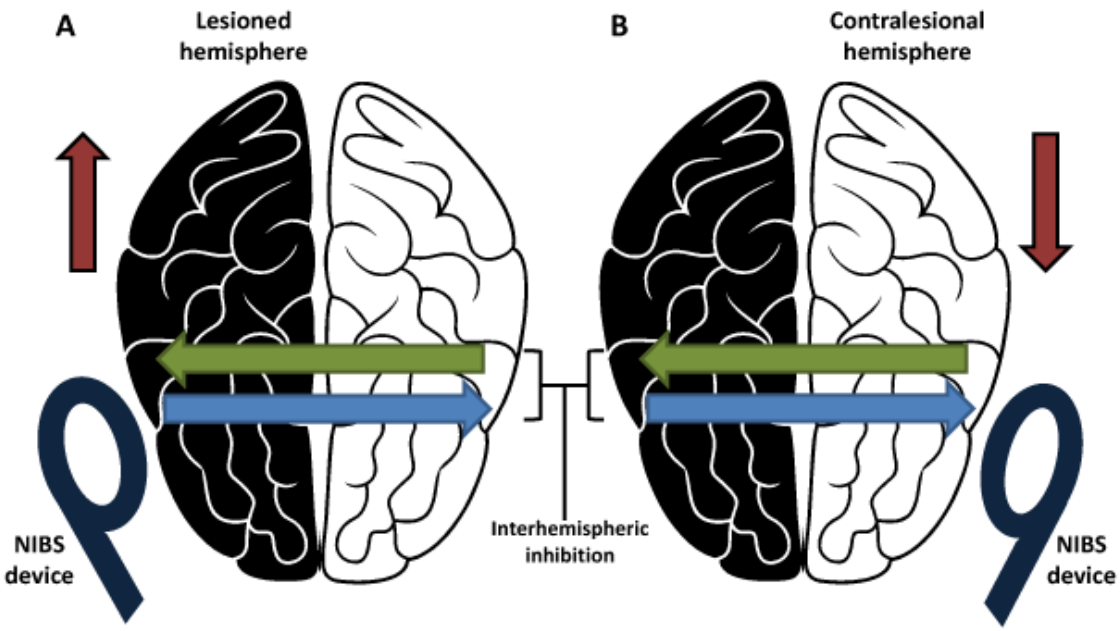


Figure 4. Principles of non-invasive brain stimulation (NIBS) in post-stroke recovery. Normalizing interhemispheric inhibition between the two motor cortices is achieved either by: (A) up-regulation of cortical excitability in the lesioned hemisphere; or (B) down-regulation of cortical excitability in the contralesional hemisphere, using either excitatory or inhibitory stimulation respectively, with repetitive transcranial magnetic stimulation or transcranial direct current stimulation.

5.2.1.1 Up-regulation of excitability in the ipsilesional hemisphere

Two non-invasive strategies have been used to increase excitability in the affected hemisphere: anodal tDCS and rapid-rate rTMS. The facilitated ipsilesional M1 may improve paretic hand function by increasing excitability of descending projections to alpha-motoneurons (Ackerley et al., 2010; Boggio et al., 2007; Bradnam et al., 2013; Kim et al., 2010; Stagg et al., 2012). Neuroimaging correlation using fMRI has shown increased cerebral blood flow over the lesioned hemisphere following NIBS that may be associated with the facilitation of neural plasticity after stroke (Takeuchi and Izumi, 2012)**.**

Anodal tDCS delivered to M1 of the lesioned hemisphere was studied in patients with chronic stroke in sham-controlled double-blind crossover experimental designs. These studies showed improvements in the performance of motor tasks with treatment but not with sham stimulation that lasted for more than 30 minutes after the end of the stimulation period. Behavioural gains were accompanied by enhanced cortical excitability and reduced intracortical inhibition within the lesioned hemisphere, suggesting the involvement of glutamatergic and GABA-ergic neurotransmission as possible operating mechanisms (Hummel et al., 2005; Hummel and Cohen, 2006). Moreover, repeated sessions over several days prolonged the behavioural gains (Boggio et al., 2007; Khedr et al., 2009). Khedr and colleagues applied rTMS daily over the affected M1 combined with customary rehabilitative treatment for 10 days within the first 2 weeks after stroke, and reported motor improvements with treatment relative to sham that lasted for at

least 10 days after the end of the therapy (Khedr et al., 2005). Furthermore, it was demonstrated that functional rTMS (during voluntary muscle contraction) produced greater cortical hyperexcitability compared to resting rTMS (Massie et al., 2012).

5.2.1.2 Down-regulation of excitability in the contralesional hemisphere

Studies on healthy individuals using inhibitory rTMS to one motor cortex resulted in improvements in motor performance in the ipsilateral hand (Kobayashi et al., 2004), illustrating the principle of reducing inhibition from one hemisphere to the other. Recently, NIBS and in particular low-frequency rTMS, has been used to promote functional recovery of stroke patients by suppressing the contralesional intact motor cortex, thus reducing interhemispheric inhibition. Studies on well-recovered stroke patients have revealed improvements in hand function (Boggio et al., 2007; Fregni et al., 2005; Khedr et al., 2009; Kim et al., 2010; Mansur et al., 2005; Nowak et al., 2008; Takeuchi et al., 2005) especially when delivered prior to physical therapy (Avenanti et al., 2012), whilst results were more equivocal in those with more severe impairments (Ackerley et al., 2010; Bradnam et al., 2013; Talelli et al., 2012; Theilig et al., 2011), with some studies even showing a deterioration in motor performance of their paretic limb, suggesting that contralesional activity may be functional relevant for some patients, especially those with significant disruption to ipsilesional corticospinal tract integrity and poorer recovery (Ackerley et al., 2010; Bradnam et al., 2012). In particular, the prevailing view of interhemispheric imbalance and the need to suppress contralesional excitability to redress this imbalance, has not considered effects on output pathways other than transcallosal

projections (Bradnam et al., 2013) as well as potential deleterious effects by suppressing ipsilateral cortico-reticulo-propriospinal pathways from the contralesional M1 that may be important for functional recovery in those patients with more damaged ipsilesional corticospinal tracts.

5.2.1.3 Bihemispheric non-invasive brain stimulation

Some studies have suggested that bilateral NIBS is more effective than unilateral stimulation of either the ipsi- or contralesional motor cortices alone (Takeuchi and Izumi, 2012). Sham-controlled bihemispheric tDCS in chronic stroke patients with residual moderate to severe hemiparesis has shown significantly greater improvements in motor function in the treatment group with simultaneous physical therapy compared with a control group receiving physical therapy alone (Lindenberg et al., 2010).

5.2.1.4 Clinical factors and implications

There remains uncertainty in regards to factors that may influence the effectiveness of NIBS. These include (Hummel and Cohen, 2006; Takeuchi and Izumi, 2012; Ward et al., 2006): (i) the age of the stroke patient; (ii) the stage of stroke – whether NIBS is more effective in the acute or chronic stages as recent studies have suggested that inhibitory NIBS over the contralesional M1 did not facilitate recovery in acute stages (Seniow et al., 2012); (iii) lesion site and the reorganized neural network structure – uncertainty pertaining to delivery depending on cortical or subcortical as well as integrity of corticospinal pathways and patterns of network activation; (iv)

severity of impairment – it is not certain whether NIBS is beneficial across all severities of stroke, as most studies had recruited patients with only mild to moderate disability; and (v) the timing of NIBS delivery in conjunction with physical or other rehabilitation strategies. Such variability highlights the importance that NIBS is not a "one size fits all" treatment. In addition, the optimal stimulation parameters such as the intensity, duration and frequency of stimulation need further clarification, which may in turn address the transient nature of clinical responses observed in most studies using NIBS.

5.2.2 Constraint-induced movement therapy

Constraint-induced movement therapy (CIMT) is centred on 4 principles: the non-use hypothesis, massed practice, shaping and behaviourally-relevant treatment settings (Meinzer et al., 2012). Non-use of an affected (paretic) limb develops during the first months post-stroke when physiologic damage results in depression of function and failure to effectively use the affected extremity (non-use hypothesis) (Taub et al., 2006). This non-use can be overcome by creating situations that induce patients to reuse a paretic limb. In CIMT, this is achieved by constraining the use of the unaffected limb by a sling or splint over an extended time period and training of the affected limb is "induced" over a period of 2 or more consecutive weeks for several hours a day (massed practice principle). Moreover, depending on treatment success, the difficulty of the required motor activity is gradually enhanced (shaping principle) and the training is performed in a behaviourally

relevant setting, in which patients are trained in activities relevant to their everyday life.

CIMT has shown promise with 2 weeks of therapy producing gains that remained significant for 2 years in both acute and chronic stroke patients (Park et al., 2008; Weiss et al., 2011; Wolf et al., 2006; Wolf et al., 2008). Several lines of evidence have identified the induction of functionally relevant reorganization of brain networks supporting motor functions following CIMT. Using TMS it was demonstrated that motor performance of chronic stroke patients improved substantially, together with an increase in motor output area size and MEP amplitudes, indicating enhanced neuronal excitability in the damaged hemisphere (Liepert et al., 1998). On a neuroimaging level, positive correlations between paretic handgrip strength and increased activation in ipsilesional motor and secondary somatosensory areas were observed using fMRI after CIMT (Johansen-Berg et al., 2002a; Kononen et al., 2012; Rijntjes et al., 2011), whilst other studies have shown shifting of activity towards contralesional sensorimotor cortices in chronic patients, suggesting that decreased use of the unaffected limb (in CIMT) may contribute to a relative increase in representation of the paretic limb in contralesional regions (Kopp et al., 1999; Levy et al., 2001; Schaechter et al., 2002; Schaechter, 2004). Other imaging studies have demonstrated significant increases in grey matter bilaterally in sensorimotor areas in CIMT groups compared with controls (Gauthier et al., 2008).

5.2.3 Paired associative stimulation

Somatosensory stimulation via the stimulation of peripheral nerves of the affected limb has been associated with improved motor function (Celnik et al., 2007; Chae et al., 2008; Conforto et al., 2002; Embrey et al., 2010; Floel et al., 2004). The combining of peripheral nerve stimulation with NIBS delivered over the affected cortex (paired associative stimulation, PAS) can increase excitability in the target cortex by LTP-like mechanisms associated with motor gains outlasting the treatment sessions and promotes the effect of rehabilitation training (Celnik et al., 2007; Dancause and Nudo, 2011; McKay et al., 2002; Stefan et al., 2002; Uy and Ridding, 2003; Uy et al., 2003).

5.2.4 Robot-assisted devices

Several studies examining the use of robot-assisted devices in mildly impaired stroke patients in the acute and subacute stages have shown mild to moderate gains (Kwakkel et al., 2008; Takahashi et al., 2008; Volpe et al., 2005). However, in a more recent study, high intensity robot therapy over 12 weeks in chronic stroke patients with moderate to severe upper limb impairment, results did not differ significantly in motor function compared to usual care or intensive physical therapy, although over 36 weeks it showed modest improvements compared to usual but not intensive therapy groups (Lo et al., 2010). This argues for the potential long-term benefits of intensive rehabilitation in patients with moderate to severe impairment even years after stroke. Robot-assisted therapy offers potential advantages in that they can be active without fatigue for long periods, can be

performed in a consistent and precise manner, be programmed and have capacity to measure a range of behaviours.

5.2.5 Pharmacological modulation of neuroplasticity

Pharmacological interventions that act on neurotransmitter systems may promote neural plasticity and potentially enhance the effectiveness of post-stroke motor therapies.

Psychomotor stimulants such as amphetamine or nicotine are known to stimulate changes in cortical and subcortical circuits in the normal brain including increased synapse formation in prefrontal cortex, stratum and motor cortex (Kolb and Teskey, 2010), increased axonal plasticity (Goldstein, 2009) and enhance basic forms of motor training in humans (Butefisch et al., 2002). Nicotine has been shown to facilitate recovery in laboratory animals and this functional improvement is correlated with synapse growth in the motor cortex, angiogenesis and axonal plasticity (Chen et al., 2007; Cui et al., 2010; Gonzalez et al., 2006). Additionally, amphetamines appear to facilitate the induction of activity-driven LTP in the cortex (Stroemer et al., 1998). Recently, the use of fluoxetine, a selective serotonin reuptake inhibitor, has been associated with better recovery in combination with physiotherapy, presumably through anti-inflammatory effects and neurogenesis (Chollet et al., 2011). Other agents that have shown promise include levodopa (Scheidtmann et al., 2001), inosine (Chen et al., 2002), anti-Nogo-A , growth factors such as NGF, FGF-2, VEGF, GCSF, BDNF, IGF-1 (De Smedt et al., 2011; Kolb and

Teskey, 2010; Minnerup et al., 2009; Paciaroni and Bogousslavsky, 2011; Schabitz et al., 2007; Sun et al., 2003), as well as agents that act to inhibit GABA-A (Clarkson et al., 2010) and phosphodiesterase-5 (Zhang et al., 2005; Zhang et al., 2002).

METHODOLOGY

1 CORTICAL EXCITABILITY STUDIES USING TRANSCRANIAL MAGNETIC STIMULATION

1.1 Hardware requirements

Two high-power magnetic stimulators that were connected via a BiStim device (Magstim Co., Whitland, South West Wales, UK), were used to generate magnetic currents and delivered over the patient's head through a 90mm circular coil. Conventional non-polarizable 5mm Ag-AgCl surface EMG electrodes (ConMed, Utica, USA) were used for recording, with the recorded signals being passed through a pre-amplifier and filter (3Hz-3KHz) and amplified using a Grass ICP511 AC amplifier (Grass-Telefactor, Astro-Med Inc., West Warwick, RI, USA). Signals were then sampled at 10KHz with an IBM-compatible personal computer that was fitted with a 16-bit data acquisition card (National Instruments PCI-MIO-16E-4, Austin, TX, USA) (Figure 1).

1.2 Software Requirements

Both data acquisition and stimulus delivery were controlled by a computerized threshold-tracking program, QTRAC software version 0.9.7 (©Hugh Bostock, Institute of Neurology, Queen Square, London, UK).

1.3 Stimulating and recording technique

The motor cortex of participants was stimulated using a 90mm circular coil oriented over the scalp to induce a current flow in a posterior-anterior direction if the left hemisphere (right APB muscle) was studied. Conversely, the current flow was in an anterior-posterior direction if the right hemisphere (left APB muscle) was studied. The coil was initially placed over the vertex of the scalp, and subsequently adjusted in both anterior-posterior and lateral planes in order for the optimal site of stimulation to be determined. This position over the scalp will require the least amount of magnetic current stimulation to evoke an MEP from the APB muscle of maximal amplitude.

Magnetic currents were generated by two-high power magnetic stimulators connected via a BiStim device so that both conditioning and test stimuli could be independently set and delivered through the one coil.
Contraindications to testing included individuals with implanted metallic objects or devices such as cardiac pacemakers, defibrillators or intracranial clips.

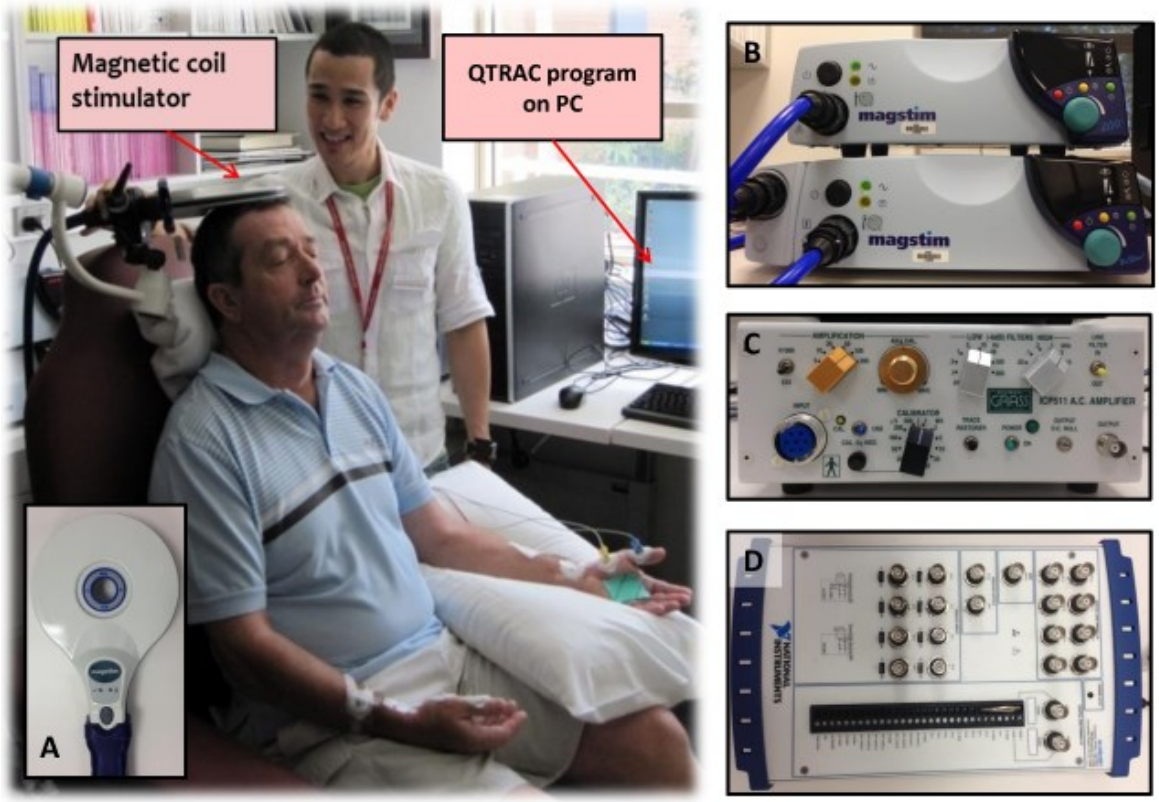


Figure 1. Experimental design and setup for paired-pulse threshold-tracking transcranial magnetic stimulation (TMS). Subject was seated in comfortable chair, with magnetic circular coil (A) placed over the vertex of the head. (B) BiStim machine; (C) Grass ICP511 AC amplifier; (D) 16-bit data acquisition card.

1.4 Cortical excitability parameters

1.4.1 Resting motor threshold

In the conventional TMS technique using a constant stimulus paradigm, resting motor threshold (RMT) was defined as the minimum stimulus intensity, measured as the % of the maximum stimulator output (MSO) that was required to produce a predetermined amplitude in at least 5 out of 10 trials (Rossini et al., 1999).

In the present thesis, RMT was redefined as the stimulus intensity (measures as % of MSO) that was required to evoke and maintain a target MEP response with

amplitude of 0.2±0.04mV peak-to-peak (Vucic et al., 2006). This technique was first developed by Fisher and colleagues (Fisher et al., 2002) who established that the relationship between the logarithm of MEP amplitude and stimulus intensity was close to linear, and that the target response of 0.2mV was located in the middle of the hundred-fold range of responses observed on this linear relationship.

Prior to commencement of threshold-tracking TMS as employed in this thesis, RMT was determined by repeated stimulation over the participant's motor cortex and re-adjusting the stimulus intensity until the target MEP response was consistently obtained. The subject was seated in a comfortable chair with the testing limb completely relaxed. Voluntary EMG activity was continually monitored using audio and visual feedback displayed on a projected computer screen, to ensure that these did not contaminate the recordings.

Following the determination of the subject's RMT, the threshold-tracking protocol proceeded and a defined sequence of cortical excitability parameters was acquired as per the QTRAC software protocol.

1.4.2 Short interval intracortical inhibition and facilitation

A paired-pulse TMS paradigm was developed from previous studies that investigated short-interval intracortical inhibition (SICI) (Hanajima et al., 1998; Kujirai et al., 1993). In the conventional paired-pulse technique, conditioning and test stimulus intensities were kept constant and the changes in MEP amplitudes

were recorded. In the current thesis, the target MEP response of 0.2mV was fixed and changes in the test stimulus intensity required to generate this target MEP, when preceded by a subthreshold conditioning stimuli, were measured ("threshold tracking"). This method was developed to overcome the variability in MEP amplitudes with consecutive stimuli that resulted in limitations using the constant-stimulus method (Kiers et al., 1993), and as suggested by Fisher et al, measurement of intracortical excitability at a constant MEP response using threshold-tracking methods limits the contribution of spinal and peripheral elements to the output measurement (Fisher et al., 2002).

In this experimental paradigm, a subthreshold conditioning stimulus preceded the testing stimulus at increasing interstimulus intervals (ISIs) and delivered over the motor cortex, as follows: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, 10, 15, 20, 25, and 30ms (Vucic et al., 2006). The subthreshold conditioning stimulus intensity was set to 70% RMT and was previously validated by other studies as the intensity at which maximal intracortical inhibition occurs (Kujirai et al., 1993; Vucic et al., 2009). This intensity was also too weak to evoke a response.

Stimuli were delivered sequentially as a series of three channels (Figure 2): channel 1 tracked the stimulus intensity required to produce the unconditioned test response (i.e., RMT, such that the RMT was continuously monitored throughout the testing to ensure it remained stable); channel 2 monitored the subthreshold conditioning stimulus (70%RMT) so as to ensure that an MEP response was not

produced and that the subject remained relaxed; and channel 3 tracked the stimulus required to evoke the target MEP when conditioned by a subthreshold stimulus equal in intensity to that on channel 2.

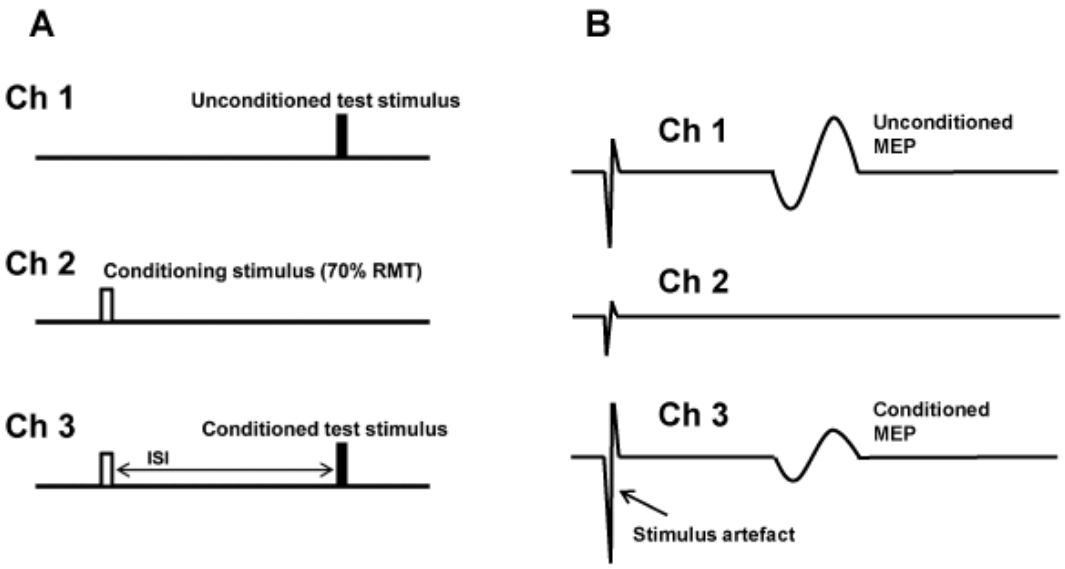


Figure 2. Experimental paradigm and configuration of stimulus patterns for measurement of short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF). Cortical excitability was assessed by measuring changes in stimulus intensity required to generate a target motor evoked potential (MEP) response of 0.2 mV. *Channel 1*, unconditioned test stimulus, measuring resting motor threshold (RMT). *Channel 2*, conditioning stimulus, set to subthreshold (70% RMT). *Channel 3*, conditioned test stimulus at different interstimulus intervals (ISIs).

Tracking was deemed acceptable when the test stimulus produced two consecutive MEP responses that were within 20% of the target response (0.2 mV) or consistently oscillated about the target. The three channels were applied sequentially and the computer advanced to the next ISI (interstimulus interval) only

when tracking met the target criteria. Stimuli were delivered approximately every 5 seconds and was limited by the charging capability of the BiStim system.

Intracortical inhibition (SICI) induced by a conditioning stimulus was measured as the increase in test stimulus required to evoke the target MEP of 0.2mV, and typically occurs when the ISI was less than 7ms. Conversely, intracortical facilitation (ICF) typically occurred with ISI greater than 7ms and was measured as the reduction in test stimulus required to evoke the target MEP (Figure 3). The percentage threshold change was therefore calculated as (Fisher et al., 2002):

[(Conditioned test stimulus intensity – RMT) / RMT] X 100

1.4.3 Stimulus-response curve

Using a single-pulsed protocol, the magnetic stimulus-response (SR) curve was constructed by determining the mean MEP amplitude evoked at each level of stimulation with the stimulus intensity increasing sequentially as follows: 60, 80, 90, 100, 110, 120, 130, 140, and 150% of RMT. Three stimuli were delivered at each intensity, and the maximum MEP amplitude (mV) and MEP onset latency (ms) recorded.

1.4.4 Cortical silent period

The cortical silent period (CSP) was evaluated using a single-pulsed protocol and same pattern of stimulus intensity delivery as for the SR curve. The recorded response however, was assessed whilst the subject performed a weak voluntary

contraction of the APB muscle, broadly estimated by the investigator as representing approximately 10-30% of maximum voluntary contraction. CSP duration (ms) was measured as the interval between onset of the facilitated MEP to resumption of continuous EMG activity (Cantello et al., 1992; Inghilleri et al., 1993), with the mean CSP duration at each level of stimulation calculated using the mean of the three obtained recordings at each intensity (Figure 4).

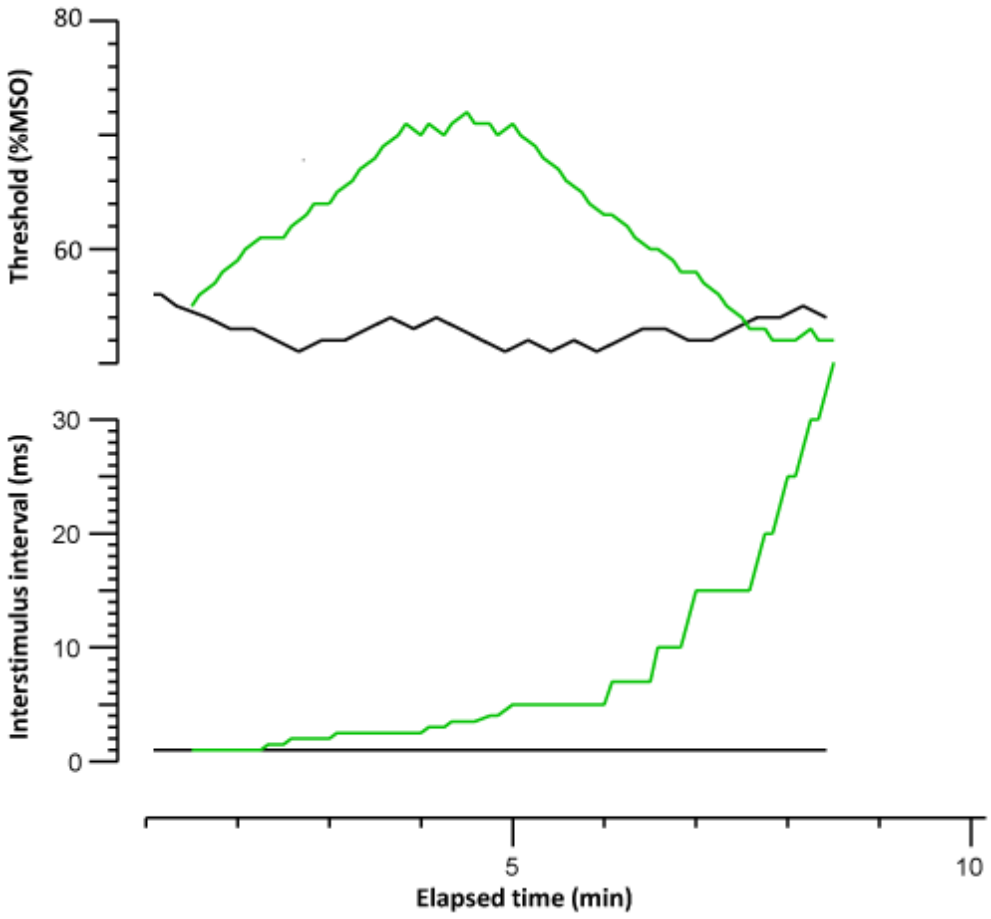


Figure 3. Screen-capture demonstrating threshold-tracking paired-pulse protocol recording short-interval intracortical inhibition (SICI) and facilitation (ICF). The period of time whereby the green trace (conditioned test stimulus) was above the black trace (control unconditioned stimulus) represented SICI (typically ISI between 1-7ms), and the period whereby the green trace was below the black represented ICF (typically 10-30ms).

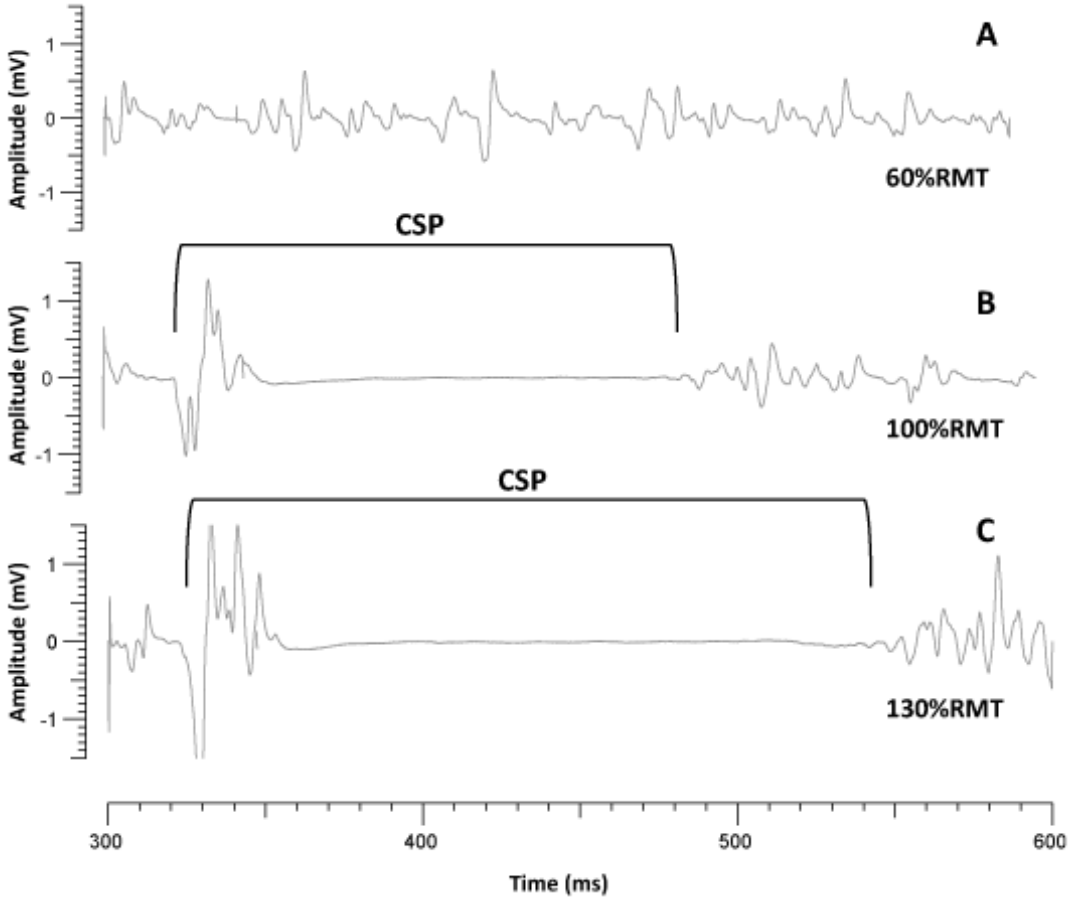


Figure 4. Screen capture demonstrating recording of the cortical silent period (CSP) protocol at different single stimulus intensities (as a proportion of the resting motor threshold, RMT), 60% RMT (A), 100% RMT (B) and 130% RMT (C). CSP duration is measured between the onset of the facilitated MEP and the resumption of EMG activity, as increases with increasing stimulus intensities.

2 PERIPHERAL NERVE EXCITABILITY STUDIES

2.1 Hardware requirements

For peripheral nerve stimulation, electrical stimuli were converted to current using an isolated linear bipolar constant current stimulator (maximum output ±50mA; DS5, Digitimer, Welwyn Garden City, UK) (Figure 5A). Conventional non-polarizable 5mm Ag-AgCl surface EMG electrodes (ConMed, Utica, USA) were used for stimulating and recording, with the recorded signals being passed through a pre-amplifier and filter (3Hz-3KHz) and amplified using a Grass ICP511 AC amplifier (Grass-Telefactor, Astro-Med Inc., West Warwick, RI, USA) (Figure 1C). Electrical noise was removed via a Hum Bug 50/60 Hz Noise Eliminator (Quest Scientific Instruments, North Vancouver, Cananda) (Figure 5B). Signals were then sampled at 10KHz with an IBM-compatible personal computer that was fitted with a 16-bit data acquisition card (National Instruments PCI-MIO-16E-4, Austin, TX, USA) (Figure 1D).

2.2 Software Requirements

Data acquisition and electrical stimulus delivery were controlled by a computerized threshold-tracking programme, QTRAC software version 0.9.7 (©Hugh Bostock, Institute of Neurology, Queen Square, London, UK) using the TRONDNF protocol.

2.3 Stimulating and recording technique

Before stimulation, the skin over the stimulation site was prepared to minimize skin resistance, and the skin temperature around the stimulation site was maintained at approximately 32°C (Kiernan et al., 2001a). The median nerve was stimulated over the wrist with the cathode placed at the proximal wrist crease between the pulmaris longus and flexor carpi radialis (FCR) tendons and the anode positioned approximately 10cm proximally over the lateral forearm. Prior to commencing the excitability testing protocol, the optimal site of stimulation at which the least amount of stimulus current to evoke a compound muscle action potential (CMAP), was determined. The resultant CMAPs were recorded over the motor point of the APB muscle and the reference electrode placed 4cm distally in a muscle-belly-tendon montage.

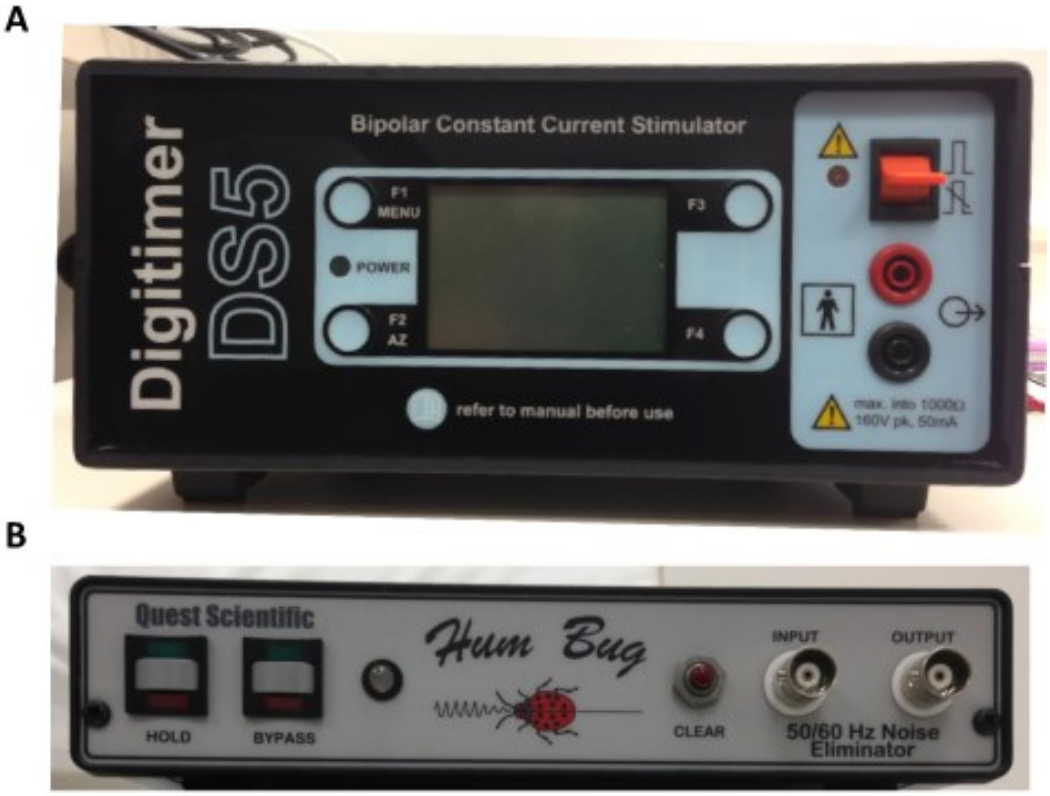


Figure 5. Additional hardware equipment used for peripheral nerve excitability studies. (A) linear bipolar constant current stimulator (maximum output ±50mA); (B) Hum Bug 50/60 Hz Noise Eliminator.

2.4 Nerve excitability protocol and parameters

Axonal excitability studies provide information about membrane potential and axonal ion channel function, and can be investigated using threshold tracking techniques (Bostock et al., 1998; Kiernan et al., 2000). "Threshold" refers to the stimulus current that is required to evoke a target potential (Burke et al., 2001) (see below). In this technique, the resting threshold is measured and nerve excitability altered by changing the nerve environment by applying a conditioning polarizing current. The threshold tracking software (QTRAC) consisted of an automated

tracking system such that the test stimulus intensity was automatically increased or decreased in percentage steps following each response, depending on differences between the recorded and target responses.

2.4.1 Stimulus-response curve

The initial stage of the nerve excitability protocol was to establish the SR curve by incremental stimulation of the motor nerve. This recorded the unconditioned responses as the stimulus was increased from zero to where it produced supramaximal potentials. From this the maximal CMAP amplitude, measured from baseline to negative peak, was obtained and a target response was determined. This target potential corresponded to the steepest point on the fitted SR curve and approximated 40% of the maximal CMAP amplitude (Bostock et al., 1998), and was used for the remainder of the nerve excitability protocol to assess different aspects of axonal excitability and a series of recorded parameters (as outlined below).

2.4.2 Strength-duration properties

The changes in stimulus current required to produce the defined target response using four different stimulus current durations (0.2, 0.4, 0.8 and 1.0ms) were used to assess strength-duration properties. The strength-duration time constant, τ_{SD}, and rheobase, Rh, was calculated in accordance with Weiss' Law that defines a linear relationship between stimulus intensity and stimulus duration (Mogyoros et al., 1996; Weiss, 1901), based on decrease in current required to produce a target potential as the stimulus duration was increased:

$$Q = Rh\ (t + \tau_{SD})$$

Q denotes stimulus charge and t representing stimulus duration.

Based on this equation, τ_{SD} is the x-intercept of the linear relationship between stimulus charge and duration (Figure 6). Rheobase is defined as the threshold current for a target response when the stimulus is of infinitely long duration and represents the slope of this linear relationship (Mogyoros et al., 1996). Both these parameters are properties of the nodal membrane, specifically the persistent Na^+ conductance (Bostock et al., 1998; Burke et al., 2001).

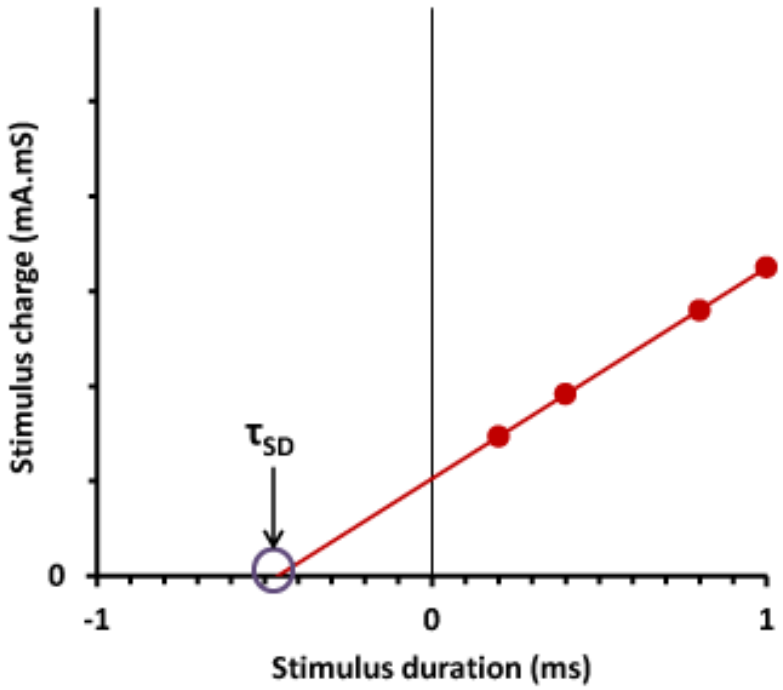


Figure 6. Plot of stimulus charge versus stimulus duration, illustrating the determination of strength–duration time constant (τ_{SD}) using Weiss' law. Strength–duration time constant is determined as the negative intercept on the x-axis of the line measured using stimuli of varying duration (0.2, 0.4, 0.8 and 1ms).

2.4.3 Threshold electrotonus

In the threshold electrotonus (TE) protocol, changes in axonal excitability were measured in response to the delivery of prolonged subthreshold depolarizing (+40% of controlled threshold current) and hyperpolarizing (-40%) conditioning stimuli of 100ms in duration (Bostock et al., 1998; Burke et al., 2001; Kiernan et al., 2000). TE measures the threshold changes that occur in response to these subthreshold polarizing pulses used to alter the potential difference across the internodal and nodal axonal membrane (Bostock et al., 1998). Changes in threshold were measured at 26 time points during and after the conditioning pulses, and 3 stimulus combinations were tested sequentially: test stimulus alone (measured control threshold), test stimulus + depolarising current, and test stimulus + hyperpolarizing current. Stimulus combinations were tested repeatedly until 3 valid estimates were recorded within 15% of the target response (Figure 7).

2.4.4 Recovery cycle

Changes in axonal membrane excitability following the delivery of a supramaximal conditioning stimulus of 1ms duration, are depicted in the recovery cycle. Eighteen conditioning-test stimulus intervals were studied from 200 to 2ms, and 3 stimulus combinations were recorded: unconditioned test stimulus (1ms duration), supramaximal conditioning stimulus alone, and conditioning and test stimulus together. Each of these combinations was repeated until 4 valid threshold estimates were acquired (Figure 8).

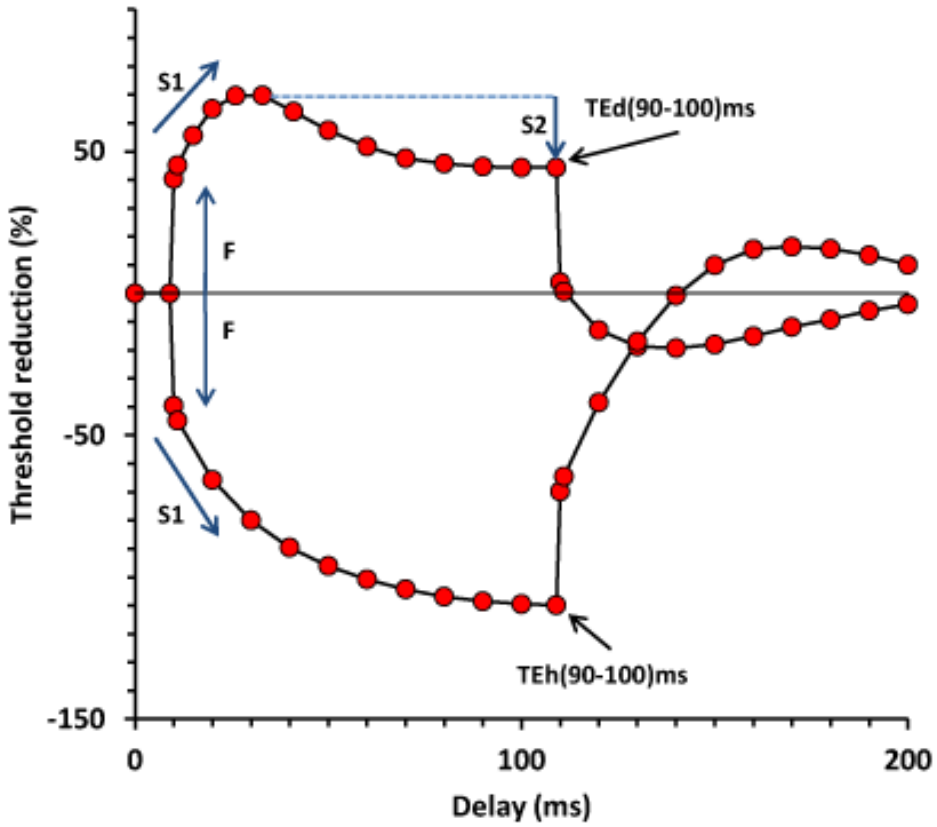


Figure 7. Threshold electrotonus (TE) waveform (depolarizing direction plotted upwards and hyperpolarizing downwards] demonstrating response of the axon to 100ms subthreshold polarizing currents (40% of threshold), with an initial fast (F) phase in response to initial polarization followed by a slow (S1) phase due to the gradual spread of current into the internode. The S2 phase of accommodation to depolarization is related to the activation of slow K+ channels. Conditioning-test interval corresponding to hyperpolarizing and depolarizing TE at 90-100ms, TEh(90-100) and Ted(90-100), are depicted. TEh(90-100) reflects inward rectification towards threshold produced by slow activation of I_H by hyperpolarization (Bostock et al., 1998; Pape, 1996).

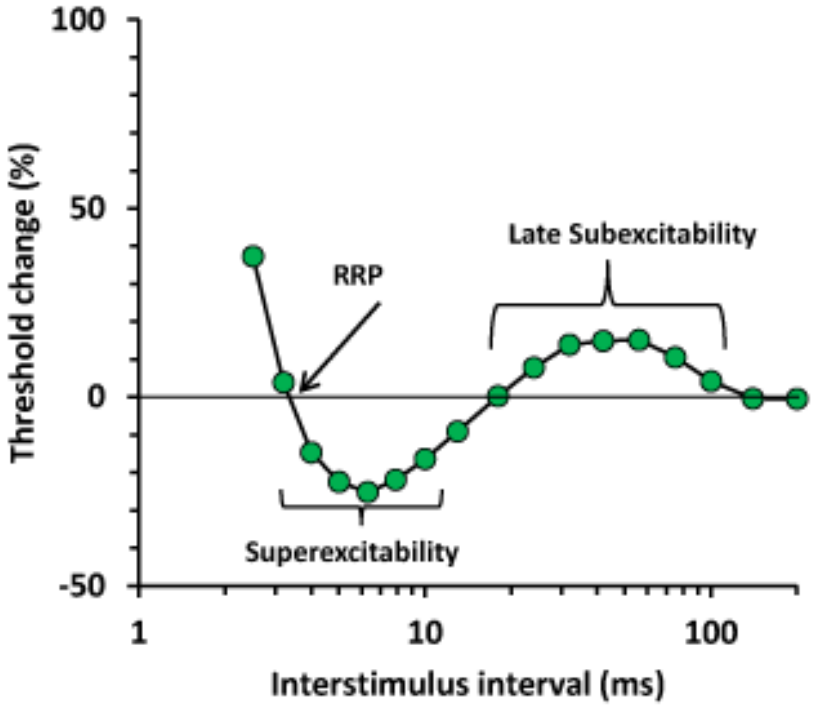


Figure 8. Recovery cycle plot illustrating the relative refractory period (RRP, measure of nodal Na^+ channel inactivation), superexcitability (measure of paranodal fast K^+ conductance) and late subexcitability (measure of nodal slow K^+ channel) (Bostock et al., 1998; Krishnan et al., 2009).

2.4.5 Current-threshold (I/V) relationship

The final stage of the protocol recorded the I/V relationship which is dependent on the rectifying properties of the nodal and internodal axolemma (Kiernan et al., 2000). This was obtained by tracking threshold changes following the delivery of 1ms duration test pulses that were preceded by 200ms-long subthreshold polarizing currents. Subthreshold currents were altered sequentially from +50% (depolarizing) to -100% (hyperpolarizing) of the controlled threshold in a 10% step-wise fashion (Figure 9). The slope of the I/V relationship during depolarizing currents represent outward rectifying K^+ channel conduction, whilst the gradient during the hyperpolarizing phase reflect inwardly rectifying conductance I_H (Pape, 1996).

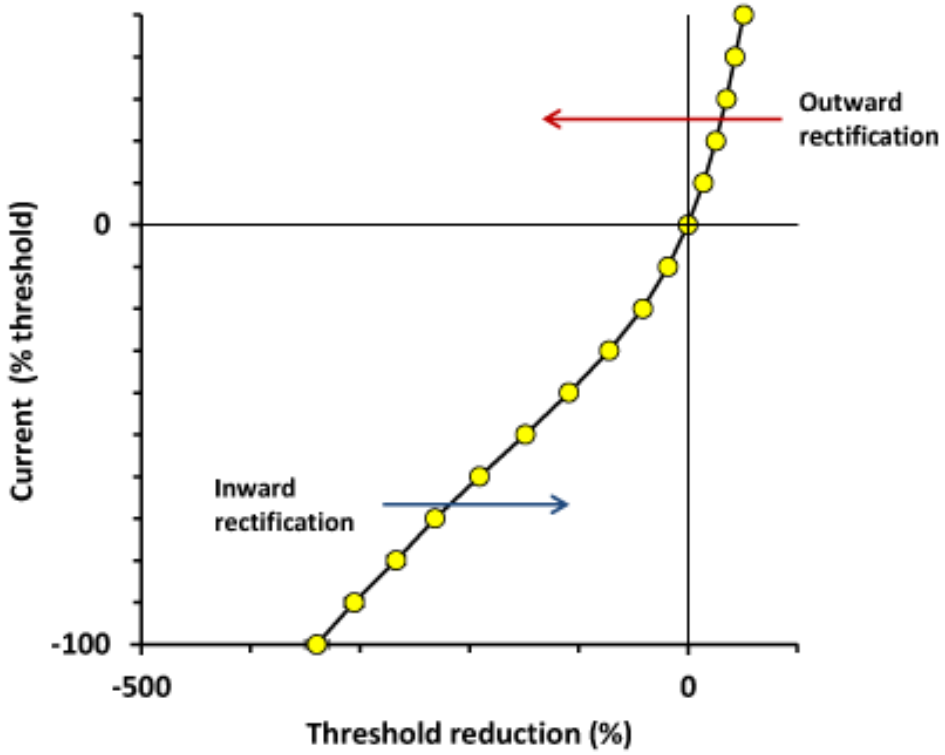


Figure 9. The current-threshold (I/V) relationship reflects the rectifying properties of the axon, with outward rectification resulting from fast and slow K^+ channel activation causing a steepening of the curve toward the top right, and inward rectification (I_H) as illustrated by the steepening toward the bottom left reflecting accommodation to hyperpolarizing currents (Kiernan et al., 2000).

3 NERVE CONDUCTION STUDIES

Standard nerve conduction studies were undertaken to record the CMAP response over the APB muscle whilst stimulating the median nerve at the wrist, to determine the maximum CMAP amplitude, CMAP latency and *F*-wave latencies such that certain TMS parameters can be obtained. These include the MEP amplitude as a proportion of the CMAP amplitude, as well the central motor conduction time (CMCT) calculated using the formula (Claus, 1990):

$$CMCT = [\ MEP\ latency - (\ (\ F\text{-wave latency} - CMAP\ latency\ )\ -1\ )\ ]\ /\ 2$$

The median nerve was stimulated over the wrist at the proximal wrist crease between the tendons of the palmaris longus and flexor carpi radialis muscles using a hand-held bipolar probe consisting of both the cathode and anode electrodes, with the cathode oriented 4cm distally to the anode (Figure 10). Currents of 0.1ms duration were used and the intensity (in mA) increased until a supramaximal response was obtained. The active recording electrode was placed over the motor point of the subject's APB muscle with the reference recording electrode about 4cm distally in a muscle-belly-tendon montage (Bastron and Lambert, 1960; Jones, 2012) (Figure 10). Both stimulation and recording were controlled using Medlec Synergy (Oxford Instruments, UK) and the Synergy software (Viasys Healthcare, UK).

The CMAP onset latency (ms) was determined from the stimulus artefact to the onset of the negative potential (positive deflection), whilst the CMAP amplitude was determined from the baseline to the peak of the negative potential (Falck and

Stalberg, 1995) (Figure 10). *F*-wave studies were undertaken using a series of 10 supramaximal stimuli and the minimal *F*-wave latency was used to calculate the CMCT.

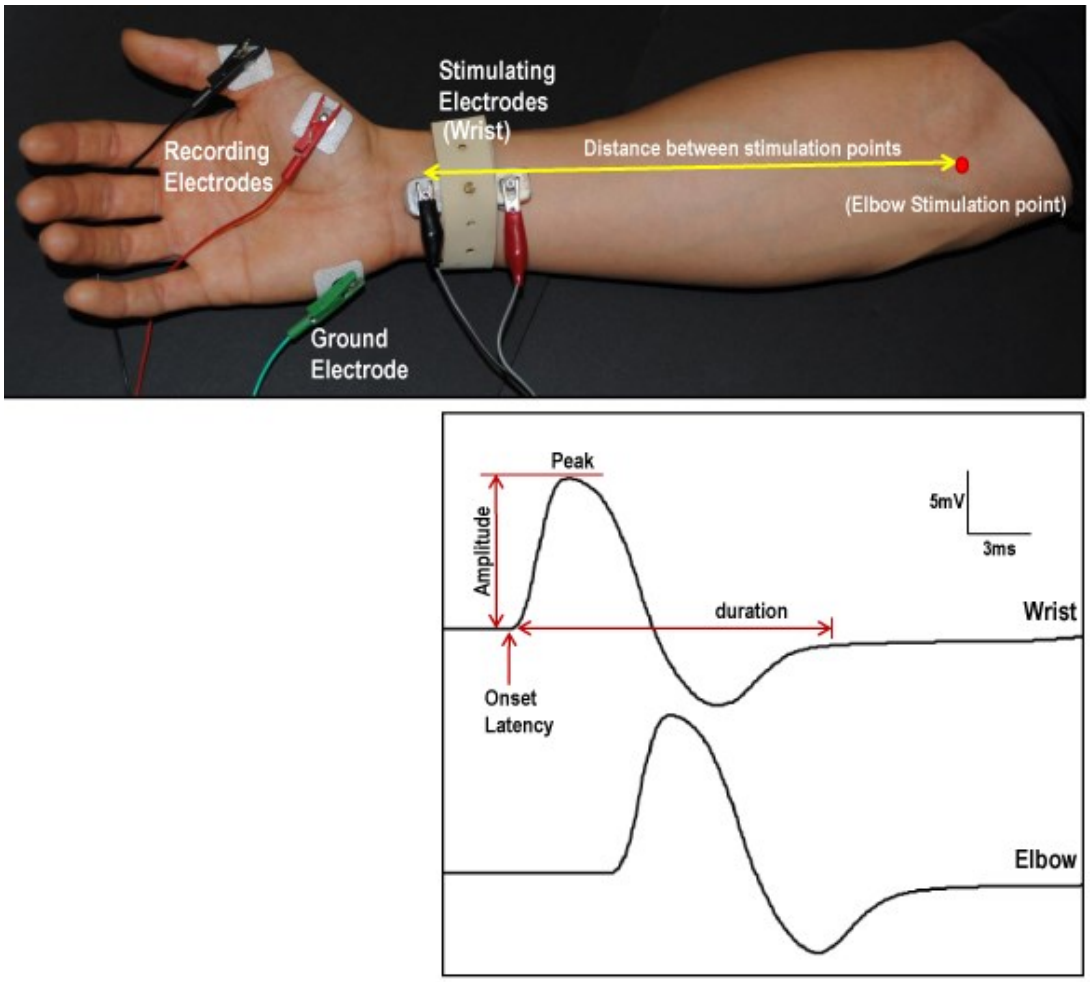


Figure 10. Stimulation and recording setup for conventional nerve conduction studies (above) with stimulation of the median nerve over the wrist and recording over the APB muscle. The recorded compound muscle action potential (CMAP) waveforms are shown (below) with parameters including onset latency, amplitude and duration of response.

CHAPTER 1

The effects of large artery ischemia and subsequent recanalization on nerve excitability

SUMMARY

While neurological symptoms during limb ischaemia are common, the mechanisms remain poorly defined. A 46-year-old woman presented with a history of right upper limb paraesthesia as a result of large artery thrombosis. Neurophysiological studies before and after recanalisation, were undertaken using nerve excitability techniques to provide insights into the pathophysiology of nerve dysfunction. To determine whether changes between the studies were reflective of true alterations after revascularization and not intra-individual variability, further studies were also performed on ten controls subjects at different time points to assess the reproducibility of excitability parameters. Results demonstrated that the ischaemic axons were relatively depolarized when compared to reperfused nerves. This chapter provides novel insight into the mechanisms of axonal dysfunction underlying "pure" nerve ischaemia in vivo, and offers a pathophysiological explanation for the neurological symptoms experienced in patients with arterial disease. The results have also demonstrated reproducibility of nerve excitability parameters such that these techniques can be used reliably in longitudinal assessment of changes in peripheral nerve excitability following stroke in the later chapters of this thesis.

1 INTRODUCTION

Peripheral arterial disease causing chronic limb ischaemia is a relatively common and debilitating process (Mousa et al., 2005). While neurological symptoms including paraesthesia and pain are common, a pathophysiological explanation for these symptoms remains poorly defined.

Novel techniques assessing nerve excitability may now enable measures of axonal membrane ion channel function to be undertaken in vivo (Bostock et al., 1998; Kiernan et al., 2000; Kiernan et al., 2005a; Kiernan et al., 2005b; Nodera and Kaji, 2006). These techniques have been previously used to identify mechanisms that underlie ischaemic conduction failure, a contributing factor to the development of peripheral neuropathies due to diseases such as diabetes (Krishnan and Kiernan, 2005), (Farrar et al., 2010; Lin et al., 2008). Specifically, excitability studies have demonstrated that nerve ischaemia induces membrane depolarization secondary to paralysis of the electrogenic Na+/K^+ ATPase pump (Bostock et al., 1994; Grosskreutz et al., 1999; Han et al., 2008; Kiernan and Bostock, 2000; Krishnan et al., 2005; Stohr, 1981). For this reason, it is important to demonstrate that the changes in biophysical properties of peripheral axons in neuropathic nerves reflect those changes that would occur in axons experiencing ischaemia in vivo. To date, there have been no studies examining the effects of pure nerve ischaemia in an in vivo disease setting.

In the present case, excitability techniques were utilized during chronic upper limb ischaemia from an arterial thrombosis and were compared before and after endarterectomy, to provide novel insight into symptom generation.

2 CASE HISTORY

A 46-year-old female presented with a 6 month history of right upper limb sensory symptoms comprising of distal paraesthesia, pain and reduction in skin temperature together with pallor. There was no associated muscle weakness. Clinical examination demonstrated a relatively pale and cool right upper limb compared to the left with a weaker distal radial pulse. She complained of subjective numbness with persistent pins and needles in the limb distal to the elbow region although no objective sensory discrepancies were apparent on examination. Power and reflexes were preserved.

CT angiogram demonstrated a filling defect consistent with a thrombus within the right brachiocephalic artery extending to its bifurcation, with extension of the thrombus into the subclavian artery (Fig. 1.1A). Consequently the patient underwent an endarterectomy with removal of the adherent thrombus (Fig. 1.1B). Right upper limb sensory symptoms immediately improved following the revascularization procedure and completely resolved over the ensuing days.

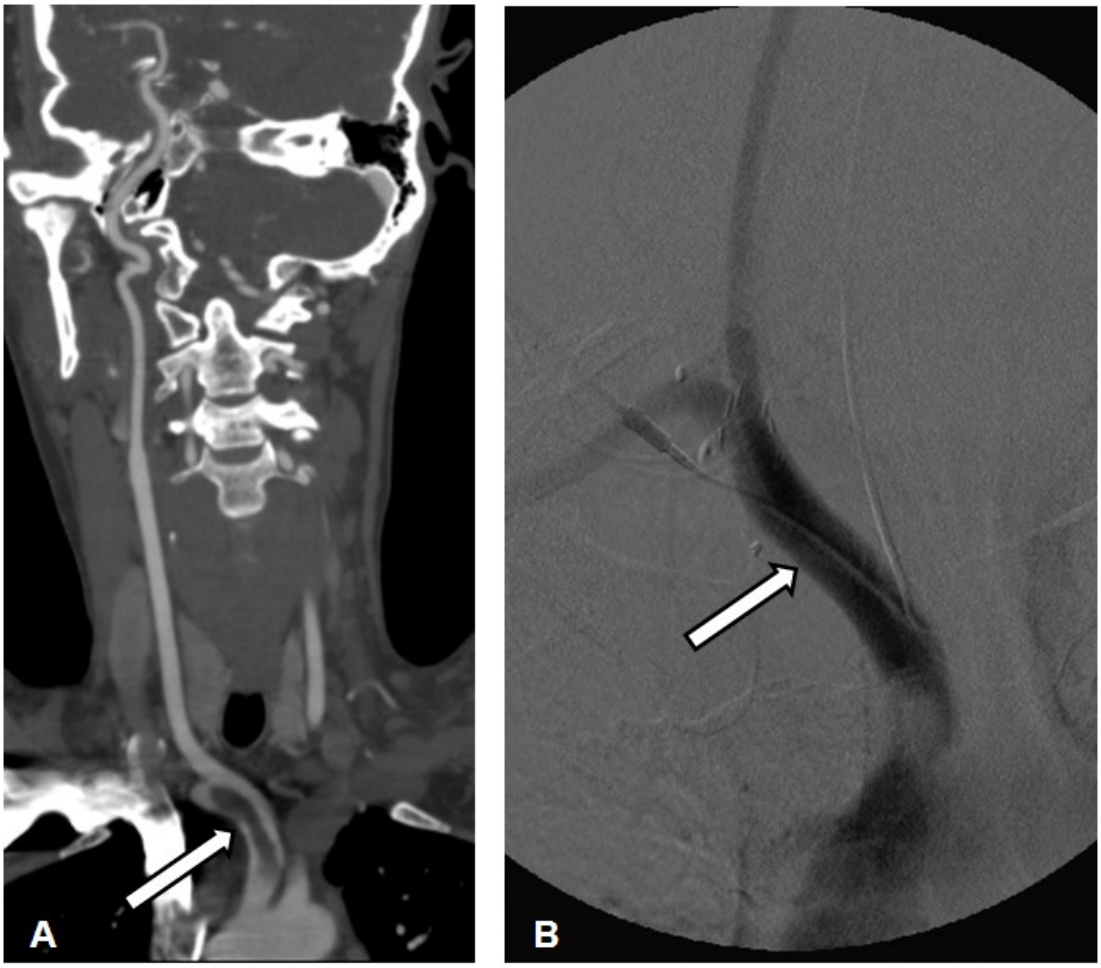


Figure 1.1. (A) CT angiogram demonstrating thrombus in the right brachiocephalic artery at the bifurcation extending into the right subclavian artery (arrow). (B) Digital subtraction angiogram showing patency and revascularization of the right brachiocephalic artery following endarterectomy.

3 METHODS

Motor nerve excitability studies were undertaken on the median nerve-APB of the affected limb of the patient before and after surgical revascularization, according to previously described methodology (page 67). The patient gave informed consent to the procedures, which was approved by the institutional Ethics Committee.

Skin temperature close to the site of stimulation was maintained >32°C (Kiernan et al., 2001a) on both occasions (33°C pre- and 34°C post-surgery). Serum electrolyte levels were measured at the time of study and were all within the normal range (Kiernan et al., 2002). Results were compared to 10 controls without underlying conditions affecting the peripheral nerve, undertaken on 2 separate occasions, to assess the reproducibility and intra-individual variability of excitability indices, and determine the significance of the excitability changes observed in the patient.

Differences in excitability parameters between the 2 test occasions were calculated and 95% confidence limits were obtained using Predictive Analytical Software (PASW) version 18 (©SPSS Inc, Chicago, Illinois).

4 RESULTS

Compared to postsurgical values, presurgical results demonstrated a lower threshold current to stimulate 50% of maximal CMAP (pre: 2.26 mA ; post: 3.50 mA), increased latency to peak (pre: 6.75 ms; post: 6.01ms), and a smaller peak CMAP amplitude(Fig. 1.2A). The slope of the stimulus-response curve was less (3.0)

in the ischaemic limb compared to the postsurgical limb (4.0). The strength-duration time constant (τ_{SD}) which provides an indirect measure of nodal persistent Na^+ conductance, was higher in the ischaemic limb compared to the reperfused limb (pre: 0.546; post: 0.462)(Fig. 1.2B).

There were differences in threshold electrotonus (TE) curves (Fig. 1.2C) pre- and post-thrombus removal. In the depolarizing direction, the mean threshold change at the end of the 100ms current in the ischaemic limb was lower compared to the revascularised recordings [TEd (90-100ms): pre 45.52%; post 52.77%]. There was also a difference in accommodation to hyperpolarizing currents with a mean maximal threshold change smaller [TEh (90-100ms)] in the ischaemic limb compared to the reperfused limb (pre: -116.93%; post: -133.84%). Of relevance, the changes in the current-threshold relationship were consistent with membrane depolarization, with the resting I/V slope (reflection of membrane potential (Han et al., 2008)) being higher in the presurgical ischaemic (0.558) limb compared to the postsurgical value (0.461).

Assessment of recovery cycle parameters demonstrated significant differences between pre- and post-surgical values (Fig 1.2D). Refractoriness, a marker of the recovery from inactivation of nodal transient sodium channels, was greater in the presurgical recordings (31.31%), compared to postsurgical values (10.48%). This was accompanied by reductions in superexcitability (pre: -25.34%; post: -28.23%), related in part to the activity of juxtaparanodal potassium channels. There were

also changes in late subexcitability (pre: 12.8%; post: 11.1%), which is a surrogate marker of the activity of nodal potassium channels of slow kinetics.

The reductions in depolarizing and hyperpolarizing TE in the ischaemic limb, is suggestive of a more depolarized membrane potential compared to the waveform post-clot removal (Baker and Bostock, 1989), with TEd(90-100ms) being a sensitive and accurate parameter of changes in membrane potential (Kiernan and Bostock, 2000).

In total, the reductions in depolarizing and hyperpolarizing threshold electrotonus, combined with alterations in recovery cycle measures (namely greater refractoriness and reduced superexcitability), and changes in stimulus-response parameters (lower threshold and increased latency) are consistent with axonal membrane depolarization prior to surgery, with normalization of axonal function in post-surgical recordings.

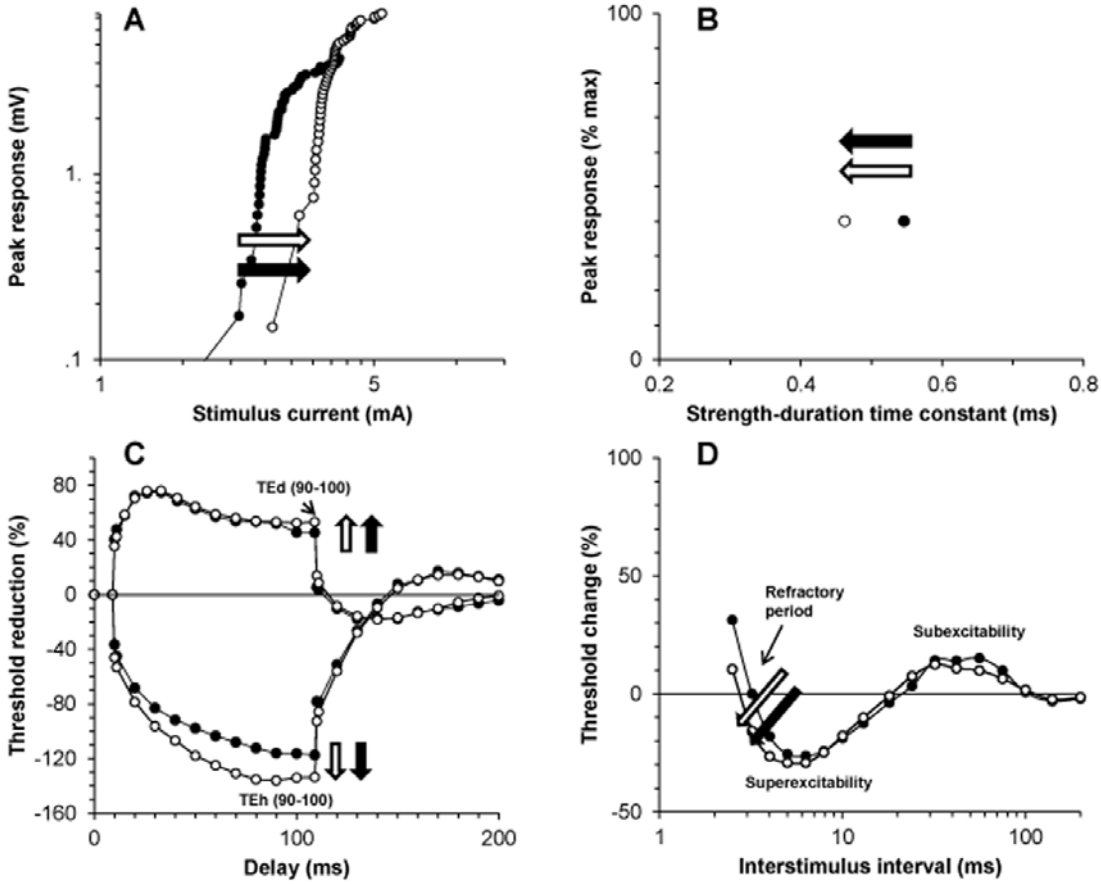


Figure 1.2. Excitability data for the patient pre- (closed circles) and post-endarterectomy (open). White arrows, direction of change in the patient following revascularization; Black arrows, direction of change post-ischaemia observed in experimental models (Bostock et al., 1994; Kiernan and Bostock, 2000; Krishnan et al., 2005). (A) Absolute stimulus-response curves. (B) Strength-duration time constants. (C) Threshold electrotonus. (D) Recovery cycle.

Excitability parameters in controls measured on 2 separate occasions were reproducible and demonstrated little variability (Fig. 1.3). The differences in excitability parameters pre- and post-endarterectomy were outside the 95% confidence limits determined by the control values (Table 1.1), indicating that changes observed in the patient were true alterations in biophysical axonal properties following reperfusion of the ischaemic limb rather than being related to intra-individual variability.

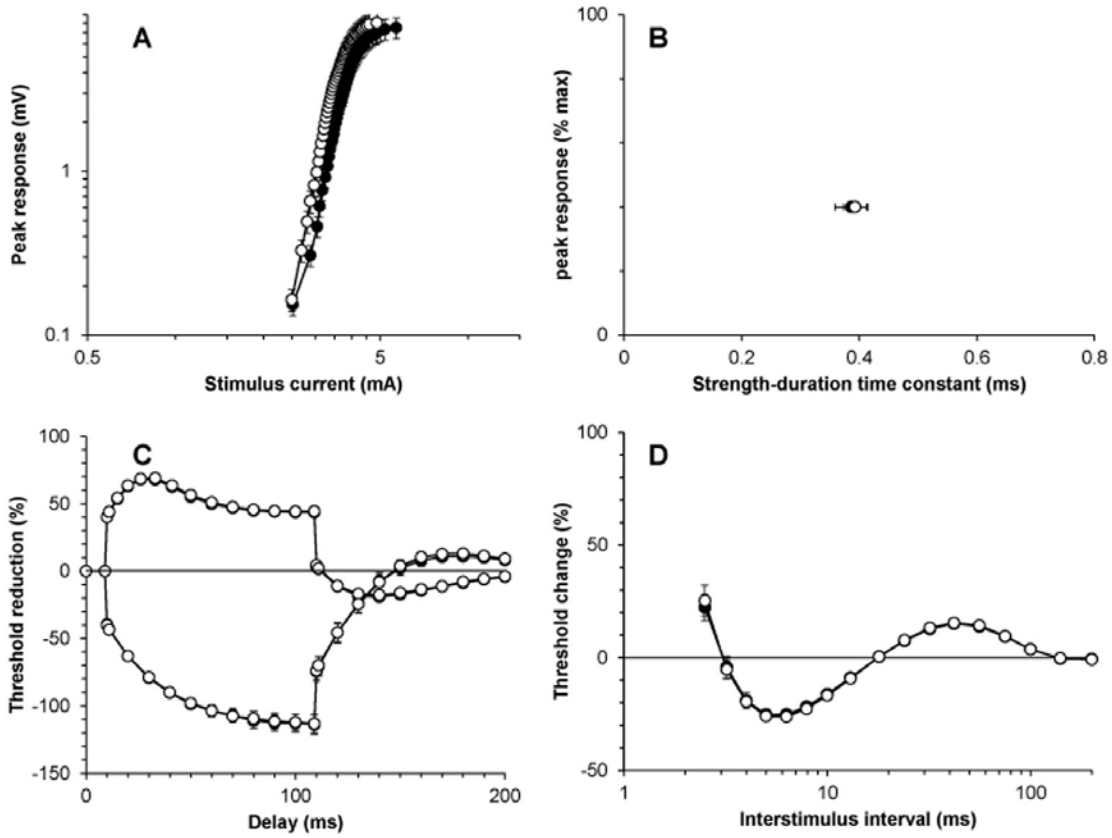


Figure 1.3. Excitability data for 10 controls studied on 2 separate occasions (1st recording: closed circles; 2nd recording: open circles), demonstrating reproducibility between the two recordings. (A) Absolute stimulus-response curves. (B) Strength-duration time constants. (C) Threshold electrotonus. (D) Recovery cycle.

	Patient	**Controls**	
	Change post-surgery	95% Confidence Intervals (mean of differences)	
Excitability Parameter		Lower	Upper
Threshold current 50% CMAP (mA)	1.24	-0.78	0.43
Latency (ms)	-0.74	-0.45	0.20
CMAP (mV)	3.18	-0.13	1.55
S-R Slope	0.94	-0.36	0.80
τ_{SD} (ms)	-0.084	-0.05	0.063
TEd (90-100) (ms)	7.25	-1.52	3.01
TEh (90-100) (ms)	-16.91	-3.89	5.13
Resting I/V slope	-0.097	-0.078	0.021
Refractoriness (%)	-20.83	-16.57	9.32
Superexcitability (%)	-2.89	-2.38	0.60
Subexcitability (%)	-1.7	-1.6	2.6

Table 1.1. Change in excitability parameters post-surgery in patient compared with the 95% confidence interval obtained from the mean of the differences between the two separate test occasions in controls.

5 DISCUSSION

Comparison of nerve excitability changes in a chronically ischaemic limb before and after restoration of perfusion has established that ischaemia may induce a sustained membrane depolarization. Furthermore, surgical treatment resulted in normalization of axonal properties and restoration of normal impulse conduction. These results are consistent with previously reported experimental models that used a sphygmomanometer to induce limb ischaemia (Fig. 1.2)(Bostock et al., 1994; Grosskreutz et al., 1999; Kiernan and Bostock, 2000; Krishnan et al., 2005).

An initial consideration was whether the changes in excitability parameters in this study reflected true abnormalities rather than intra-individual variability. Previous studies examining the reproducibility of excitability indices have demonstrated that there is relatively little variability within subjects, with refractoriness and supernormality being least variable (Mogyoros et al., 2000; Tomlinson et al.). The fact that there was a 200% change in refractoriness between the pre and post ischaemic recordings (>52% being considered as a significant change within the same individual (Mogyoros et al., 2000)), and the relatively consistent changes in all excitability parameters (Kiernan et al., 2000), confirmed that the changes in this study were significant in the ischaemic state and also following reperfusion.

The increase in CMAP amplitude post-surgery may be explained by a resolution of depolarising conduction block that was previously present in the ischaemic axons. As a result of this difference in CMAP amplitudes, different populations of motor

axons may have been studied pre and post revascularisation. This may have partly contributed to the change in hyperpolarizing TE, but would not explain the changes seen in other excitability parameters following resolution of ischaemia. In addition, a series of studies examining the excitability properties of different population of motor axons (different sized CMAP amplitudes) have demonstrated differences occurring only in hyperpolarizing TE between smaller and larger CMAP amplitudes but not in other parameters: TEd(90-100)ms, recovery cycle indices (refractory period, superexcitability, subexcitability) and strength-duration time constant (Shibuta et al., 2010).

Peripheral nerve ischaemia is a common contributing mechanism to the development of peripheral neuropathies, including diabetic neuropathy(Nukada, 1992). The present study has demonstrated that ischaemia caused membrane depolarization, presumably related to impairment of the energy-dependent electrogenic Na^+/K^+ pump that normally maintains membrane polarization by extruding 3 Na^+ ions out for every 2 K^+ entering the cytoplasm (Bostock et al., 1991; Bostock et al., 1994; Kiernan and Bostock, 2000).

Tissue acidosis as a result of ischaemia may lead to alterations in pH which in turn may affect persistent Na^+ conductances and cause membrane potential shifts. However, acidosis is associated with a decrease in strength-duration time constant due to blockade of the Na^+ channels by H^+ ions (Baker and Bostock, 1999; Mogyoros et al., 1997). This is not consistent with the findings of the present study which

demonstrated a longer strength-duration time constant in the ischaemic compared to the revascularised limb. Therefore, Na^+/K^+ pump failure from energy depletion rather than tissue acidosis is the more likely pathophysiological mechanism for the membrane depolarization observed in the ischaemic limb.

Taken in total, the depolarised state of the axons during ischaemia would serve to induce a greater tendency for sensory fibres to discharge ectopically, generating sensory symptoms, such as paraesthesia and pain, as experienced by the present patient during her period of limb ischaemia.

CHAPTER 2

Longitudinal plasticity across the neural axis in acute stroke

SUMMARY

With the advent of novel brain stimulation techniques aimed at improving functional outcome, understanding post-stroke plasticity becomes critical for the appropriate selection of patients, and optimal timing to introduce neuromodulatory interventions. As such, to better define the nature and temporal evolution of neuroplastic changes in stroke during the recovery process and its clinical implications, the present study combined detailed clinical assessment with novel central and peripheral threshold-tracking techniques to assess the changes that develop throughout the neural axis. Transcranial magnetic stimulation, peripheral nerve excitability and clinical assessments were undertaken in 31 acute stroke patients, followed longitudinally over 3-months from time of ictus, comprising a total of 384 clinical studies. During the hyper-acute phase (<7 days), short-interval intracortical inhibition (SICI) was significantly reduced in lesioned [4.3±1.3%] and contralesional hemispheres [3.6±1.9%] compared with controls [11.4±1.3%, P=0.001]. There were also significant alterations in accommodative properties of motor axons in the affected limb. At follow-up, SICI remained suppressed in both hemispheres in the context of significant clinical improvement. Simultaneous assessment of central and peripheral motor pathways has identified bilateral plastic changes that develop throughout the neural axis in acute stroke patients. It is proposed that these changes represent an adaptive response, and that the persistent bihemispheric reduction in SICI during the first 3-months may act to promote stroke recovery, through cortical reorganization.

1 INTRODUCTION

Despite improvement in acute stroke management, many patients are left with residual motor deficits affecting function and independence. In part, this variability in outcome reflects differences in patterns of motor reorganization (Westlake and Nagarajan, 2011). Specifically, unilateral lesions result in disruption of the complex interplay between inhibitory and excitatory influences responsible for the balance of excitability between hemispheres (Grefkes and Fink, 2011; Westlake and Nagarajan, 2011; Ziemann, 2005). Debate remains concerning the nature, evolution and clinical significance of cortical excitability changes that develop in both hemispheres (Butefisch et al., 2003; Cicinelli et al., 2003; Liepert et al., 2000e; Manganotti et al., 2008; Shimizu et al., 2002; Swayne et al., 2008; Wittenberg et al., 2007), particularly regarding the role of the unaffected hemisphere in terms of stroke recovery. While some investigators have reported persistence in contralesional cortical hyperexcitability in association with poor functional recovery (Boyd et al., 2010; Manganotti et al., 2008; Murase et al., 2004; Swayne et al., 2008), others have reported positive correlations with recovery (Butefisch et al., 2003; Butefisch, 2004; Takeuchi et al., 2010).

In part, this discrepancy may be attributed to heterogeneity in duration from time of stroke to assessment, as well as the cross-sectional design of most studies. Further elucidating the nature of excitability changes and its potential influences on functional recovery may have therapeutic implications, especially when assessing the effects of emerging strategies that utilize different modalities (pharmacological

and electrophysiological) to neuromodulate the balance in cortical excitability between hemispheres in an attempt to improve stroke recovery (Chollet et al., 2011; Lindenberg et al., 2010). Clearly, the optimal timing of such interventions will need to be better defined, to avoid detrimental effects particularly if delivered during the phase, when the balance between the hemispheres is adaptive for functional recovery after stroke.

Furthermore, peripheral changes have been identified in motor axons in the affected limb of chronic stroke patients (Jankelowitz et al., 2007). Of further relevance, ipsilateral ("unaffected") limbs have demonstrated impairment in grip strength and dexterity using kinetic and kinematic measures in the subacute (Nowak et al., 2007) and chronic phases (Quaney et al., 2005) of stroke patients. These studies provide evidence for plasticity involving the peripheral motor pathways bilaterally, but again, given the cross-sectional nature of these studies, the timing and clinical significance of these changes post-stroke has yet to be established.

As such, the present study combined clinical assessment, with central and peripheral motor threshold-tracking techniques using paired-pulse transcranial magnetic stimulation and nerve excitability studies, to assess the entire neural axis (bilaterally) in acute stroke patients from time of ictus, followed longitudinally over 3-months. The aim was to define the nature and temporal evolution of post-stroke excitability changes in central and peripheral motor pathways, and their association

with functional improvement during the acute recovery process. Further, we hypothesized that cortical disinhibition, particularly over the contralesional hemisphere, will become less prominent in the later stages following an acute stroke, as access to alternative neural networks become better established. Secondly, we also hypothesized that nerve excitability changes may develop in peripheral motor axons immediately following an acute stroke in response to the changes occurring in the central nervous system.

2 METHODS

2.1 Subjects

Patients diagnosed with a first-time unilateral ischemic stroke and motor deficit were recruited for the study in the acute period (within 7 days post-ictus), from a specialized acute stroke unit. Acute stroke was confirmed by diffusion-weighted MRI. Patients with disorders affecting the central and/or peripheral nervous system or taking medications that could potentially affect these and thereby influence excitability parameters, or patients with contraindications to transcranial magnetic stimulation, were excluded. Written informed consent was obtained from the participants and the study was approved by the local Human Research Ethics Committee.

The study cohort consisted of 31 stroke patients (15 males, 16 females; aged 29-90, mean 69.4±15.0 years) recruited within the acute period (mean 6.3 days). Subcortical lesions were evident in 52% of patients, 32% had cortical lesions, 9%

had embolic lesions involving both cortical and subcortical regions, and 7% had pontine lesions. Clinical, functional and electrophysiological assessments were performed within the same session on patients within 1 week of the acute stroke, and repeated at 3-month follow-up (range 85-109 days; mean 96.5 days. Thirty controls (18 males; aged 43-92 years; mean = 60.4±15.9 years) were also studied, with cortical stimulation over the left hemisphere and peripheral stimulation of the right hand. No side to side differences in cortical and peripheral excitability parameters in controls, have been demonstrated by our laboratory and previous studies (Butefisch et al., 2003; Cicinelli et al., 2003; Jankelowitz et al., 2007).

2.2 Clinical assessment

Formal clinical and functional impairment was assessed using the National Institute of Health Stroke Scale (NIHSS), the modified Rankin scale (mRS), the upper limb component of the Fugl-Meyer score (FM), and the Barthel index (BI). The measure of reflex activity was excluded from the FM assessment, to form a modified FM score with a maximum of 60, giving a more unidimensional assessment, as previous studies found that the reflex items reflected a different motor control construct than the other items (Woodbury et al., 2008).

2.3 Cortical function

Measures of cortical excitability and thereby function were assessed using threshold-tracking transcranial magnetic stimulation according to previously described methodology (page 58). Both stroke affected and unaffected

hemispheres were assessed separately with recordings measured over the contralateral abductor pollicis brevis muscle (APB).

2.4 Peripheral nerve function

Peripheral nerve excitability studies were performed on affected (AL) as well as unaffected (UL) upper limbs of stroke patients, using previously described methodology (page 67). Motor axons of the median nerve were electrically stimulated at the wrist and the compound muscle action potential (CMAP) obtained from APB was recorded.

2.5 Statistical analysis

Group data are expressed as mean ± SEM and compared using either Student's *t*-test or Mann-Whitney *U*-test depending on normality of data distribution, and applied when analyzing patient against control data. Likewise, side-to-side paired data for each considered variable obtained at baseline were compared using either paired *t*-test or Wilcoxon test. A repeated measures analysis of variance (ANOVA) with time as the within-group factor, and study side as the between-group factor, and follow-up pair-wise comparisons, were performed to test the evolution of neurophysiological data. The Bonferroni correction was applied. Clinical data between the study time-points were compared using the Wilcoxon test. Correlation between excitability indices and clinical scores were analyzed by Spearman's rank test. A P-value of <0.05 was regarded as statistically significant. It is noted that in our control data for SICI with conditioning stimulus at 70%RMT (Vucic et al., 2006),

no age-related changes were evident between younger and older subjects. Peripheral nerve excitability measurements were compensated for age and temperature before statistical analysis, using relations obtained in controls (Kiernan et al., 2000; Kiernan et al., 2001a; Kiernan et al., 2001b).

3 RESULTS

3.1 Acute stroke baseline

3.1.1 Clinical assessment

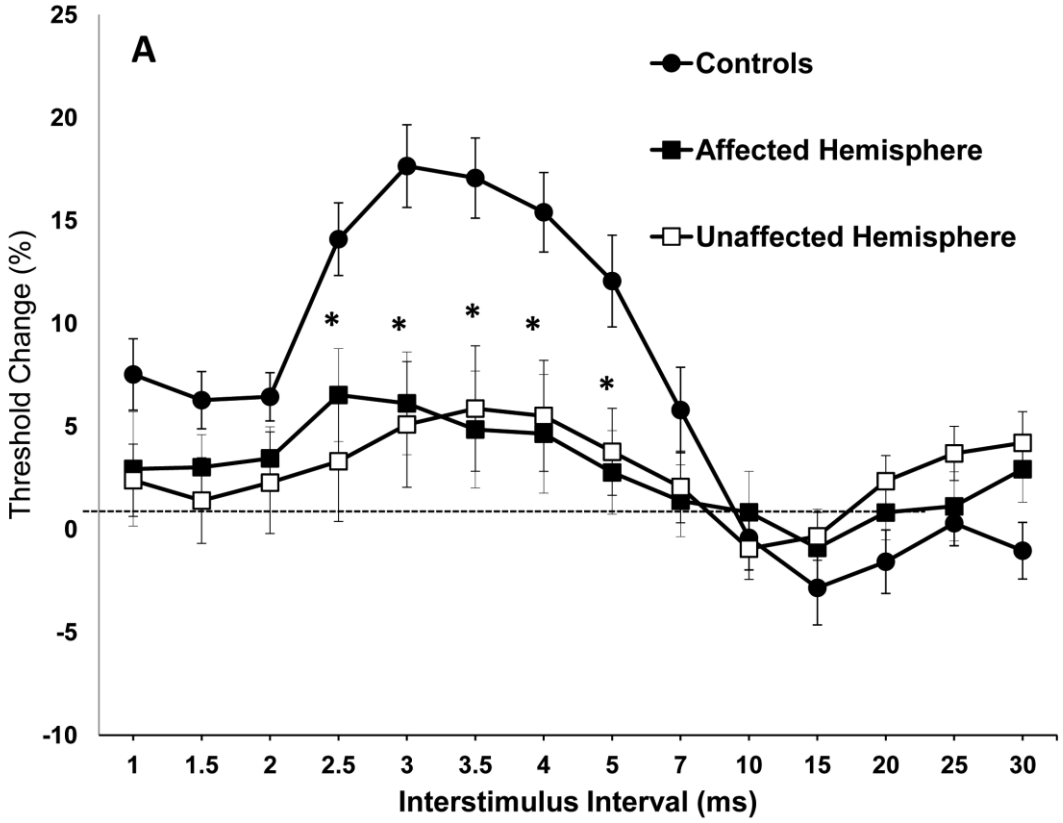
Clinical scores of patients ranged from mild to severe, with inability to achieve independence in 35%. The mean for FM score was 46.3 (range 0-60), NIHSS 5 (0-19), BI 65.8 (0-100) and mRS 2.9 (0-5), translating to moderate levels of motor, global neurological and functional impairment.

3.1.2 Cortical Function

Affected hemisphere motor cortices were inexcitable in 19% of patients (absent target MEP response at maximum stimulator output), all of whom exhibited moderate-severe functional impairment (mean FM=28, NIHSS=9, BI=36.7, mRS=4.2). In the remainder of patients, RMT was not significantly different in controls (59.8±1.8%) when compared to affected (59.4±3.4%,*P*=0.89) and unaffected hemispheres (58.1±1.9%,*P*=0.5), nor between hemispheres (*P=0.32*). Assessment of intracortical excitability established that averaged SICI (over 1-7ms interstimulus-intervals), was significantly reduced in both affected (4.3±1.3%, *P*=0.001) and unaffected (3.6±1.9%, *P*=0.001) hemispheres compared to controls

(11.4±1.3%) (Fig. 2.1), without side-to-side differences (*P*=0.86). Averaged ICF was not different in affected (1.1±1.2%, *P*=0.19) and unaffected (1.2±1.0%, *P*=0.13) hemispheres when compared to controls (-1.4±1.3%), and between hemispheres (*P*=0.72).

Assessment of other parameters of cortical function established no significant differences in CSP duration (controls=215.8±5.2ms; AH=221.5±10.6ms, *P*=0.59; UH=218.7±6.1ms, *P*=0.23) and maximum MEP amplitude (expressed as percentage of CMAP amplitude) (controls=24.6±3.5%; AH=38±8.1%, *P*=0.98; UH=44.2±6%, *P*=0.72) between hemispheres in stroke patients and controls, as well as between hemispheres (CSP: *P*=0.79; MEP amp: *P*=0.39).



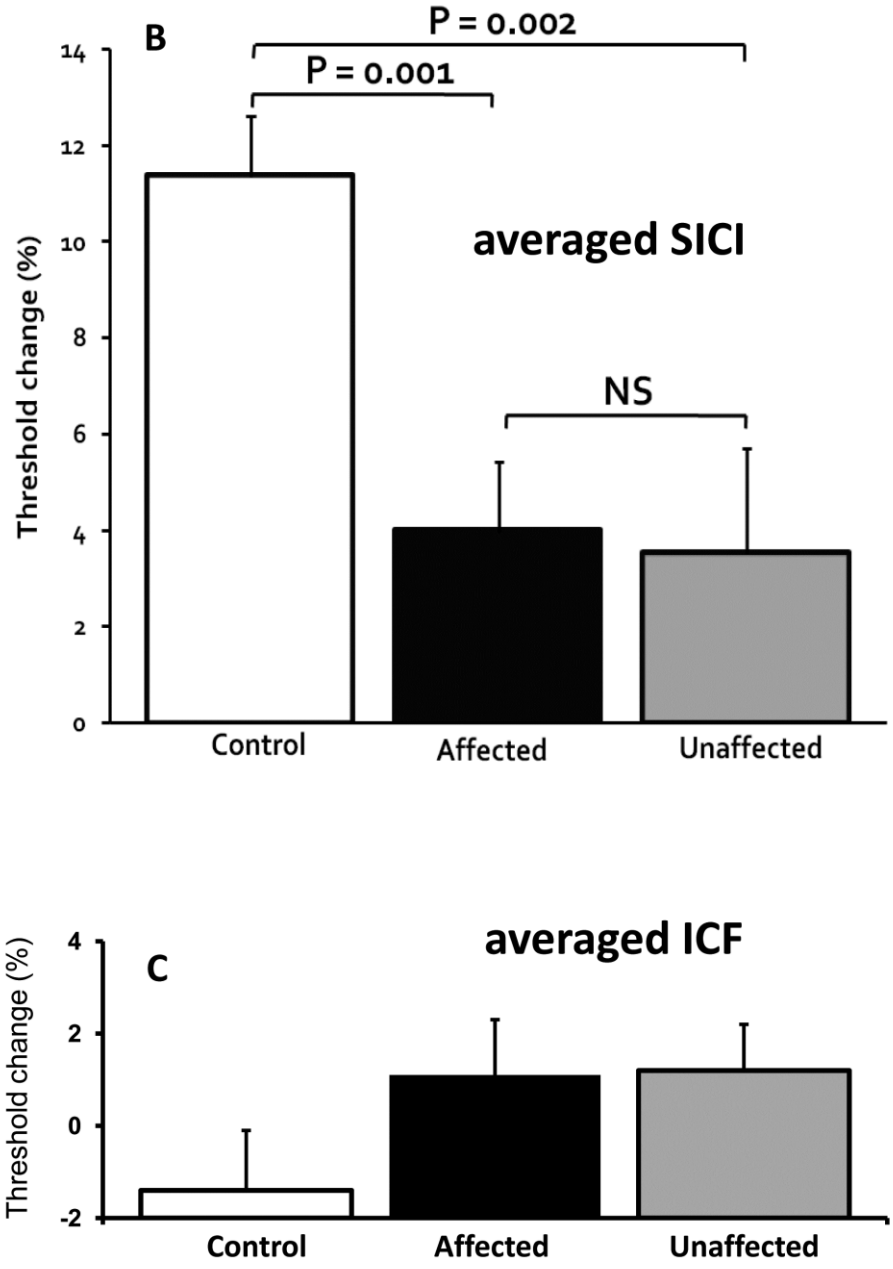


Figure 2.1. Baseline paired-pulse subthreshold conditioning TMS demonstrating (A)reduction in short-interval intracortical inhibition (SICI) in affected and unaffected hemispheres; (B)significant reductions in averaged SICI (between interstimulus-intervals of 1 to 7ms) in affected and unaffected hemispheres compared to controls; (C)no significant differences in averaged intracortical facilitation (ICF) between affected, unaffected hemispheres and controls. **P*<0.01, NS = not significant. Threshold change is the difference in test stimulus intensity required to evoke the target motor evoked potential (MEP) calculated as: [Conditioned test stimulus intensity - RMT]/RMT x 100.

3.1.3 Peripheral nerve function

There was a significantly greater change to hyperpolarizing currents observed in affected (AL) [TEh(90-100ms): -132.9±4.5%] compared with unaffected limbs (UL) (-121.6±3.6%, *P*=0.03) as well as to controls (-112±3.2%, *P*=0.003) (Fig. 2.2A). The difference between unaffected limbs and controls, however, was not statistically significant (*P*=0.13).

The findings in TE were accompanied by significant reductions in hyperpolarizing I/V gradient in affected (0.26±0.01) when compared to unaffected limbs (0.29±0.14, *P*=0.005) and controls (0.32±0.11, *P*=0.001) (Fig. 2.2B). The difference between unaffected limbs and controls did not reach statistical significance (*P*=0.088). The greater threshold change to hyperpolarizing currents observed in TE accompanied by the lower gradient in hyperpolarizing I/V slope demonstrated in affected limbs, suggests impaired accommodation to hyperpolarizing currents reflecting reduction in inward-rectifying (I_H) conductance of motor axons (Kiernan et al., 2000). There were no differences in depolarizing threshold electrotonus between affected and unaffected limbs of patients [TEd(90-100ms): AL 47.6±0.7%, UL 46.5±1.1%, *P*=0.49], although affected limbs showed larger threshold changes compared to controls (44.3±0.6%, *P*<0.001) whilst there was a trend for this difference between unaffected limbs and controls (*P*=0.06)(Fig. 2.2A).

In terms of other peripheral functional parameters, CMAP amplitudes were no different between stroke patients and controls (AL=5.7±0.6mV, *P*=0.30;

UL=5.4±0.5mV, *P*=0.12; controls=6.9±0.6mV;) or when comparing the limbs (*P*=0.82) In addition, $\tau_{SD,}$ which reflects nodal persistence Na^+ conductance (Bostock and Rothwell, 1997) was also comparable between the 3 groups (AL=0.48±0.02ms, *P*=0.09; UL=0.45±0.02ms, *P*=0.40; controls=0.43±0.02ms; paired, *P*=0.29). There were no differences in recovery cycle measures between limbs of patients compared to controls. Specifically, the relative refractory period was similar between groups (AL=3.3±1.0ms, *P*=0.24; UL=3.1±1.0ms, *P*=0.051 ; controls=3.1±1.0ms; paired, *P*=0.30), as was superexcitability (AL=-22.0±1.0%, *P*=0.27; UL=-21.6±1.1%, *P*=0.24; controls=-23.1±1.0%; paired, *P*=0.79) and late-subexcitability (AL=14.3±1.0%, *P*=071.; UL=13.6±0.7%, *P*=0.48; controls=14.5±0.6%; paired, *P*=0.67). The absence of differences in τ_{SD} and recovery cycle parameters suggests comparable resting membrane potentials in affected and unaffected limbs of stroke patients (Burke et al., 2001).

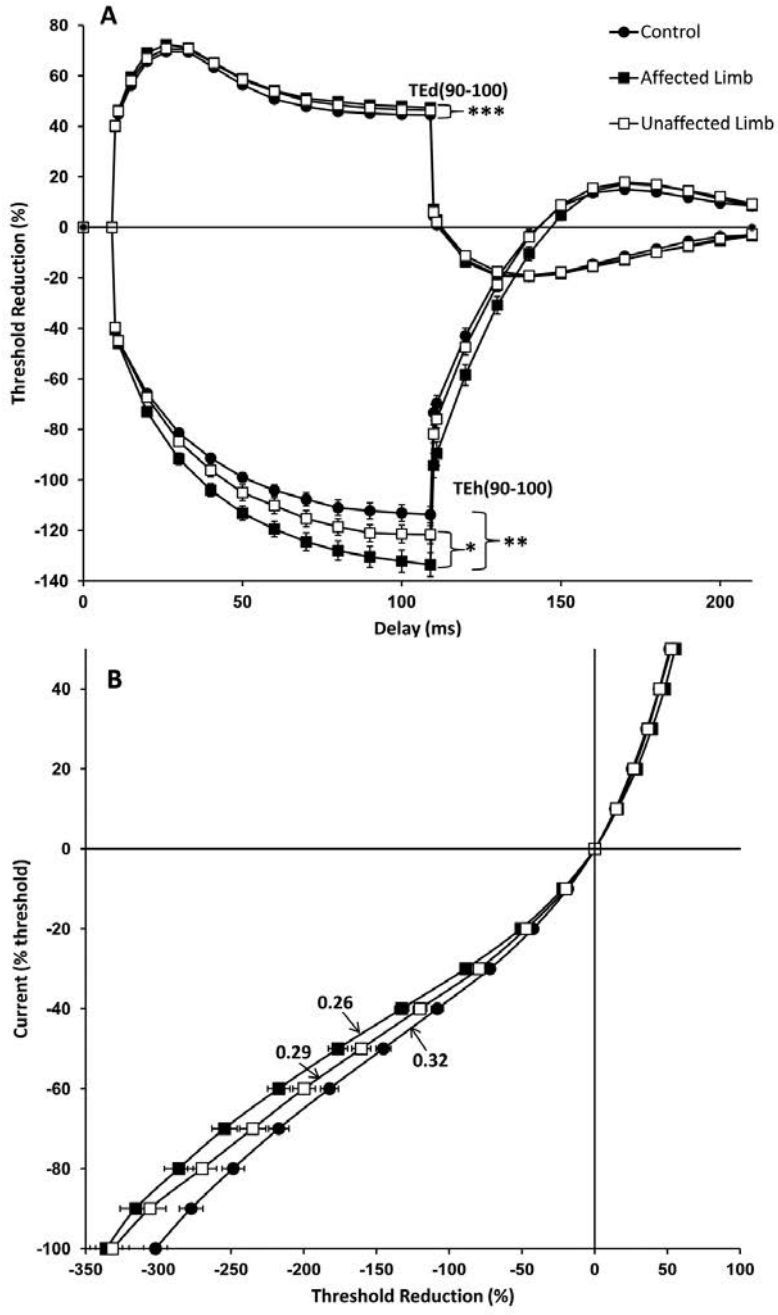


Figure 2.2. (A)Threshold electrotonus demonstrating significant reductions in accommodation to hyperpolarizing currents [TEh(90-100)]in affected limbs compared to both unaffected limbs and controls. Affected limbs also showed greater change to depolarizing currents [TEd(90-100)] compared to controls. (B)Current-threshold relationship demonstrating significantly reduced hyperpolarizing slope in affected limbs compared to unaffected limbs and controls. **P*<0.05,***P*<0.01,****P*<0.001. TEh(90-100), threshold change produced by prolonged subthreshold hyperpolarizing currents measured at 90-100ms delay; TEd(90-100), threshold change produced by prolonged subthreshold depolarizing currents measured at 90-100ms delay.

3.2 Longitudinal 3-month stroke follow-up

3.2.1 Clinical assessment

At 3 month follow-up, there were significant improvements in all clinical scores compared to baseline assessments (Fig. 2.3A-D) reflecting functional independence in all patients at follow-up.

3.2.2 Cortical function

Averaged SICI remained suppressed in both hemispheres compared to controls at 3-months (AH: 4±2.3%, UH: 5±2.3%, P=0.006) but were no different compared to baseline recordings and to each other (ANOVA: $F_{1,24}$=0.04; P=0.84) (Fig. 2.4). Of the cohort of stroke patients with inexcitable affected hemisphere cortices at baseline, all had normalization of cortical excitability (RMT=60.9±8%), associated with improvement in clinical scores from moderate-severe levels of disability to functional independence (mean FM= 54.25; NIHSS=2.25; BI=86.25; mRS=2.5).
All other parameters including ICF, RMT, CSP duration and MEP amplitude remained unchanged in both hemispheres compared to baseline.

3.2.3 Peripheral nerve function

Changes in nerve excitability in limbs of stroke patients persisted at 3-months. In particular, indices that were significantly different compared to controls at baseline showed little change at follow-up.

3.3 Clinical correlations

At baseline and 3-months, correlations were examined between cortical and peripheral measures, and clinical assessment. There were no relationship between SICI in both hemispheres and clinical scores, nor were there correlations between peripheral excitability parameters and clinical measures. However, RMT and CMAP of the affected sides correlated with all functional scores (FM, NIHSS, BI and mRS) (Table 2.1). In other words, better clinical scores were associated with lower RMT and higher CMAP amplitude. Correlations were sought between central and peripheral measures to explore the relationship between changes in upper and lower motor neuron pathways. This demonstrated a negative correlation between RMT and CMAP (r=-0.51, *P*=0.036). Together, these suggest that corticomotoneuronal dysfunction following an ischemic lesion is associated with disruption to impulse conduction downstream and consequently greater functional impairment.

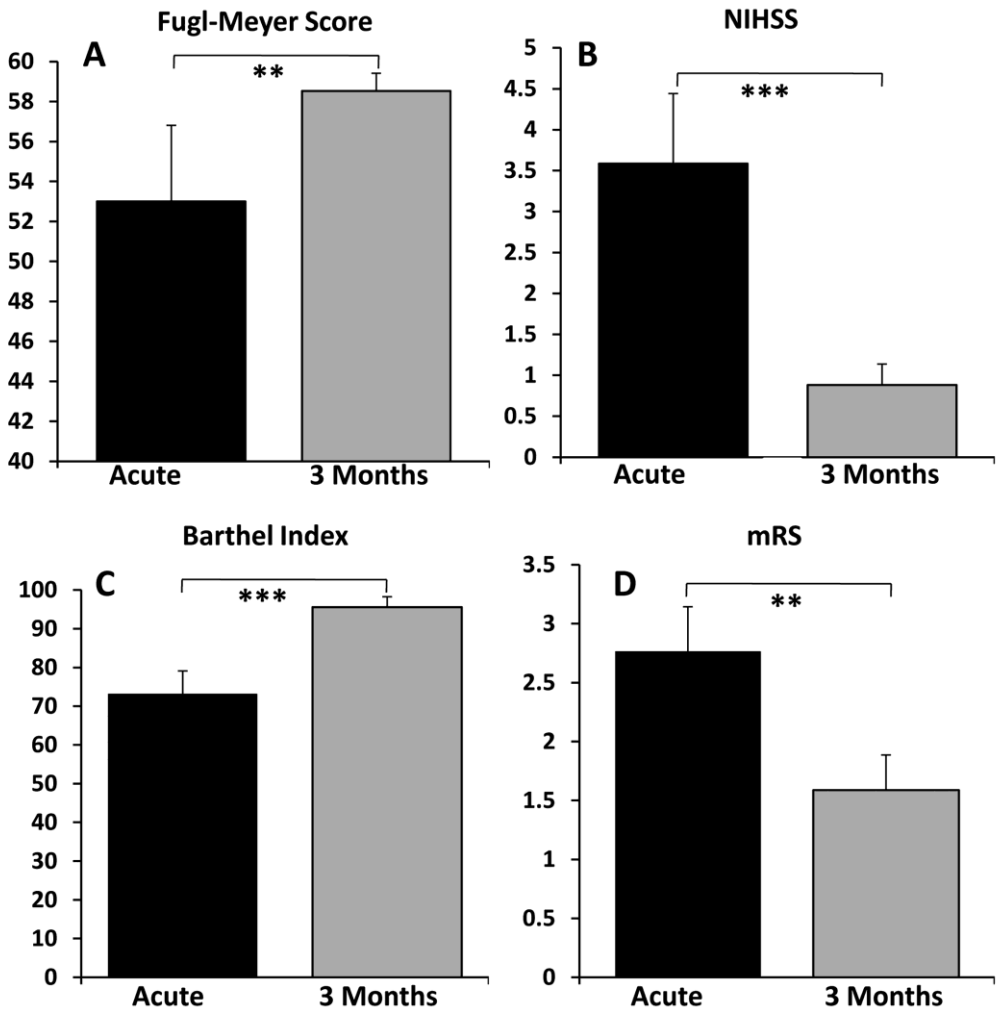


Figure 2.3. Significant improvements in all clinical scores (A-D) at 3-months follow-up compared to baseline. ***P*<0.01,****P*<0.001.

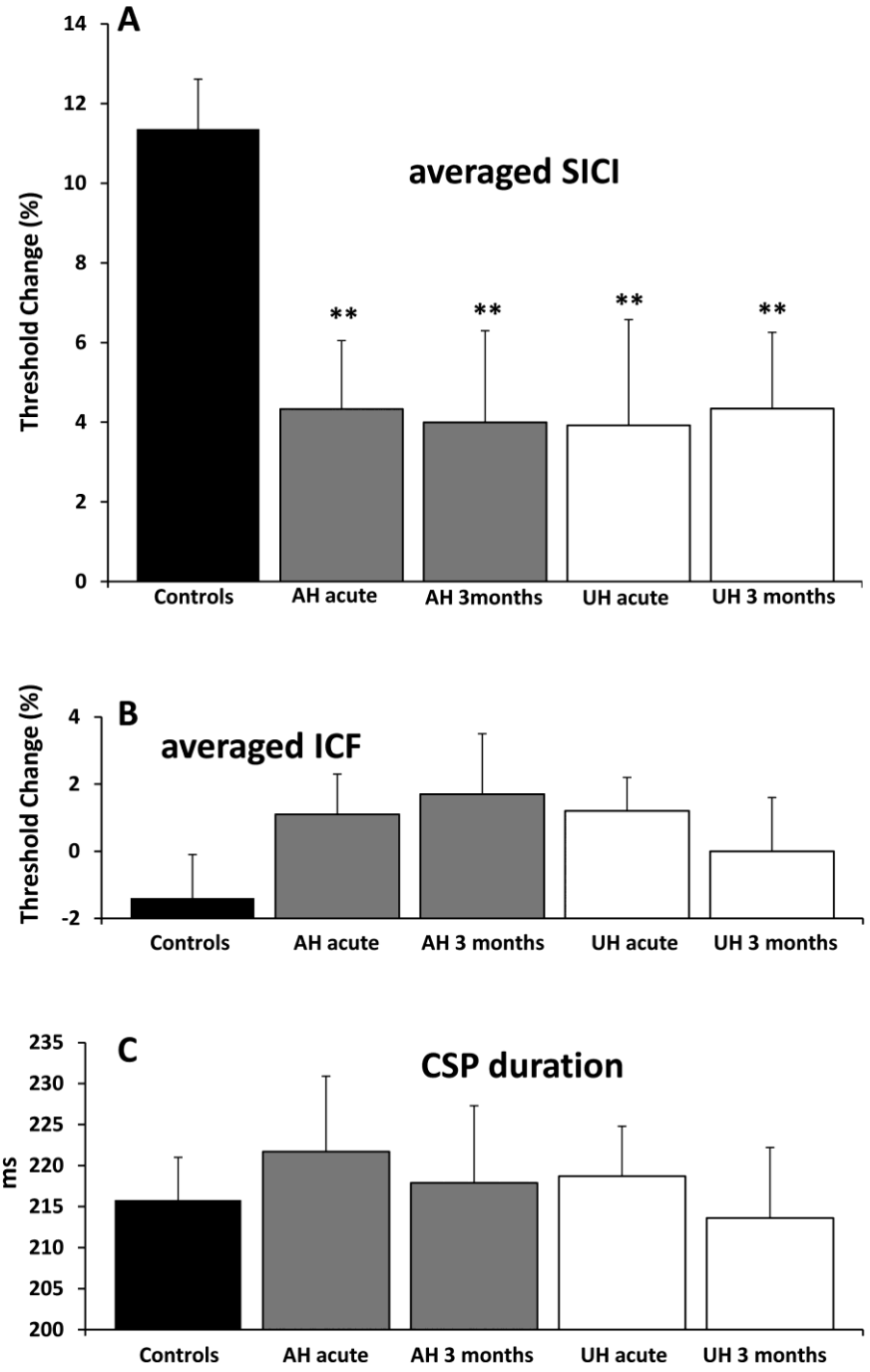


Figure 2.4. (A)Persistently suppressed averaged SICI (short-interval intracortical inhibition) in affected (AH) and unaffected (UH) hemispheres at 3-months. (B) and (C) No significant differences in averaged ICF (intracortical facilitation) and CSP (cortical silent period) at 3-months compared to baseline and controls. ***P*<0.01. Threshold change is the difference in test stimulus intensity required to evoke the target motor evoked potential (MEP) calculated as: [Conditioned test stimulus intensity - RMT]/RMT x 100.

Functional scores	RMT		CMAP	
	r	**P-value**	**r**	**P-value**
FM	-	-	0.637	<0.001
NIHSS	0.573	0.016	-0.621	<0.001
BI	-0.503	0.04	0.650	<0.001
mRS	0.515	0.035	-0.646	<0.001
CMAP	-0.510	0.036	-	-

Table 2.1. Correlations between functional scores, central and peripheral studies at baseline, demonstrating significant correlations between central (RMT) and peripheral (CMAP) electrophysiological parameters and clinical scores. r= correlation coefficient.

4 DISCUSSION

Using a novel combination of threshold tracking techniques, combined with clinical assessment to simultaneously investigate central and peripheral neuronal excitability in acute stroke patients, the present study has identified evolution of neuroplastic changes that develop at central and peripheral levels shortly after stroke-onset. Persistent reductions in SICI in both affected and unaffected hemispheres, combined with persistent peripheral nerve excitability abnormalities, followed by significant improvements in functional measures over 3-months, suggest an adaptive process promoting recovery after stroke.

Whilst similar results were also observed by previous investigators that utilized a cross-sectional design, our longitudinal study was able to critically determine the evolution of these changes. In addition, the present study has identified significant functional improvements in a cohort of acute stroke patients who initially exhibited inexcitable motor cortices, suggesting that the absence of ipsilesional responses in the hyperacute period does not always correlate with poor functional outcome (Escudero et al., 1998; Pizzi et al., 2009). Furthermore, it has been proposed more recently, that the presence of an MEP response is more useful for predicting outcome when combined with clinical assessment as well as novel imaging modalities such as diffusion tensor MRI to better define the structural integrity of the corticomotor pathways (Stinear, 2010; Stinear et al., 2007). In contrast to previous studies by Swayne and colleagues (Swayne et al., 2008), the acute disinhibition observed over the contralesional cortex in our study was not demonstrated in their cohort, and may be related to the difference in sample size with a larger population size used in our present study.

4.1 Cortical plasticity

Stroke alters the balance between cortical excitatory and inhibitory circuits, and the excitability changes occurring bihemispherically may shift over time in association with stroke recovery (Bashir et al., 2010). The present study has identified reductions in intracortical inhibition (SICI) in both the ipsilesional and contralesional hemispheres in the immediate period (<1 week) following acute stroke. These changes persisted at 3 months, in the context of significant improvements in all

clinical scores, suggesting that persistent disinhibition during the first 3-months of acute stroke is associated with functional recovery.

The reduction in SICI, a reflection of down-regulation in GABAergic circuits via $GABA_A$ receptors (Bashir et al., 2010), enables the unmasking of functionally latent networks, permitting cortical reorganization critical in stroke recovery (Bashir et al., 2010; Murase et al., 2004; Westlake and Nagarajan, 2011). Specifically, the inhibitory neurotransmitter GABA is critical for cortical plasticity as blockade of GABAergic signaling are associated with changes in cellular excitability including long-term potentiation that mediates learning and memory (Butefisch, 2004). Focal and ischemic cortical lesions in rat models have demonstrated down-regulations in $GABA_A$-receptor function and associated decreases in GABAergic intracortical inhibition in both lesioned and contralesional motor cortices (Neumann-Haefelin and Witte, 2000; Witte, 1998). Accompanying this, rat models have also demonstrated axonal sprouting, dendritic growth and pruning as well as synaptogenesis and enhanced synaptic efficiency in both hemispheres (Bury and Jones, 2002; Jones et al., 1996; Luke et al., 2004; Stroemer et al., 1995). Of further relevance, bihemispheric reorganization of cortical networks may result in enhanced inter-hemispheric connectivity (Grefkes and Fink, 2011), thereby contributing to a recovery of motor function. Moreover, the role of $GABA_A$-mediated processes in stroke recovery is further supported by the findings that reducing $GABA_A$-mediated inhibition using an antagonist resulted in improvements in stroke outcome in mice (Clarkson et al., 2010), as well as worsening of stroke

deficits in patients who were administered midazolam, which increases GABAergic inhibition (Lazar et al., 2002).

Whilst decreased cortical inhibition in the affected hemisphere appears consistent in both the acute and chronic periods following functional improvement (Marshall et al., 2000; Murase et al., 2004; Ward et al., 2003b), the evolution of excitability changes in the unaffected hemisphere as a function of recovery, remains complex. The balance between excitability changes is controlled by mutual interhemispheric inhibition, and although contralesional hyperexcitability occurs acutely after stroke, the concern remains whether persistent hyperexcitability may result in potentially detrimental inhibitory effects on the ipsilesional hemisphere (Murase et al., 2004; Ziemann, 2005). Previous reports have demonstrated chronic stroke patients with poor recovery have increased interhemispheric inhibition from the contralesional cortex, and that improved clinical scores were obtained following training and associated with reductions in this transcallosal inhibition (Harris-Love et al., 2011). One study however, demonstrated that the contralesional hyperexcitability was not associated with an increase in interhemispheric inhibition from the contralesional to ipsilesional motor cortex (Butefisch et al., 2008), but the study were performed on stroke patients within 6 weeks of ictus and the interhemispheric influences may change over time. Other cross-sectional studies have reported discrepancies regarding the prognostic influence of persistent contralesional cortical disinhibition on ultimate recovery, with some advocating a return to normal inhibition in association with recovery (Boyd et al., 2010; Manganotti et al., 2008; Murase et al.,

2004; Swayne et al., 2008), and others demonstrating the converse (Butefisch et al., 2003; Takeuchi et al., 2010). Some investigators have found that patients with good recovery of hand function had reductions in inhibition in the contralesional hemisphere whilst those with poor recovery did not (Butefisch et al., 2003). Furthermore, the contralesional hyperexcitability occurred regardless of alterations in interhemispheric inhibition from the ipsilesional cortex, suggesting that this may represent changes in neurotransmission associated with stroke recovery, as opposed to a release phenomenon (Butefisch et al., 2008).

The role of the unaffected hemisphere in stroke recovery may be multifactorial. Specifically, it may act as a relevant anatomical substrate for functional reorganization by recruitment of latent ipsilateral corticospinal tracts to the paretic limb to aid in motor recovery (Boyd et al., 2010). Such a hypothesis is supported by evidence for ipsilateral MEPs evoked in stroke patients using TMS (Caramia et al., 1996). The transcallosal transfer of information from the intact to lesioned hemisphere, may change from inhibitory to facilitatory, to aid adaptive plastic changes, as well as to facilitate perceived difficulty in movement of the paretic limb (Werhahn et al., 2002).

4.2 Plasticity of peripheral motor axons

The present study demonstrated significant changes in nerve excitability parameters in the affected limb of patients in the immediate period after ictus. The impaired accommodation to hyperpolarizing currents, as reflected by alterations in

TEh(90-100ms) and hyperpolarizing I/V slope, were suggestive of reductions in I_H conductances. However, there were also significant differences in accommodation to depolarizing currents [TEd(90-100ms)]observed in the affected limb compared to controls suggesting an additional biophysical alteration in motor axons (reduction in slow K^+ conductance) following stroke.

The subclinical involvement of the unaffected limb may be explained by downstream changes in response to contralesional hemispheric disinhibition, as well as involvement of ipsilesional uncrossed fibers. The exact mechanisms for the observed biophysical changes may not be as easily elucidated as those that occur in response to peripheral nerve insult, in particular those observed in peripheral nerve ischaemia (chapter 1), but may provide a window of insight into events occurring in the central nervous system.

Potential limitations to the present study include the non-randomized delivery of interstimulus intervals during the paired-pulse pulse paradigm by non-blinded investigators that may have resulted in a systematic effect in the MEP response. Whilst the cortical silent period (CSP) duration was measured from the onset of the facilitated MEP to return of EMG activity as per convention (Cantello et al., 1992; Daskalakis et al., 2003; Inghilleri et al., 1993; Vucic et al., 2006), this may differ from other investigators that report CSP duration as measured from the onset of stimulus.

5 CONCLUSIONS AND CLINICAL IMPLICATIONS

The present study demonstrated bihemispheric reductions in SICI from time of ictus to 3 months after stroke associated with functional recovery, suggesting that persistent bilateral disinhibition during this period may be associated with a process promoting ongoing utilization of additional networks, to thereby aid stroke recovery. A critical question remains to address when the balance in cortical excitability transforms from being adaptive to become maladaptive. Furthermore, in keeping with the hypothesis that the role of the contralesional hemisphere may become less critically important during stroke recovery, longitudinal studies extending beyond 3-months will be required to establish when the excitability changes begin to normalize. This becomes more critical with the advent of novel neuromodulating approaches aimed at reducing the excitability of the intact hemisphere, while increasing that of the affected hemisphere, such as non-invasive brain stimulation with repetitive-TMS and transcranial direct current stimulation (tDCS) (Lindenberg et al., 2010; Mansur et al., 2005).

Separately, bilateral alterations in peripheral motor axonal function confirm the presence of plasticity occurring in the peripheral nervous system evident immediately after stroke onset. Taken in total, the present study provides further insight into mechanisms of neuroplasticity that occur following an acute stroke, with implications for neuromodulation to facilitate stroke recovery.

CHAPTER 3

Botulinum toxin modulates cortical maladaptation in post-stroke spasticity

SUMMARY

Maladaptive plasticity involving the unaffected hemisphere (UH) in stroke patients may contribute to post-stroke deficits including spasticity. We investigated central and peripheral effects of botulinum toxin in post-stroke spasticity to determine whether there is modulation of cortical processes in the UH. Transcranial magnetic stimulation and peripheral nerve excitability studies were undertaken in 5 stroke patients with upper-limb spasticity, prior (T1) and 6-weeks post (T2) botulinum injection. TMS demonstrated inexcitable motor cortices of the affected hemisphere at T1 and T2, and short-interval intracortical inhibition (SICI) in the UH was significantly reduced at T1. At T2, SICI in the UH significantly increased compared to T1, normalizing to controls, and associated with clinical improvements in spasticity. Peripheral excitability parameters were unchanged following injection. Cortical excitability changes were demonstrated in UH, suggesting that clinical benefits of botulinum toxin relate to modulation of abnormal central reorganization (maladaptive plasticity) in post-stroke spasticity.

1 INTRODUCTION

Spasticity may form part of the spectrum of the upper motor neuron syndrome following stroke, with a prevalence rate between 17 and 46% at 1 year (Bhattacharya et al., 2005; Lundstrom et al., 2008). There is evidence to suggest that post-stroke deficits including spasticity are paralleled by reorganization of structures in the central nervous system in a way that is detrimental to stroke recovery (so-called “maladaptive plasticity”) (Pascual-Leone et al., 2005).

Cortical reorganization may occur in both hemispheres during the acute stages following stroke, (Liepert et al., 2000e; Manganotti et al., 2008; Shimizu et al., 2002) and it is proposed to represent an adaptive process to promote access to latent motor output systems. Such changes may be less required in the chronic stages of recovery, when alternative motor networks have become better established. For this reason, it is plausible to suggest that central reorganization in the chronic stages of stroke may represent a maladaptive process, not only associated with poor motor recovery, but with the emergence of other deficits such as spasticity. Specifically, it has been suggested that cortical excitability in the contralesional hemisphere should normalize during the chronic stages of stroke in those patients with good outcomes, while those with poor recovery have tended to maintain the contralesional changes (Murase et al., 2004; Swayne et al., 2008).

Patients with impaired recovery tend to have more substantial damage and disruption to the integrity of the ipsilesional cortex and its motor pathways and hence a greater reliance on recruitment of contralesional networks (Ward, 2006). These patients also have the greatest disability and highest risk of developing post-stroke spasticity (Ward, 2012). Hence, persistent maladaptive disinhibition in the intact hemisphere may contribute to the pathophysiology of spasticity in chronic stroke patients. In further support, recent use of 1 Hz repetitive transcranial magnetic stimulation (rTMS) over the intact hemisphere to enhance cortical inhibition resulted in improvements in spasticity (Mally and Dinya, 2008).

Botulinum toxin type A has been a major therapeutic approach for the treatment of spasticity in chronic stroke patients. The toxin acts peripherally by inhibiting acetylcholine (ACh) release from presynaptic nerve terminals at the neuromuscular junction, resulting in weakness of muscles (Curra et al., 2004). Limited evidence has also suggested that the toxin may alter peripheral axonal Na^+ conductances (Yerdelen et al., 2007). Recent studies however, have identified electrophysiological and functional changes within the central nervous system following peripheral intramuscular injection of botulinum toxin for movement disorders in association with clinical improvement (Byrnes et al., 1998; Gilio et al., 2000; Kojovic et al., 2011; Pauri et al., 2000; Senkarova et al., 2010; Thickbroom et al., 2003; Tomasova et al., 2011).

While it is generally thought that the clinical benefits of botulinum toxin depend primarily on peripheral mechanisms, the results of recent studies have also suggested the possibility for a central modulatory effect. These central mechanisms may be mediated directly through the transport of the toxin into the central nervous system, or alternatively by altering sensorimotor integration within the central nervous system (Allam et al., 2005; Filippi et al., 1993; Wiegand et al., 1976).

Studies of central and peripheral electrophysiological effects of botulinum toxin in spasticity developing after stroke, remain lacking. As such, this study aimed to investigate the effects of peripheral intramuscular injection of botulinum toxin on cortical and peripheral motor nerve excitability in chronic stroke patients with disabling limb spasticity. Studies of peripheral motor nerve excitability using novel neurophysiological techniques provide information regarding motor axonal ion channel function *in vivo* (Kiernan et al., 2000), while paired-pulse transcranial magnetic stimulation provides information on intracortical inhibitory and excitatory function in addition to corticomotoneuronal excitability (Vucic et al., 2006) . It was hypothesized that botulinum toxin may exert modulating effects on abnormal cortical reorganization, particularly over the stroke unaffected hemisphere, in spastic patients post-stroke, in conjunction with changes in peripheral motor nerve function.

2 METHODS

2.1 Subjects

Chronic stroke patients were screened, and the inclusion criteria used to select participants for the study included: (i) duration greater than 2 years since unilateral stroke to ensure that potential cortical plastic changes are not associated with ongoing functional stroke recovery; (ii) severe unilateral paresis with Fugl-Meyer score <15; and (iii) inexcitable ipsilesional motor cortex as determined by TMS. The last 2 criteria were chosen to select patients with significant disruption of the corticospinal tract and hence likely to be associated with persistent changes in cortical reorganization over the contralesional motor cortex.

A total of 40 electrophysiological studies were performed in a longitudinal design on 5 chronic stroke subjects with significant functional disability and unilateral upper limb spasticity (3 men, 2 women; aged 63.2 ±5.6 years, range 47-81) (Table 3.1). The time from stroke onset to study entry ranged from 3.5 to 5 years (mean 4.4 years). In general, the patients were naïve to botulinum toxin therapy or had recently commenced treatment. Specifically, patient 4 had 1 previous treatment with botulinum toxin to the affected upper limb, and patient 1 had 2 previous treatments. Botulinum toxin type A was injected into the affected forearm flexor muscles including the flexor carpi radialis (FCR), flexor carpi ulnaris (FDU), and flexor digitorum profundus (FDP) at a dose ranging from 25 to 37.5 mouse units in each muscle depending on the severity of the spasticity.

Clinical and electrophysiological assessments were performed on patients within 3 days prior to botulinum toxin injection (mean 1.4±0.4 days, range 1-3) and were repeated 6 weeks after injection when the clinical effects were maximal. In addition, 1 patient underwent clinical and electrophysiological studies multiple times over 3-months following botulinum toxin injection (1, 3, 5, 7, 9, and 11 weeks). Electrophysiological results were compared with 10 aged-matched controls (mean 61.3±3.4 years, range 48-83). Informed consent was obtained from the participants, and the study was approved by the local Human Research Ethics Committee.

	Age (y)	**Gender**	**Time since stroke (y)**	**Stroke location**	**mAS**		**Tardieu scale†**	
					Pre*	Post*	Pre*	Post*
1	68	M	5	MCA cortical	2	1	48	26
2	47	F	3.5	MCA cortical	3	1	30	0
3	81	M	4.5	MCA subcortical	2	0	33	0
4	61	F	4	Internal capsule	2	0	22	0
5	59	M	5	Internal capsule	2	1	16	10

Table 3.1. Patient characteristics. †Units are measured in degrees from neutral wrist position following a fast passive movement. *Pre- and post-botulinum toxin injection. MCA, middle cerebral artery.

2.2 Clinical assessment

Baseline motor function was assessed using the upper limb component of the Fugl-Meyer score (FM). Spasticity of the affected upper limb forearm flexors were evaluated pre- and post-botulinum toxin injection using the modified Ashworth Scale (mAS) (Bohannon and Smith, 1987) and the fastest component of the Tardieu scale (Haugh et al., 2006) in accordance with Lance's definition of velocity dependence in spasticity (Lance, 1980). In the latter, patients were assessed in a seated position, and a goniometer was used to determine the angle of catch measured from neutral wrist position (at 0°), following a fast passive movement of the joint from its flexed position. All assessments were performed by the same investigator (W.H.).

2.3 Cortical excitability

Measures of cortical excitability were assessed according to previously described methodology (page 58). Both stroke-affected and unaffected hemispheres were assessed separately, and a motor evoked potential (MEP) was obtained from the contralateral abductor pollicis brevis (APB) muscle.

2.4 Peripheral nerve excitability

Peripheral motor nerve excitability studies using previously described methodology (page 67), were performed on affected spastic (AL) and unaffected (UL) upper limbs of the chronic stroke patients.

Stimulating and recording sites for the FCR muscle were similar to that described by Jankelowitz (Jankelowitz and Burke, 2009). Motor axons of the median nerve were stimulated electrically at the elbow medial to the brachial artery, and the FCR compound muscle action potential (CMAP) was obtained with the active recording electrode over the body of the muscle at approximately the site recommended for needle electromyography that produced the largest amplitude. Skin temperature was monitored close to the stimulation site and was maintained above 32°C using towels.

2.5 Statistical analysis

Group data are expressed as mean ± SEM. Within-group clinical and electrophysiological data before and after botulinum toxin were compared using paired Wilcoxon signed rank tests, and results between controls and patients were compared using either Student *t*-tests or Mann-Whitney *U*-tests depending on normality of data distribution. A *P*-value of <0.05 was regarded as statistically significant.

3 RESULTS

3.1 Clinical assessments

All post-stroke patients had significant motor dysfunction with an upper-limb FM score of 5.6±1.4 (range 1-9). Measures of spasticity prior to botulinum toxin injection were 2.2±0.2 on mAS, and 29.8±5.4 degrees from neutral wrist position using the fastest component of the Tardieu method. Significant improvements in

spasticity were achieved following injection (mAS: 0.6±0.2, *P*=0.038; Tardieu: 7.2±5.1 degrees, *P*=0.042) (Table 3.1).

3.2 Cortical excitability

As per the inclusion criteria, all patients had relatively inexcitable motor cortices on transcranial magnetic stimulation (defined as an absent target MEP response at maximum stimulator output) over the stroke-affected hemisphere, in keeping with the severity of their functional disability (Escudero et al., 1998) . This was unchanged following botulinum toxin injection.

Over the contralesional hemisphere, averaged SICI prior to injection was significantly reduced (2.1±2.5%) compared to controls (15.1±2.1%,*P*=0.001). Following injection, there was a significant increase in averaged SICI (12.0±1.4%, *P*=0.02) normalizing to control values (*P*=0.712) (Fig. 3.1). Other parameters of cortical excitability over the contralesional hemisphere were unchanged following botulinum toxin injection and were no different from controls (RMT: before 68.7±4.8%, after 65.3±4.9%, *P*=0.07; controls 60.4±3.7%, *P*= 0.12; MEP amplitude: before 1.8±0.3 mV, after 2.7±0.5mV, *P*=0.08; controls 1.8±0.4mV, *P*=0.54; CSP: before 205.7±13.2ms, after 210.6±7.8ms, *P*=0.76; controls 215.7±8.3ms, *P*=0.25) (Table 3.2).

In the patient who was studied at multiple time points following injection, SICI demonstrated normalization that peaked at 7 weeks post-botulinum toxin, which

then trended towards pre-injection levels at 11 weeks. This was paralleled by changes in clinical spasticity that appeared to precede the changes observed in SICI (Fig. 3.2).

Patient	Averaged SICI (%)		RMT (%)		MEP amplitude (mV)		CSP duration (ms)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
1	6.72	16.04	78.4	77.4	1.18	1.04	179.6	229
2	-3.07	11.66	77.3	74.8	1.27	2.84	178.2	182.9
3	9.50	10.35	58.2	54.4	2.5	3.05	234.2	209
4	-1.19	11.86	73.8	65.5	2.36	4.36	239.6	210.8
5	-1.46	10.09	55.9	54.4	1.69	2.21	196.9	221.3
mean	2.1±2.5	12.0±1.4*****	68.7±4.8	65.3±4.9	1.8±0.3	2.7±0.5	205.7±13.2	210.6±7.8

Table 3.2. Cortical excitability parameters pre- and post-botulinum toxin injection. *Significant increase in averaged (short-interval intracortical inhibition) SICI post botulinum toxin injection. RMT, resting motor threshold; MEP, motor evoked potential; CSP, cortical silent period.

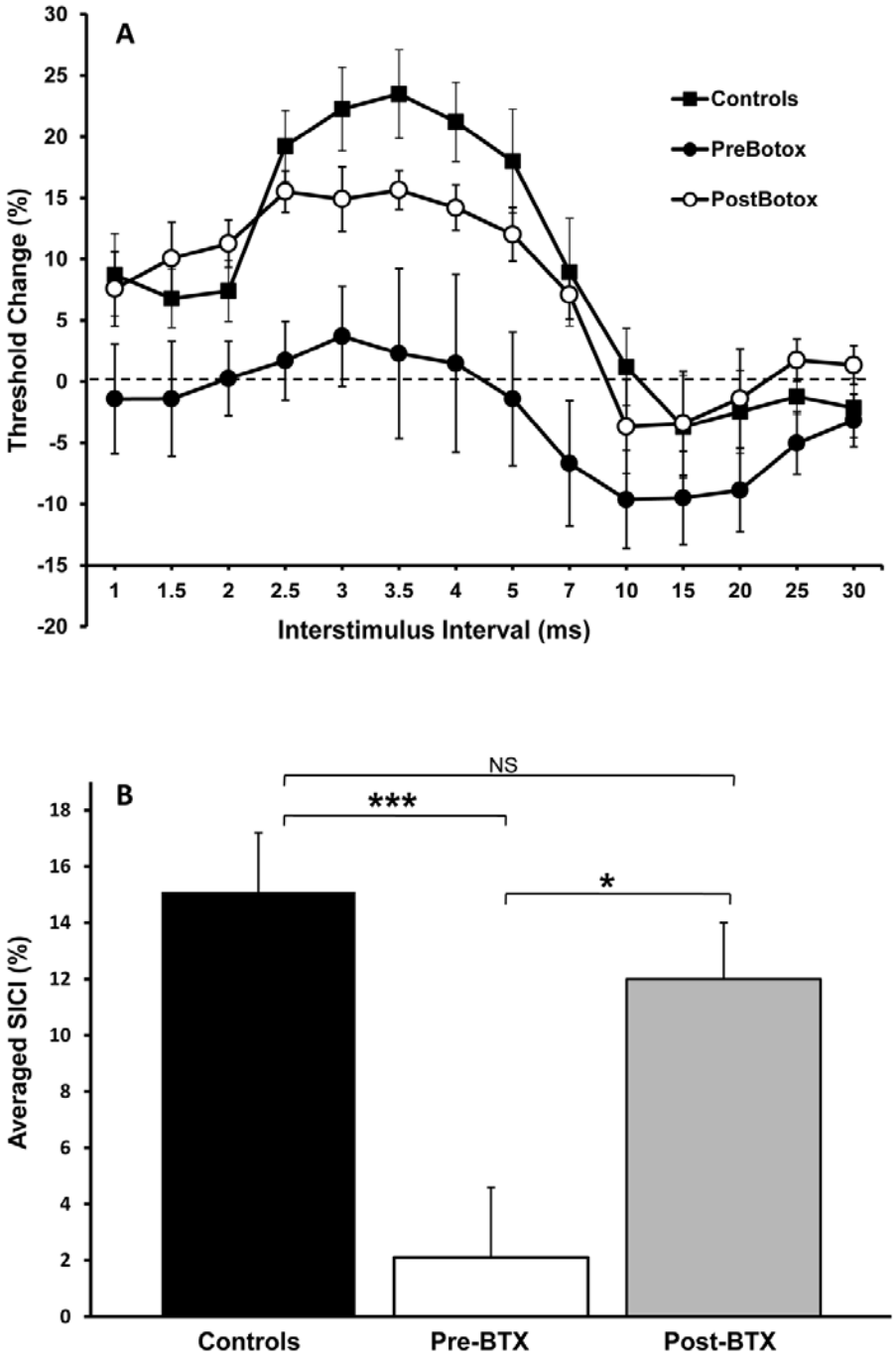


Figure 3.1. (A) Paired-pulse subthreshold conditioning transcranial magnetic stimulation demonstrating threshold change at different interstimulus intervals comparing contralesional motor cortices in patients pre- and post-botulinum toxin (BTX) injection with controls. (B) Averaged short-interval intracortical inhibition (SICI) was significantly reduced in the contralesional hemisphere of post-stroke patients with spasticity prior to peripheral BTX injection into the spastic limb. At 6 weeks after injection, there was a significant increase in averaged SICI, normalizing to control levels. NS = non-significant, * = <0.05, *** = <0.001.

3.3 Peripheral nerve excitability

Results are shown in Table 3.3. CMAP amplitudes of the injected limb post botulinum toxin (1.5±1.5mV) were significantly reduced compared to baseline values (7.56±1.12mV, *P*=0.04). This is an expected finding given the well documented action of botulinum toxin at the neuromuscular junction (Palomar and Mir, 2012). Other parameters of the SR curve reflecting properties of the nodal membrane, specifically the persistent Na^+ conductances, were unchanged (stimulus threshold: before 10.8±1.2mA, after 10.9±1.2mV, *P*=0.9; τ_{SD}: before 0.47±0.02, after 0.50±0.05, *P*=0.67). There were also no differences in measures of threshold electrotonus (a marker of internodal axonal membrane function), current-threshold relationship (a measure of nodal and internodal rectifying conductances), and recovery cycle parameters, in the injected spastic limb before and after botulinum toxin. Recovery cycle parameters include the relative refractory period (RRP) due to inactivation of nodal transient Na^+ channels, superexcitability (dependent on paranodal fast K^+ channels) and late subexcitability (determined in part by nodal slow K^+ channels). All nerve excitability parameters for the unaffected non-injected limb remained unchanged before and after botulinum toxin injection into the affected side.

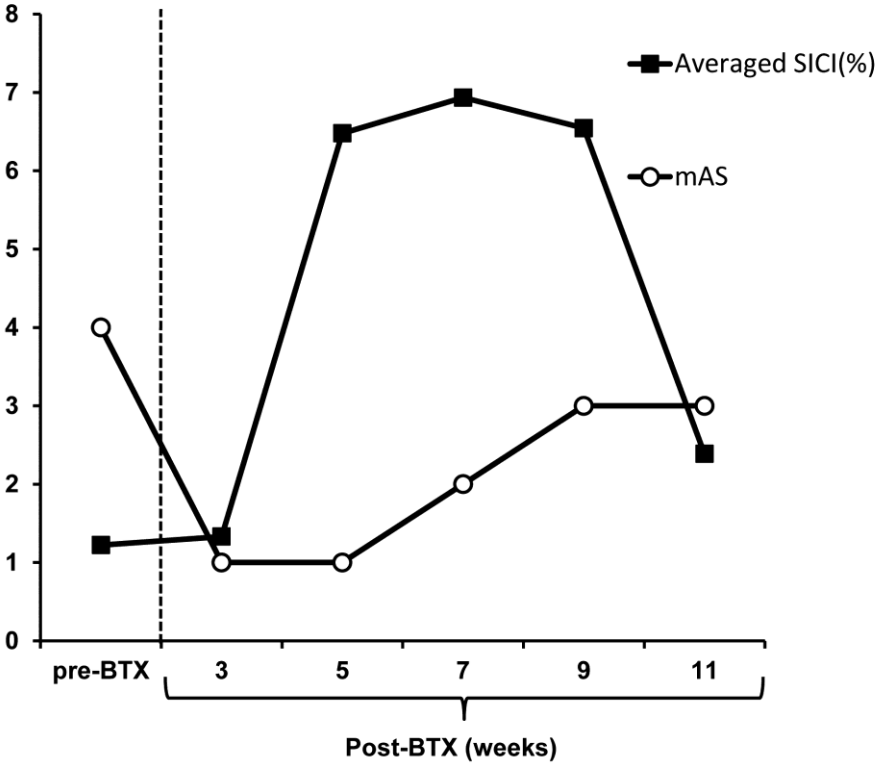


Figure 3.2. Changes in clinical spasticity as measured using modified Ashworth scale (mAS), and averaged short-interval intracortical inhibition (SICI) over contralesional hemisphere in a single patient studied at multiple time-points following intramuscular injection of botulinum toxin (BTX) into the spastic limb. Changes in SICI appear to lag behind clinical changes in spasticity.

Nerve excitability parameter	Injected limb			Non-injected limb		
	Before	**After**	***P***	**Before**	**After**	***P***
Stimulus-response						
CMAP amplitude (mV)	7.56±1.12	1.51±1.51	0.04*	6.43±1.12	7.01±1.14	0.64
Threshold current (mA)	10.78±1.17	10.93±1.18	0.90	9.45±1.25	9.89±1.16	0.85
τ_{SD} (ms)	0.47±0.02	0.49±0.05	0.67	0.45±0.04	0.49±0.02	0.37
Threshold electrotonus						
S2 accommodation (%)	15.26±2.0	11.86±2.47	0.39	15.67±1.93	15.13±2.21	0.84
Depolarizing threshold at 90- 100ms (%)	46.49±3.53	58.11±10.3	0.44	45.11±3.03	50.3±1.82	0.19
Hyperpolarizing threshold at 90-100ms (%)	-122.3±3.03	-116.2±6.63	0.29	-115.5±4.35	-114.9±1.36	0.86
Maximal depolarizing threshold change (%)	61.76±2.42	69.97±8.71	0.46	60.78±2.49	65.43±1.55	0.16
Current-threshold relationship (I/V)						
Resting I/V slope	0.64±0.04	0.60±0.05	0.90	0.61±0.08	0.55±0.02	0.44
Minimum I/V slope	0.17±0.03	0.21±0.01	0.40	0.19±0.02	0.21±0.02	0.51

Table 3.3. Nerve excitability parameters for the injected and non-injected upper limb before and after botulinum toxin. The only significant change in nerve excitability parameters was a reduction in CMAP amplitude in the injected spastic limb following botulinum toxin (*). Results indicate that there were no significant alterations in biophysical properties of motor axons in both injected and non-injected limbs after intramuscular botulinum toxin administration.

4 DISCUSSION

This study has identified reductions in intracortical inhibition over the intact hemisphere in patients with post-stroke spasticity that normalized following injection of botulinum toxin into the affected limb, at a time when the therapeutic effects of the injection were maximal. Furthermore, intracortical inhibition tended to revert to pre-injection values, in conjunction with the return of spasticity, as the clinical effects of the toxin wore off. This suggests that pre-existing maladaptive

plastic responses, present in the intact hemisphere, may contribute to the development of spasticity in the chronic stages of stroke.

Recently, fMRI studies on upper limb spasticity in chronic stroke patients, have identified increased activation in both stroke-affected and unaffected hemispheres, with subsequent botulinum toxin treatment-induced reductions in activation in both hemispheres associated with clinical improvements in limb spasticity (Senkarova et al., 2010; Tomasova et al., 2011). Moreover, the activation changes reverted to pre-botulinum toxin patterns at 3-months (Tomasova et al., 2011). The imaging changes demonstrated may be analogous to the cortical excitability changes observed in this study, particularly over the contralesional hemisphere. Impairment of intracortical inhibitory circuits may be 1 of the mechanisms through which spasticity is evoked and maintained in chronic stroke patients, and the amount of intracortical inhibition appeared to correlate with the degree of spasticity such that lower levels of inhibition correlated with higher degrees of spasticity (Marconi et al., 2011). The subsequent treatment-induced up-regulation in inhibitory circuits may facilitate remodeling of remaining connections in motor networks, with these neurophysiological changes potentially serving as potential functional substrates for reduction in spasticity (Marconi et al., 2011).

The changes in cortical excitability following intramuscular botulinum toxin administration may have several mechanisms. Of interest, TMS studies in normal human subjects have also demonstrated elevations in intracortical inhibition

following peripheral botulinum toxin injections (Kim et al., 2006). It has been proposed that botulinum toxin may have direct actions at the central level after hematogenous dissemination via the blood-brain barrier, or alternatively retrograde axonal transport through motor fibers and propriospinal pathways (Gilio et al., 2000) to both ipsi- and contralateral motor cortices, given that ipsilateral motor pathways have been suggested to be involved in functional reorganization after stroke.

Propriospinal pathways consisting of crossed and uncrossed fibres, have been demonstrated to be of importance in upper limb movement and control (Welberg, 2012) and are involved in reorganization following lesions of the central nervous system (CNS) (Cote et al., 2012). About 10% of corticospinal tracts are uncrossed (Davidoff, 1990; Hoyer and Celnik, 2011; Jang, 2009) and are hypothesized to act as a mechanism of motor recovery following brain injury (Hoyer and Celnik, 2011; Jang, 2009; Yeo and Jang, 2012). Their persistent involvement into the chronic stroke period may be associated with poorer recovery and function (Jang, 2007), which may be the case for chronic stroke patients with significant spasticity. While not performed in this study, other investigators have demonstrated ipsilateral MEPs when stimulating the contralesional hemisphere of stroke patients (Misawa et al., 2008), further supporting the involvement of ipsilateral motor pathways in stroke. Following retrograde transport into the CNS, the toxin may then inhibit the release of acetylcholine and other neurotransmitter systems involved with GABA (the latter which mediates intracortical inhibitory circuits).

This study also demonstrated a lack of change in peripheral motor nerve excitability parameters of the injected limb following botulinum toxin (apart from the expected reduction in distal CMAP amplitudes). Intramuscular botulinum toxin injection resulting in functional denervation may be expected to trigger changes in motoneuronal excitability due to alterations in retrograde signaling. Studies on rats however, demonstrated that acetylcholine (ACh) release and its associated Ach-receptor activation continued at a low level following botulinum toxin injection that was sufficient to enable retrograde regulation and maintenance of motoneuron excitability properties (Nakanishi et al., 2005).

The question is whether the changes observed in intracortical excitability over the intact hemisphere in this study represent a cause or effect following clinical improvement with botulinum toxin injection. Some investigators postulate that injected botulinum toxin not only acts on extrafusal muscle fibers but also on the intrafusal (gamma motoneuron) neuromuscular junction (Filippi et al., 1993). The subsequent fusimotor denervation results in reduced Iα muscle spindle afferent input (Byrnes et al., 1998; Filippi et al., 1993; Thickbroom et al., 2003) and functional changes in central motor mechanisms followed by changes in cortical excitability via reorganization of inhibitory interneuronal circuits (Kim et al., 2006).

In support of this, our patient who was studied at multiple time points over 3-months after botulinum toxin injection demonstrated changes in clinical spasticity

that preceded the changes observed in SICI (Fig. 3.2). Peripheral excitability testing in this study only assessed motor fibers and hence may not reflect possible changes in peripheral sensory nerve fibers. In addition, more recent studies using rTMS to upregulate $GABA_A$-mediated inhibition over the intact hemisphere have resulted in the release of spasticity (Kakuda et al., 2011; Mally and Dinya, 2008), suggesting that the modulation of intracortical inhibition causes the clinical improvements. For this reason, we hypothesize that the clinical benefits of botulinum toxin in post-stroke spasticity are related to its effects at both the peripheral and central level.

In conclusion, the results of this study emphasize the transitory nature of cortical reorganization over the contralesional hemisphere in post-stroke spasticity in a cohort of patients with severe motor impairment and significant disruption to ipsilesional corticospinal tract integrity. The findings suggest that the maladaptive central plastic response may be maintained by an ongoing alteration in sensory feedback from spastic muscles, and this may be modulated by the effects of peripheral botulinum toxin administration. In addition, the results also demonstrated that intramuscular botulinum toxin injection had no effect on the biophysical properties of peripheral motor axons.

CHAPTER 4

Motor Cortex Excitability in Acute Cerebellar Infarct

SUMMARY

Limited evidence to date has demonstrated changes in excitability that develops over the contralateral motor cortex after a cerebellar infarct. As such, the present study investigated changes in excitability over the contra- (contraM1) and ipsilateral motor cortices (ipsiM1), in patients with acute cerebellar infarct, to determine whether the changes may have functional relevance. Paired-pulse transcranial magnetic stimulation, combined with detailed clinical assessment, was undertaken in ten patients presenting with acute unilateral cerebellar infarct. Studies were undertaken within 1 week of ictus and followed longitudinally at 3-, 6-, and 12-month periods. Comparisons were made with 15 age-matched controls. Immediately following a stroke, short-interval intracortical inhibition (SICI) was significantly reduced over the contraM1 in all patients ($P = 0.01$), while reduced over the ipsiM1 in those with severe functional impairment ($P = 0.01$). Moreover, ipsiM1 SICI correlated with impairment ($r = 0.69$, $P = 0.03$), such that less SICI was observed in those patients with most impairment. Cortical excitability changes persisted over the follow-up period in the context of clinical improvement. Following an acute cerebellar infarct, excitability abnormalities develop over both motor cortices, more prominently in patients with severe functional impairment. The cortical changes, particularly over the ipsilateral motor cortex, may represent a functionally relevant plastic process that may guide future therapeutic strategies to better facilitate recovery.

1 INTRODUCTION

The cerebellum plays a vital role in the regulation of motor tasks, particularly relating to coordination, timing and dexterity. Afferent connections from the motor cortex via the cerebro-ponto-cerebellar pathways process the sensory outcome of motor commands, with efferent corrections conveyed back to the cortex through cerebello-thalamo-cortical projections (Haines and Dietrichs, 2012; Sultan et al., 2012). Because of this intricate communication between motor cortex and cerebellum, lesions involving the cerebellum may induce secondary changes in motor areas, either as a consequence of the damage or as an adaptation to the functional deficits.

To date, limited evidence has suggested altered excitability in the contralateral motor cortex following a unilateral cerebellar stroke, that may evolve over time (Farias da Guarda et al., 2010; Liepert et al., 2004). However, there has been a lack of studies utilizing prospective longitudinal design in a homogenous cohort of cerebellar stroke patients. Furthermore, ipsilateral motor cortical excitability changes have not been adequately explored, particularly considering the growing evidence of bilateral hemispheric changes following unilateral motor strokes, interpreted as adaptive plastic changes associated with stroke recovery (Butefisch et al., 2003; Liepert et al., 2000e; Takeuchi et al., 2010).

To address these deficiencies, the present study combined clinical assessment with threshold-tracking techniques using paired-pulse transcranial magnetic stimulation,

to assess the motor cortices of patients following an acute unilateral cerebellar stroke, from time of ictus with longitudinal follow-up over 1 year at multiple time points. The aim was to identify excitability changes that may develop not only in the contralateral but also the ipsilateral motor cortex following an acute cerebellar stroke, and how these changes may relate to motor adaptation and the functional severity of stroke.

2 METHODS

2.1 Subjects

Ten patients (aged 34-96 years, mean 65.3±16.8) diagnosed with a first-time unilateral cerebellar infarct were recruited for the study in the acute period (2-7 days, mean 4.1±1.7), from a specialized acute stroke unit. Acute stroke was confirmed through changes on diffusion-weighted MRI. Seven patients had lesions involving the posterior inferior cerebellar artery (PICA), two had lesions of the superior cerebellar artery (SCA), and one had lesions involving both the PICA and SCA. The size of the infarct was measured as the maximum dimension using a digital measuring tool within the imaging software. The mean infarct size was 18.3mm (range 6.3-45.5mm). There was no clinical or radiological evidence of motor tract involvement. Patients with disorders affecting the central or peripheral nervous system or prescribed medications that affect nerve function were excluded. Written informed consent was obtained from the participants and the study was approved by the local Human Research Ethics Committee.

Clinical, functional and neurophysiological assessments were undertaken within the same session at baseline (acute), and repeated at 3, 6 and 12-months follow-up. Results were compared to 15 age-matched controls (aged 43-92 years; mean 66.9±17.2), with cortical stimulation over the left hemisphere. No differences in cortical excitability parameters between hemispheres in controls have been demonstrated by our laboratory or previous studies (Butefisch et al., 2003; Cicinelli et al., 2003).

Formal clinical and functional impairment was assessed using the National Institute of Health Stroke Scale (NIHSS), the modified Rankin scale (mRS), the upper limb component of the Fugl-Meyer score (FM), and the Barthel index (BI). The measure of reflex activity was excluded from the FM assessment, to form a modified FM score with a maximum of 60, giving a more unidimensional assessment, as previous studies found that the reflex items reflected a different motor control construct than the other items (Woodbury et al., 2008). In addition, the degree of ataxia was assessed using the Scale for the Assessment and Rating of Ataxia (SARA). The BI and SARA were used to correlate with neurophysiological parameters, given the wide range of scores in this cohort of stroke patients. Moreover, use of BI is relevant as neuroplasticity generally operates at a network or systems level that reflects the overall functional adaptation to the post-stroke disability. Patients were further subdivided according to functional disability with a BI>70 being mild to moderate (5 patients), and BI</=70 being severe (5 patients).

2.2 Cortical excitability

Measures of cortical excitability were assessed according to previously described methodology (page 58) and performed over the motor cortices contralateral (contra-M1) and ipsilateral (ipsi-M1) to the cerebellar infarct separately with recordings measured over the contralateral (to the motor cortex) abductor pollicis brevis muscle (APB).

2.3 Statistical analysis

Group data are expressed as mean ± SEM and compared using either Student's *t*-test or Mann-Whitney *U*-test depending on normality of data distribution, and applied when analyzing patient and control groups. Likewise, side-to-side paired data for each considered variable obtained at baseline were compared using either paired *t*-test or Wilcoxon test. A repeated measures analysis of variance (ANOVA) with time as the within-group factor and post-hoc pair-wise comparisons, were performed to test the evolution of neurophysiological data. The Bonferroni correction was applied. Correlation between excitability indices and clinical scores were analyzed by Spearman's rank test. A P-value of <0.05 was regarded as statistically significant.

3 RESULTS

3.1 Clinical assessments

At baseline clinical evaluation, the mean Barthel index in the cerebellar stroke cohort was 66.5 (range 15-100), Fugl-Meyer 56.9 (range 51-60), NIHSS 2.4 (range 1-5), mRS 3 (range 1-4), and SARA 14.3 (range 0-30). As these scores suggest, there was a wide range of functional disability from mild to severe, whilst the motor impairments were generally mild, reflecting the impact of cerebellar ataxia on function despite the absence of motor weakness.

Improvements in all clinical scores occurred during the period of clinical follow-up over 1 year in the cerebellar stroke patients, with the most significant changes within the first 3 months following the acute event. At 3-months, the average functional disability was mild with mean BI 90, FM 58, NIHSS 1.1, mRS 1.6, and SARA 2.1. Thereafter, the clinical improvements plateaued over the remainder of the 1-year assessment period (at 6 months: mean BI 90, FM 58, NIHSS 1.1, mRS 1.6, SARA 1.4 ; at 12 months: mean BI 92, FM 59.8, NIHSS 1, mRS 1.6, SARA 0.7).

3.2 Cortical excitability

At baseline, assessment of intracortical excitability established that averaged SICI was reduced over contra-M1 (8.2±4.3%) compared with controls (15.8±0.8%, *P*=0.01), but was no different when compared to ipsi-M1 (10.3±3.7%, *P*=0.27). Ipsi-M1 SICI was reduced compared to controls but this did not reach statistical

significance (*P*=0.11) (Fig. 4.1A). Of relevance, when patients were analyzed according to functional disability, SICI over ipsi-M1 in the severe group was significantly reduced (4.1±3.1%) compared to the mild-moderate group (16.6±3.0%, *P*=0.02) as well as to controls (*P*=0.01) (Fig. 4.1B). Furthermore, there was a significant correlation between functional disability as measured by the BI and SICI over the ipsi-M1 (r=0.69, *P*=0.03) (Fig. 4.2), that suggested poor function was associated with greater reductions in SICI over ipsi-M1. There was no correlation between the clinical assessment of ataxia (SARA) and SICI over contra-M1 (r=-0.05, *P=0.9*) and ipsi-M1 (r=-0.4, *P*=0.2). In addition, the size of cerebellar infarct did not correlate with SICI over the contra-M1 (r=-0.23, *P*=0.5) or ipsi-M1 (r=-0.22, *P*=0.5).

Other TMS parameters over the contra-M1 and ispi-M1 at baseline were no different to controls (ICF: controls 0.4±2.2%, contra-M1 -1.9±0.9%, *P*= 0.43, ipsi-M1 -1.3±2.7%, *P*= 0.63; RMT: controls 63±2.1%, contra-M1 51.7±6.1%, *P*=0.07, ipsi-M1 60.8±3.0%, *P*=0.53; MEP amplitude: controls 26.1±4.5%, contra-M1 20.5±4.5%, *P*=0.42, ipsi-M1 21.8±6.9%, *P*=0.6) or between motor cortices (ICF: *P*=0.3, RMT: *P*=0.27, MEP amplitude: *P*=0.69).

Over the 1 year follow-up period there were no significant changes in cortical excitability measurements at 3, 6 and 12-months over both the contra-M1 (SICI: ANOVA, F=1.26, *P*=0.37; ICF: ANOVA, F=0.89, *P*=0.54; RMT: ANOVA, F=0.56, *P*=0.68; MEP amplitude: ANOVA, F=0.24, *P*=0.86) and ipsi-M1 (SICI: ANOVA, F=0.78, P=0.55; ICF: ANOVA, F=0.07, P=0.94; RMT: ANOVA, F=1.8, P=0.35; MEP amplitude: ANOVA,

F=4.5, P=0.18), although there was a trend for the SICI to normalize towards control levels (Fig. 4.3, Table 4.1).

		Baseline	3-months	6-months	12-months
RMT (%)	**Contra-M1**	51.7 ± 6.1	57.6 ± 5.4	62.8 ± 2.5	66.4 ± 6.1
	Ipsi-M1	60.8 ± 3.0	54.5 ± 3.8	63.3 ± 6.2	66.3 ± 2.7
MEP amplitude (%)	**Contra-M1**	20.5 ± 4.5	21.1 ± 3.8	21.1 ± 5.4	18.5 ± 7.0
	Ipsi-M1	21.8 ± 6.9	25.2 ± 5.8	26.2 ± 5.7	28.7 ± 7.9
ICF (%)	**Contra-M1**	-2.0 ± 0.9	0.9 ± 1.9	0.6 ± 2.7	-3.8 ± 6.5
	Ipsi-M1	-1.3 ± 2.7	1.7 ± 2.3	0.1 ± 1.2	0.2 ± 2.9

Table 4.1. Resting motor thresholds (RMT), motor evoked potential (MEP) amplitudes and intracortical facilitation (ICF) were not significantly different over the contralateral (contra-M1) and ipsilateral (ipsi-M1) motor cortices during the one year assessment period

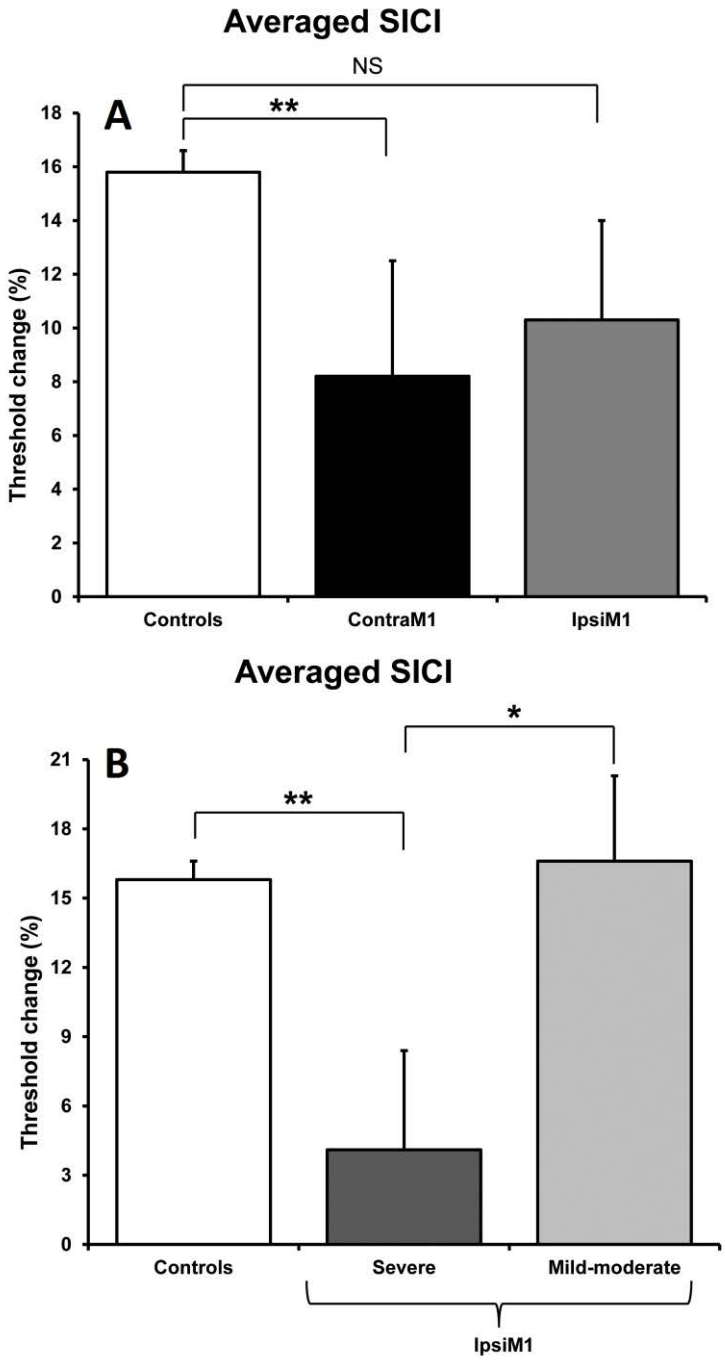


Figure 4.1. Baseline paired-pulse subthreshold conditioning TMS demonstrating (A) reduction in short-interval intracortical inhibition (SICI) in the contralateral motor cortex (contraM1) compared to controls; (B) reduction in SICI in the ipsilateral motor cortex (ipsiM1) in the severely impaired group compared to controls and the mild-moderately impaired group. **P*= 0.02, ***P*=0.01, NS = non-significant.

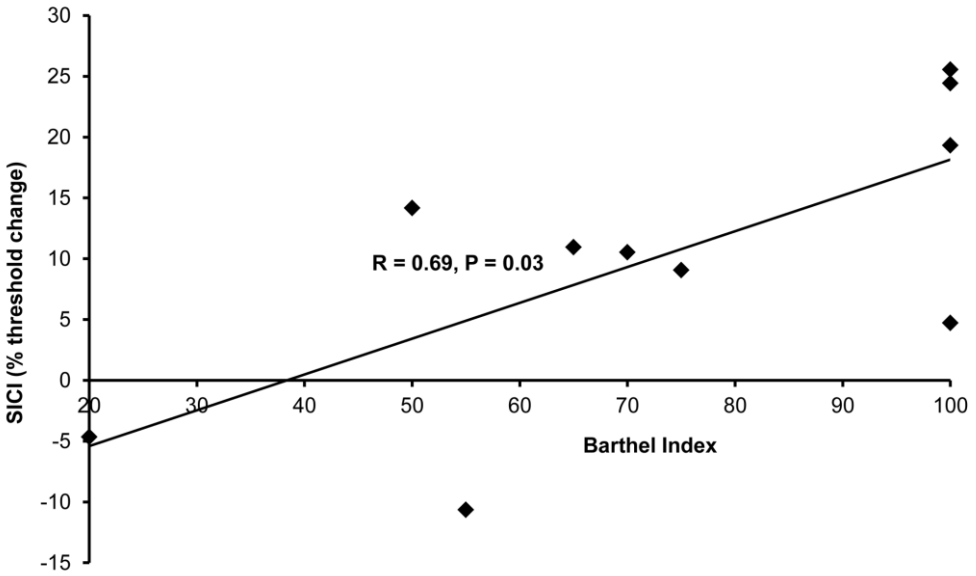


Figure 4.2. Graph demonstrating significant correlation between short-interval intracortical inhibition (SICI) over the ipsilateral motor cortex and the Barthel index of cerebellar stroke patients, such that less inhibition was observed in those with greater functional impairment.

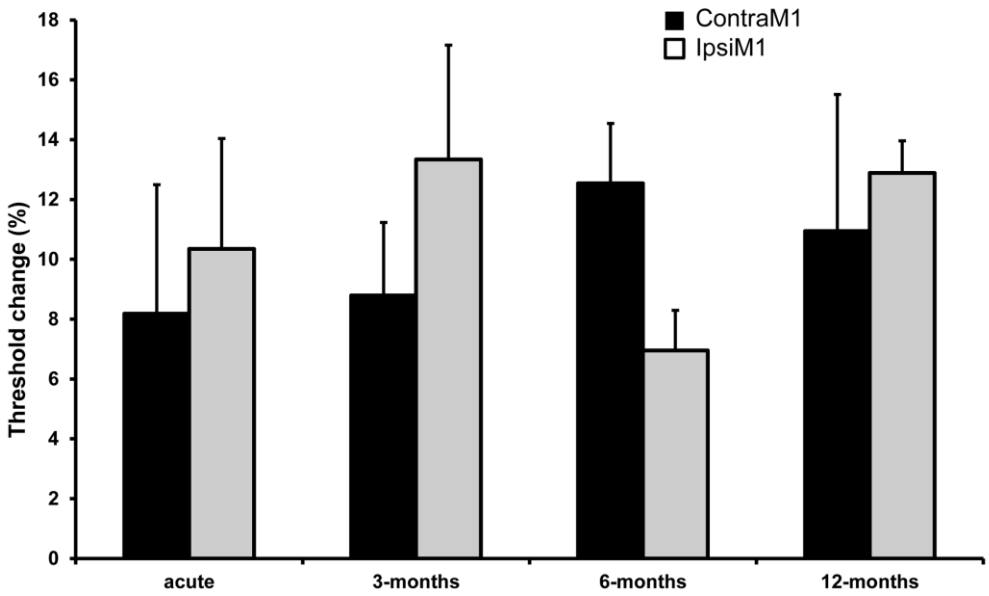


Figure 4.3. Averaged short-interval intracortical inhibition (SICI) over both the contralateral (contraM1) and ipsilateral (ipsiM1) motor cortices were not significantly different (ANOVA: contraM1 F=1.26, *P*=0.37; ipsiM1 F=0.78, P=0.55) over the 1-year follow-up period, although there was a trend towards normal.

4 DISCUSSION

The present study has identified changes in motor cortex excitability that develop shortly after onset in patients with cerebellar stroke, that likely represent neuroplastic changes. Specifically, after an acute unilateral cerebellar infarct, intracortical disinhibition developed over the contralateral motor cortex that persisted for over a year of longitudinal follow-up, associated with ongoing functional improvements. In addition, the present study established the presence of intracortical disinhibition over the ipsilateral motor cortex that correlated with functional severity, such that most cortical disinhibition developed in those patients that exhibited the greatest clinical impairment.

By means of explanation, the contralateral cortical excitability changes may reflect disruption of the inhibitory Purkinje output, resulting in an increase in excitatory influence from the deep cerebellar nuclei (DCN) to the contralateral motor cortex. The role of cerebellum in modulation of motor cortex excitability has been recently identified in rats (Luft et al., 2005). Purkinje cells of the cerebellar cortex have inhibitory connections with the DCN, which in turn, exert a tonic facilitatory effect on the motor cortex through efferent cerebello-thalamo-cortical pathways (Cruz-Martinez and Arpa, 1997; Haines and Dietrichs, 2012; Koch, 2010). This basal cerebellar inhibition on the motor cortex was demonstrated using high-voltage conditioning electrical stimuli over the lateral cerebellar hemisphere preceding a magnetic stimulus (by 5-7ms) delivered contralaterally to the motor cortex, which

proved sufficient to suppress electromyographic responses as a result of inhibition of the dentato-thalamo-cortical pathway (Ben Taib and Manto, 2009).

Further support for such an hypothesis may be derived from connectivity between the cerebellum and GABAergic inhibitory neurons in the motor cortex responsible for the generation of SICI (Daskalakis et al., 2004). However, unlike cerebellar degeneration processes, where specific cell types (such as Purkinje cells) are involved, infarcts and other vascular lesions most often affect the cerebellar cortex as well as the deep cerebellar nuclei (Farias da Guarda et al., 2010; Liepert et al., 2000c).

Alternatively, the changes may represent an adaptive plastic process from the motor cortex acting through afferent cerebro-ponto-cerebellar pathways, to facilitate functional recovery and adapt to the deficits in motor control, a role previously undertaken by the pre-lesioned cerebellum. It has been demonstrated that motor system plasticity, as required in stroke recovery may be associated with an increase in cortical excitability (Luft et al., 2005). This enhanced excitability may represent an electrophysiological adjustment of the sensorimotor pathways necessary for adaptation in cortical network functioning to promote effective motor learning and thereby recovery from deficits (Johansson et al., 1993). This motor cortex hyperexcitability may enhance the efficiency of cerebello-thalamo-cortical pathways, with subsequent increase in capacity to convey cerebellar inputs to the motor cortical neurons (Luft et al., 2005).

These GABA-mediated electrophysiological changes may later be followed by longer-lasting structural plasticity that involves long-term potentiation (LTP) (Luft et al., 2005). In support, lesions involving the hemicerebellum in rats may induce formation and remodelling of corticofugal fibres that may in turn contribute to recovery (Castro and Mihailoff, 1983). Moreover, cerebellar lesions in cats have resulted in induction of significant synaptic reorganisation in the motor cortex, including the formation of new and functionally active synapses, most prominent in layers II/III (but also present in II-V) (Keller et al., 1990; Sanes and Donoghue, 2000). This may suggest that functional elimination of sensory input through the cerebello-thalamo-cortical pathway may induce a compensatory proliferation of synapses in the motor cortex, to promote learning and recovery from cerebellar damage.

As such, the present findings may seem at variance to a previous study that reported contralateral increases in cortical inhibition in the early period after an acute cerebellar infarct (Liepert et al., 2004). One possible explanation for the discrepancy is that the previous study reported cortical excitability as a ratio compared to the ipsilateral motor cortex. This method assumes an absence of change in the ipsilateral motor cortex, which according to data from the present study, is not the case. In addition, contrary to this previous report where excitability changes only developed in strokes involving the SCA, the present study demonstrated excitability abnormalities in all patients with either PICA or SCA territory strokes, consistent with the findings from another report (Farias da Guarda

et al., 2010) that investigated cortical excitability changes in chronic cerebellar stroke patients.

The present study also identified cortical disinhibition that developed over the ipsilateral motor cortex following unilateral cerebellar infarcts. Again the changes may reflect alterations induced by the lesion in the cerebellum and related pathways that connect to the ipsilateral motor cortex. In support, such ipsilateral efferent connections have been demonstrated in functional imaging studies (Sultan et al., 2012), and unilateral cerebellar lesions in cats have also demonstrated neural sprouting and synaptogenesis with formation of ipsilateral interposito-thalamo-cortical pathways (Sarkisian et al., 1990). Moreover, unilateral cerebellar lesions have been shown to be associated with impaired motor control not only in the ipsilateral hand, but also in the hand contralateral to the lesion (Anens et al., 2010; Nowak et al., 2009).

As with the contralateral cortical changes, the disinhibition observed over the ipsilateral motor cortex may represent an adaptive response to facilitate recovery through ipsilateral afferent projections to the cerebellum. Recent studies have demonstrated the presence of bilateral cerebro-thalamo-cerebellar projections responsible for carrying ipsilateral connections (Krienen and Buckner, 2009). The adaptive nature of the ipsilateral change is further supported by findings from the present study that established cortical disinhibition being most pronounced in the severely impaired group, as well as a correlation between less SICI and more

functional impairment. Furthermore, changes in the ipsilateral motor cortex may reflect recruitment of additional distributed networks in view of increasing difficulty associated with a motor task as a result of the cerebellar stroke, a finding observed in normal controls in association with increasing motor task complexity (Krakauer et al., 2004).

Whether the disinhibitory changes observed over both motor cortices represent an epiphenomenon secondary to changes in the damaged cerebellum, or a reflection of an adaptive mechanism remains speculative. Further studies that specifically address this latter hypothesis may involve investigating whether disruption of the intracortical disinhibition result in impaired recovery. This has been observed in unilateral strokes affecting the cerebral motor pathways, whereby repetitive TMS to both ipsilesional and contralesional motor cortices to temporarily reduce the excitability of the area, resulted in impairment of motor tasks that was more evident in those stroke patients with more marked impairment (Fridman et al., 2004; Johansen-Berg et al., 2002b; Ward, 2005b). This suggests that cortical hyperexcitability in this stroke phenotype reflect a neuroplastic process that facilities motor recovery in these patients, rather than a mere “release” phenomenon particularly with respect to the theorized release of interhemispheric inhibition from the lesioned to the non-lesioned motor cortex.

Bihemispheric cortical disinhibition in acute motor strokes was previously demonstrated (Butefisch et al., 2003; Liepert et al., 2000e; Manganotti et al., 2008;

Shimizu et al., 2002; Swayne et al., 2008), and thought to reflect down-regulation in GABAergic circuits via $GABA_A$ receptors (Bashir et al., 2010) that enabled the unmasking of functionally latent networks, to promote cortical reorganization (Bashir et al., 2010; Murase et al., 2004; Westlake and Nagarajan, 2011). In addition, animal models with focal cerebral ischemia have demonstrated axonal sprouting, dendritic growth and pruning as well as synaptogenesis and enhanced synaptic efficiency (Bury and Jones, 2002; Jones et al., 1996; Luke et al., 2004; Stroemer et al., 1995). As such, similar changes at a cellular and molecular level may occur in association with the cortical excitability changes following a cerebellar infarct, in order to drive the process of neural re-wiring necessary for re-establishing fine motor control and coordination. This underscores the concept that even though learning and plasticity may involve encoding different kinds of information according to the task involved, there may be a similarity in the circuit mechanisms involved in all forms of learning (Gilbert and Li, 2012).

4.1 Clinical implications for rehabilitation

The present study has demonstrated cortical disinhibition in the contralateral motor cortex following a unilateral cerebellar infarct that persisted during the 1 year follow-up period in a cohort of patients with good functional recovery. In addition, cortical disinhibition was also evident in the ipsilateral motor cortex that correlated with the degree of functional impairment, suggesting that cortical hyperexcitability may represent functionally relevant neuroplasticity. Further research is required to confirm the causal relationship between the electrophysiological changes observed

and functional recovery of patients with cerebellar stroke. As such, the current findings may have therapeutic implications for rehabilitation, particularly whether novel non-invasive brain stimulation (NIBS) over the motor cortices may be utilized to promote functional recovery in cerebellar stroke patients - a strategy that has become increasingly studied for the use in patients with motor strokes (Lindenberg et al., 2010; Takeuchi and Izumi, 2012).

Motor learning, which incorporates skill learning, consolidation and adaptation, is associated with functional changes in a distributed network that includes the primary motor cortex (M1), premotor and supplementary motor areas, the basal ganglia and cerebellum, with M1 being the key structure in this network engaged in motor sequence learning (Reis et al., 2008). For this reason, it is conceivable that following a lesion of the cerebellum, with consequent disruption to the motor learning network, changes in M1 may be necessary to drive functionally relevant neuroplastic processes in order to assist recovery of this perturbed system.

In support of such an hypothesis, recent evidence has suggested benefits of NIBS over the primary motor cortex to enhance cortical excitability, in order to facilitate motor learning contralateral to the training hand (Reis et al., 2008). Excitatory repetitive TMS (rTMS) and transcranial direct current stimulation (tDCS) applied over M1 during practice demonstrated improvements in motor sequence learning and target accuracy, sequential finger movement as well as performance in visuomotor coordination tasks (Kim et al., 2004; Vines et al., 2006). In addition, tDCS

over primary motor cortices was shown to modulate functional connectivity in areas including the cerebellum (Sehm et al., 2012), and rTMS over M1was observed to evoke an increased regional blood flow in the contralateral cerebellar hemisphere (Wessel, 2003), further supporting this intricate connectivity between the cerebellum and M1. Moreover, reducing the excitability of M1 using inhibitory NIBS has shown deleterious effects on motor adaptation (a task that involves cerebellar activation) (Hadipour-Niktarash et al., 2007) and motor skill learning (Muellbacher et al., 2002), again providing evidence for M1 playing a functionally relevant role in motor learning (Reis et al., 2008).

In various animal models, application of tDCS over the contralateral premotor (Ben Taib and Manto, 2007; Manto and Ben Taib, 2008) and motor cortices (Ben Taib and Manto, 2009), was sufficient to revert the motor cortex hypoexcitability in hemicerebellectomized rats. In addition, combination of tDCS and contralateral low-frequency repetitive stimulation (LFRS) over M1 enhanced the facilitatory drive exerted by cerebello-thalamo-cortical pathways to restore motor cortex excitability (Ben Taib and Manto, 2007; Manto and Ben Taib, 2008). Such a process may enable priming of sensorimotor cortical activity to peripheral nerve stimulation procedures, to promote motor learning (Manto and Ben Taib, 2008), and conversely, failure of this facilitatory drive may contribute to the inability of ataxic patients to learn new tasks (Ben Taib and Manto, 2007). Furthermore, abnormal levels of interhemispheric inhibition (IHI) may contribute to persistent deficits in stroke patients (Hummel and Cohen, 2006). If so, it has been demonstrated using a

combination tDCS and LFRS in hemicerebellectomized animal models, that IHI may become reduced, resulting in restoration of the capacity of the motor cortex to modulate cutaneomuscular reflexes after a sustained period of somatosensory stimulation (Manto and Ben Taib, 2008; Oulad Ben Taib and Manto, 2006; Oulad Ben Taib and Manto, 2008).

The studies discussed above however, were primarily undertaken in normal subjects or animal models, and hence the effects in human patients with a lesion such as stroke in the cerebellum may differ. In this regard, studies utilizing techniques such as repetitive TMS or transcranial direct current stimulation to down-regulate inhibition in both contra- and ipsilateral motor cortices in cerebellar stroke patients may be an area for future research, in order to elucidate the relevance of such cortical excitability changes in the functional recovery of these patients . Moreover, issues that would need to be addressed when considering such strategies include mono- and/or bi-hemispheric cortical stimulation, which subset of patients with what level of disability will derive the most benefit, and the most appropriate timing of such intervention following the acute event to ensure that delivery is facilitating and not detrimental to the natural course of recovery – questions that continue to provoke debate in stroke patients with lesions involving the motor tracts.

CHAPTER 5

Exploring the long-term changes in cortical excitability after acute stroke

SUMMARY

The evolution of changes in cortical excitability following stroke, particularly pertaining to the contralesional hemisphere, is being increasingly recognised in relation to maximising the potential for functional recovery. As such, the present study utilised a prospective longitudinal design over an 18-month period from stroke onset, to investigate the evolution of cortical excitability involving both motor cortices and their consequent effects on recovery. Comprehensive clinical assessments, specifically the Barthel index, Fugl-Meyer score, National Institute of Health Stroke Scale, and modified Rankin scale, were undertaken at stroke onset in 33 patients. Cortical function and excitability was investigated using paired-pulse transcranial magnetic stimulation. Clinical assessment and electrophysiological studies were repeated at 3, 6, 12 and 18 months. Immediately following stroke, short-interval intracortical inhibition (SICI) was significantly reduced in both the lesioned and contralesional hemispheres compared with controls that correlated with the degree of functional improvement over the subsequent 3 months. Over the follow-up period, ipsilesional SICI remained suppressed in all patient groups, whilst SICI over the contralesional hemisphere remained suppressed only in the groups with cortical stroke and more baseline functional impairment. Furthermore, contralesional SICI was reduced to a greater extent in these groups of patients compared to those with subcortical stroke location and milder baseline impairment during the longitudinal assessment period. Clinical scores demonstrated improvement over this duration, with the most significant changes occurring in the first 3 months. The results of the present chapter demonstrated that intracortical

disinhibition developed across both hemispheres immediately following acute stroke, that likely represented an adaptive change attempting to drive the recovery process. Whilst changes in the lesioned hemisphere persisted over the period of recovery in all patients, disinhibition persisted in patients with cortical strokes and those with more baseline functional impairment, suggesting that ongoing contralesional network recruitment may be necessary for those patients who have significant disruptions to the integrity of ipsilesional motor pathways. The study has demonstrated that evolution of cortical excitability, particularly over the contralesional hemisphere, may vary between patients with differing baseline stroke and clinical characteristics. Results from the present series have implications for the development of neuromodulatory brain stimulation protocols to harness and thereby facilitate stroke recovery.

1 INTRODUCTION

Improvements in the treatment and care for acute stroke patients have been paralleled by an increasing number of longer term stroke survivors. However, despite a range of rehabilitation strategies that were designed to support functional recovery, 50-60% of stroke patients continue to have some degree of motor impairment (Schaechter, 2004).

Following acute stroke, the initial recovery process revolves around resolution of reversible pathophysiological events that result from the brain injury, particularly oedema, necrotic tissue, reperfusion of the ischemic penumbra through collateral circulation, and diaschisis (Butefisch, 2004; Furlan et al., 1996). However, subsequent stages of recovery largely comprise of functional and structural reorganization at both a "motor systems" as well as at a cellular level, forming the basis of neuroplasticity.

Functional imaging studies in hemiparetic stroke patients have consistently identified task-related brain over-activation during recovery, changes that were often bilateral, involving multiple brain regions (Calautti and Baron, 2003; Chollet et al., 1991; Cramer et al., 1997; Foltys et al., 2003; Marshall et al., 2009; Rehme et al., 2011b; Ward et al., 2003a; Ward et al., 2003b; Weiller et al., 1993). Recruitment of these regions may facilitate recovery (Ward, 2005a), at least in the early stages (Rehme et al., 2011b). However, there continues to be debate regarding the role of these widespread changes during more chronic phases of recovery, particularly in

the context of incomplete or poor motor recovery (Chollet et al., 1991; Grefkes et al., 2008; Grefkes and Fink, 2012; Ward et al., 2003b).

Electrophysiological approaches, particularly utilizing transcranial magnetic stimulation (TMS), have also demonstrated changes in cortical excitability in both hemispheres after stroke (Butefisch et al., 2003; Butefisch et al., 2008; Cicinelli et al., 2003; Liepert et al., 2000d; Liepert et al., 2000e; Manganotti et al., 2008; Shimizu et al., 2002; Swayne et al., 2008), that likely reflect changes in the intrinsic cortical circuits (Swayne et al., 2008) and alterations in GABAergic neurotransmission (Glodzik-Sobanska et al., 2004; Neumann-Haefelin et al., 1998; Sacco et al., 2009). However, debate continues regarding the relationship of these changes to the timing and location of stroke, and more importantly the evolution of these plastic changes as a function of stroke recovery. The discrepancies observed between studies may in part be related to the lack of longitudinal design, as well as the heterogeneity across the stroke cohorts and timing from stroke onset.

With growing evidence supporting the use of novel non-invasive brain stimulation (NIBS) to further facilitate stroke recovery (Ackerley et al., 2010; Boggio et al., 2007; Bradnam et al., 2013; Fregni et al., 2005; Khedr et al., 2010; Kim et al., 2010; Mansur et al., 2005; Nowak et al., 2008; Stagg et al., 2012; Takeuchi et al., 2005), it seems important to clarify the nature and evolution of cortical changes, to better select patients that may benefit from such intervention, as it seems unlikely that these therapies will prove successful with a “one-size-fits-all” approach. Moreover,

some studies have demonstrated negative impacts of NIBS over the contralesional cortex, suggesting that the concept of reinstalling interhemispheric balance between motor cortices may not apply to all patient groups.

As such, to better delineate changes within motor cortices and between hemispheres, the present study utilized paired-pulse TMS to further explore the evolution of excitability changes in a cohort of acute stroke patients studied at multiple time points (acutely within 1 week, 3-, 6-, 12- and 18-months) over a period of 18 months. A further aim was to better define changes within the contralesional hemisphere, with respect to functional improvement. Analyses of subgroups by stroke location and baseline impairment underpinned the hypothesis that functionally relevant intracortical excitability changes differed in their evolution.

2 METHODS

2.1 Subjects

A total of 33 stroke patients (15 males, 18 females; aged 28-90, mean 65.6±14.9) diagnosed with acute unilateral ischemic stroke were recruited in the acute period (< 1 week) from a specialized stroke unit. Inclusion criteria for the study were (i) first-ever ischemic stroke with motor involvement; and (ii) brain MRI demonstrating acute infarct on diffusion weighted imaging (DWI) sequences. Exclusion criteria were (i) previous history of stroke; (ii) cognitive impairment or dysphasia sufficient to affect informed consent; (iii) drugs or concomitant neurological disorders that

may affect motor cortical excitability (Ziemann, 2004); and (iv) any contraindications to TMS.

All patients received standard multidisciplinary and rehabilitative care that was determined by their clinical requirements. The patients were subdivided according to lesion location into a cortical group (15 patients) and subcortical group (18 patients) as determined by MRI findings. Subcortical infarcts were defined as location within the basal ganglia, internal capsule, or corona radiata, whilst cortical infarcts were defined as wedge-shaped, superficial ischemic lesions in the territory of one of the large major cerebral arteries or lesions in a border zone that may involve the underlying white matter.

Patients were assessed clinically and electrophysiologically within the same session at baseline following recruitment (6.4±0.8 days), and repeated at several time points during the longitudinal follow-up period at 3- (26 patients), 6- (21 patients), 12- (17 patients) and 18-months (11 patients).

Results were compared to 29 control subjects (aged 47-83, mean 58.8± 9.7), with cortical stimulation over the left hemisphere. No differences in cortical excitability parameters between hemispheres in controls have been demonstrated by our laboratory or previous studies (Butefisch et al., 2003; Cicinelli et al., 2003). All patients and control subjects provided written informed consent and the study was approved by the local Health and Research Ethics Committee.

2.2 Clinical assessments

On each study occasion, patients were assessed using the Barthel index (BI), upper-limb component of the Fugl-Meyer Score (FM), national institute of health stroke scale (NIHSS), and modified Rankin Scale (mRS). The measure of reflex activity was excluded from the FM assessment, to form a modified FM score with a maximum of 60, giving a more unidimensional assessment, as previous studies found that the reflex items reflected a different motor control construct than the other items (Woodbury et al., 2008). The BI was used to correlate with neurophysiological parameters, given the wide range of scores in this cohort of stroke patients. Moreover, use of BI is relevant as neuroplasticity generally operates at a network or systems level that reflects the overall functional adaptation to the post-stroke disability. Patients were further subdivided according to functional disability with a BI>70 being mild (20 patients), and BI</=70 being moderate to severe (13 patients).

2.3 Cortical excitability

Measures of cortical excitability were assessed in accordance with previously described methodology (page 58), and performed separately over the motor cortices ipsi- and contralateral to the infarct, with recordings over the contralateral abductor pollicis brevis muscle.

2.4 Statistical analysis

Group data are expressed as mean ± standard error of the mean and compared using either Student *t* test or Mann-Whitney *U* test depending on normality of data

distribution. Multiple comparisons were Bonferroni corrected. Likewise, side-to-side data were compared using either paired *t*-test or Wilcoxon test. Changes with time of each electrophysiological parameter in each hemisphere were examined using a repeated measures analysis of variance (ANOVA) with the within-group factor being time, and between-group factors being stroke location and baseline severity when performing the subgroup analyses. Correlations between electrophysiological parameters and clinical scores were assessed by Spearman rank test. A *P* value of <0.05 was regarded as statistically significant.

3 Results

3.1 Acute stroke baseline

3.1.1 Clinical assessments

Functional severity of the acute stroke patients ranged from mild to severe at baseline, with the mean BI of 72.6 (range, 10-100), FM of 50.4 (range, 0-60), NIHSS of 4 (range, 0-17), and mRS of 2.6 (range, 0-5) (Fig. 5.1).

3.1.2 Corticomotoneuronal excitability

RMT of the ipsilesional hemisphere was 58.6±2.6%, and was not different to the contralesional hemisphere (57.5±1.7%; *P*=0.27). Both hemispheres were also similar in RMT to controls (61.5±1.6%; *P*=0.3 and *P*=0.10, respectively). MEP amplitudes were not different between controls (2.4±0.3mV) and the ipsilesional (2.2±0.3mV, *P*=0.63) and contralesional hemispheres (2.5±0.3mV, *P*=0.94), nor between hemispheres.

In addition, CSP duration was also comparable between controls (214.3±4.0ms) and the ipsilesional (227.9±8.5ms, *P*=0.11) and contralesional hemispheres (214.7±10.0ms, *P*=0.97), as well as between each hemisphere (*P*=0.28).

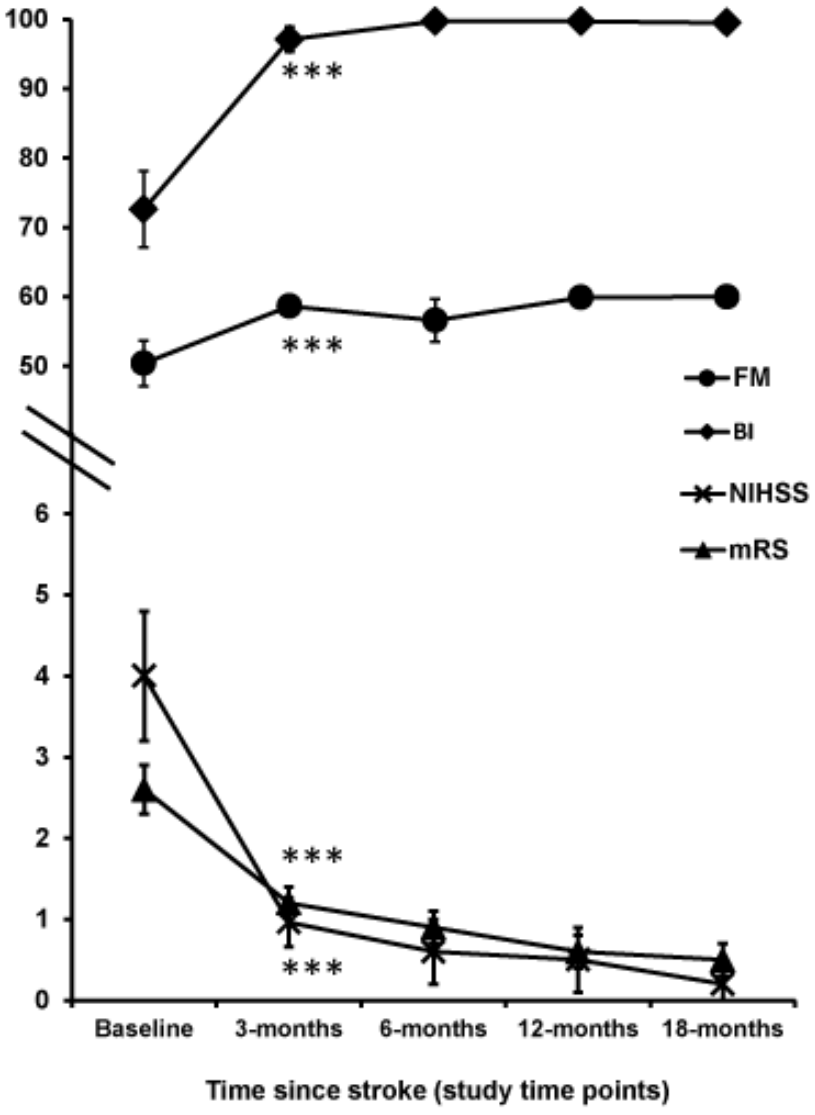


Figure 5.1. Clinical scores over the 18-months follow-up. All scales of functional and motor impairment significantly improved within the first 3 months after acute stroke, after which time plateaued thereafter. The mean scores at 18-months reflect good recovery and functional independence in all patients in the cohort. *** *P*<0.001. FM, Fugl-Meyer score; BI, Barthel index; NIHSS, National Institute of Health Stroke Scale; mRS, modified Rankin Scale.

3.1.3 Intracortical excitability

Measures of intracortical excitability established that averaged SICI was significantly reduced in both ipsilesional (6.2±1.2%, *P*=0.004) and contralesional hemispheres (3.2±1.8%, *P*<0.001) compared with controls (11.2±1.2%), without differences between the sides (*P*=0.2) (Fig. 5.2A). When analysed between groups according to stroke location, both cortical (5.4±1.9%) and subcortical groups (6.7±1.5%) in the ipsilesional hemisphere, had suppressed SICI compared with controls that was statistically more significant in the cortical group (*P*=0.04 and *P*=0.08, respectively), but were comparable with each other (*P*=1.0) (Fig.5.2B). On the contralesional side, significantly reduced SICI was evident in both cortical (2.3±3.7%) and subcortical groups (3.9±1.6%) compared to controls (*P*=0.008 and *P*=0.016, respectively), but no different to each other (*P*=1.0) (Fig. 5.2B).

Analyses according to stroke severity at baseline, revealed that SICI in the ipsilesional hemisphere was significantly reduced in the moderate-severe group (2.3±1.1%) but not in the mild group (8.1±1.4%) compared to controls (*P*=0.001 and *P*=0.27, respectively), being more suppressed in the more impaired compared to the mild group although this did not reach statistical significance (*P*=0.07) (Fig. 5.2C). On the contralesional side, both mild (3.9±2.5%) and moderate-severe groups (2.5±2.7%) had significantly reduced SICI compared with controls (*P*=0.022 and *P*=0.006, respectively), and were no different to each other (*P*=1.0) (Fig. 5.2C).

Averaged ICF on the other hand, was similar between the ipsilesional (0.4±1.2%) and contralesional hemispheres (0.3±1.0%, *P*=0.97), as they were with controls (-1.1±1.2%; *P*=0.39 and *P*=0.38, respectively).

3.2 Longitudinal assessments

3.2.1 Clinical assessments

All functional assessment scores improved over the 18-month follow-up period with the most significant being in the first 3-months. Thereafter, scores tended to plateau over the longitudinal assessment period (Fig. 5.1), with good recovery and functional independence achieved in all patients within the cohort at 18-months.

3.2.2 Corticomotoneuronal excitability

Measures of corticomotoneuronal excitability in the ipsilesional hemisphere remained unchanged (RMT, ANOVA: *F*=1.12, *P*=0.41; MEP amplitude, ANOVA: *F*=1.9, *P*=0.49; CSP duration, ANOVA: *F*=2.2, *P*=0.15) over the 18-month follow-up period. Similarly, the values pertaining to the contralesional hemisphere were also stable over the assessment period (RMT, ANOVA: *F*=0.3, *P*=0.87; MEP amplitude, ANOVA: *F*=0.8, *P*=0.56; CSP duration, ANOVA: *F*=0.07, *P*=1).

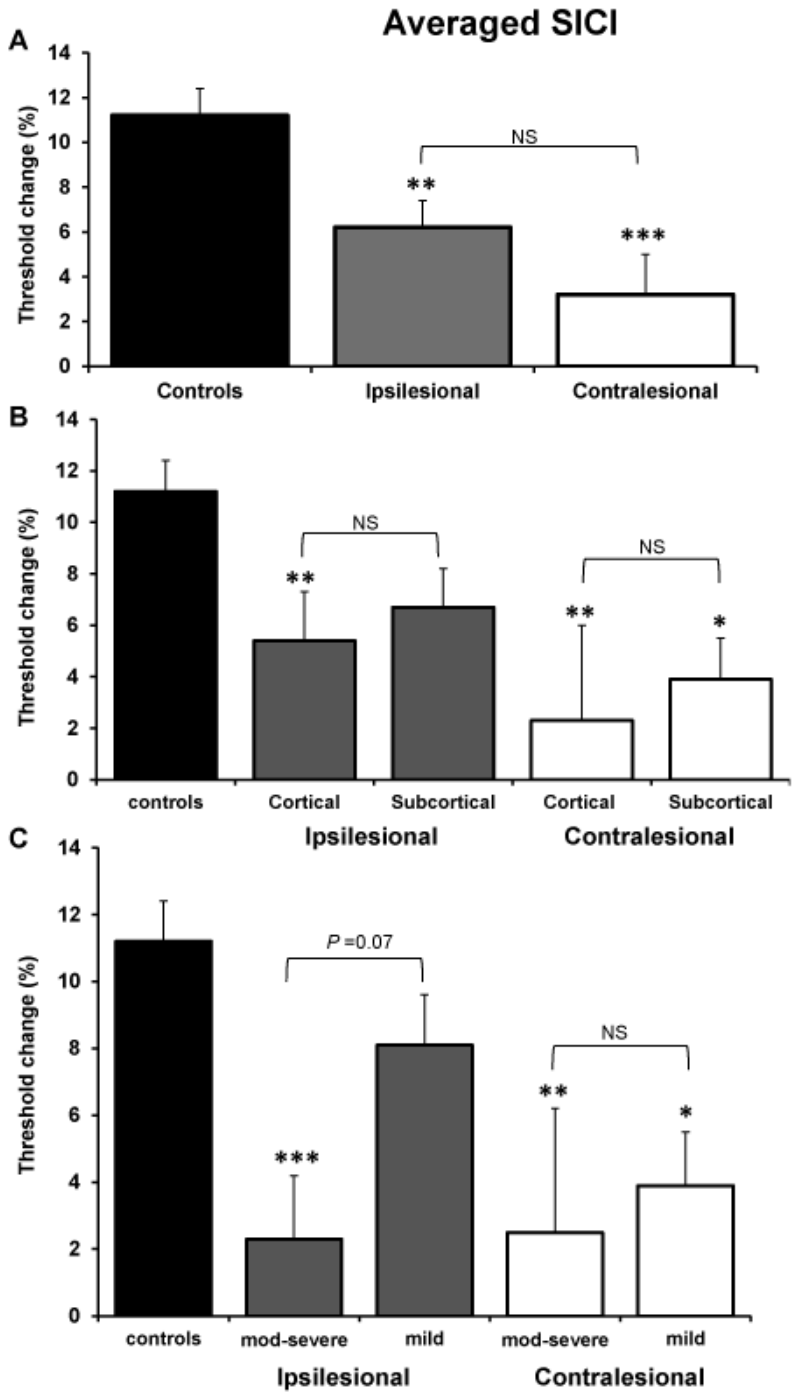


Figure 5.2. Baseline averaged short-interval intracortical inhibition (SICI). (A) Significant reductions in SICI in both ipsilesional and contralesional motor cortices compared with controls; (B) SICI was reduced in both cortical and subcortical stroke location groups in both hemispheres although there was a trend for most reductions in the cortical groups; (C) SICI reductions were more pronounced in the moderate to severe impairment groups in both hemispheres. **P*<0.05, ***P*<0.01, ****P*<0.001; NS, not statistically significant; Threshold change is the difference in test stimulus intensity required to evoke the target MEP calculated as: [conditioned test stimulus intensity – RMT]/RMT X 100.

3.2.3 Intracortical excitability

When examined as an entire cohort, there were no significant changes in the averaged SICI over the course of 18-months in both the ipsilesional (ANOVA: F=0.52, P=0.72) and contralesional hemispheres (ANOVA: F=1.04, P=0.41) (Fig. 5.3A). Subgroup analyses however, demonstrated a significant between-group effect of stroke location (ANOVA: F=11.1, P=0.045) and baseline impairment (ANOVA: F=14.56, P=0.032) over time in the contralesional but not ipsilesional hemisphere (stroke location, ANOVA: F=0.56, P=0.51; baseline impairment, ANOVA: F=2.73, P=0.20). Furthermore, there was a significant interaction between stroke location and baseline impairment over the contralesional hemisphere (ANOVA: F=10.36, P=0.049), suggesting that intracortical inhibition remained most suppressed over the contralesional hemisphere in those patients with a cortical location of stroke as well as those with the most functional impairment at baseline (Fig. 5.3C).

Statistical comparison with the control group demonstrated that in the ipsilesional hemisphere, SICI remained significantly reduced in both stroke location groups over the follow-up period. This was also the case for both groups in regards to baseline impairment (Fig. 5.3B). In the contralesional hemisphere however, SICI remained suppressed only in the cortical stroke group, whilst SICI in the subcortical group normalized to control values over the 18-months follow-up period starting at the 3-month time point. Similarly, contralesional SICI in the group with more severe baseline impairment stayed suppressed, whilst SICI in the mild baseline impairment group also normalized by 18-months (Fig. 5.3C).

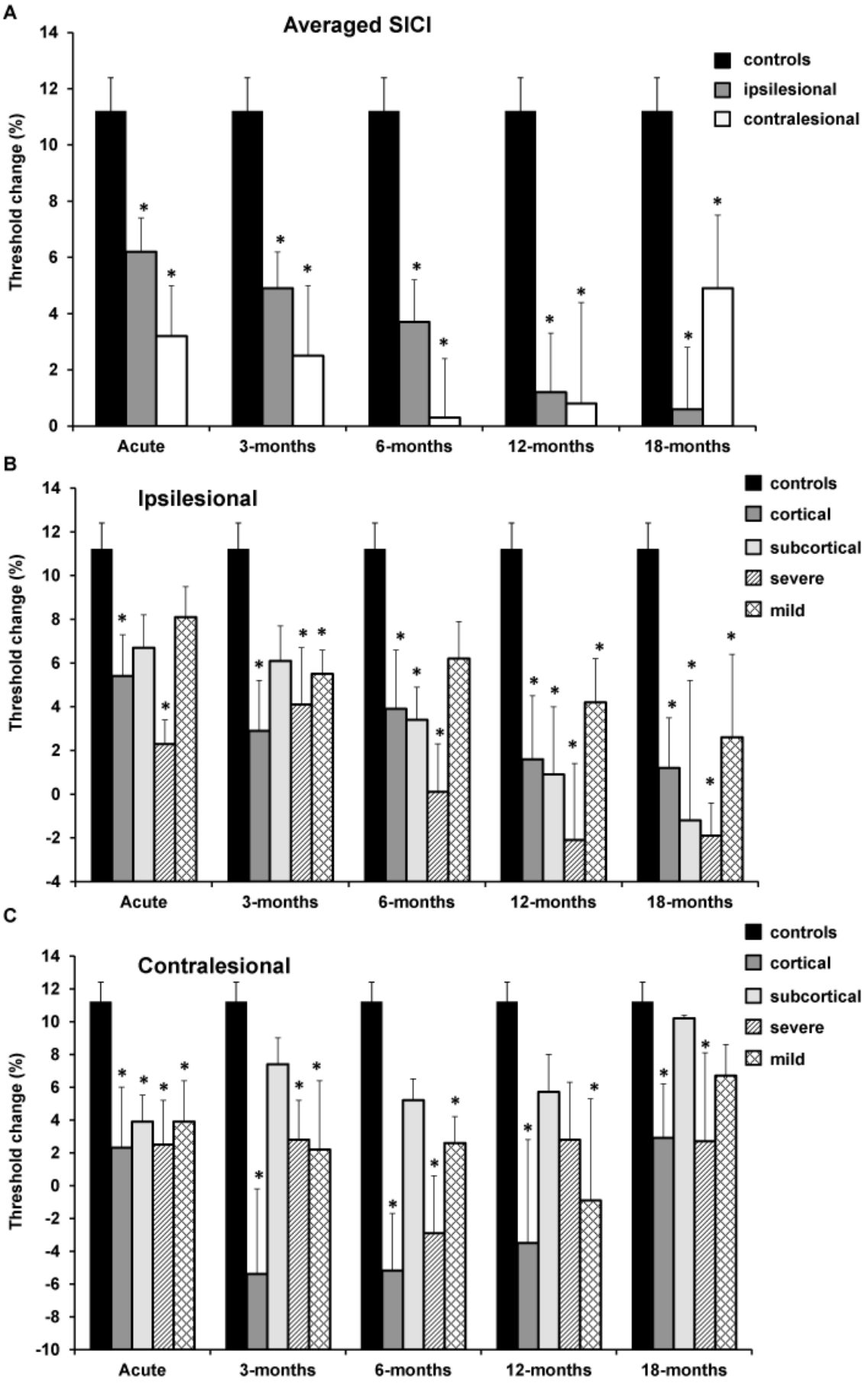

A
Averaged SICI
Threshold change (%)
14
12
10
8
6
4
2
0
controls
ipsilesional
contralesional
Acute
3-months
6-months
12-months
18-months
B
Ipsilesional
Threshold change (%)
14
12
10
8
6
4
2
0
-2
-4
controls
cortical
subcortical
severe
mild
Acute
3-months
6-months
12-months
18-months
C
Contralesional
Threshold change (%)
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
controls
cortical
subcortical
severe
mild
Acute
3-months
6-months
12-months
18-months

Figure 5.3. Longitudinal changes in averaged short-interval intracortical inhibition (SICI). (A) SICI in both ipsilesional and contralesional hemispheres remained significantly suppressed compared to controls over the 18-month period; (B) Ipsilesional subgroup changes in SICI: there were no significant between-group effects (ANOVA) for both stroke location and baseline impairment, with all groups remaining significantly different compared to controls at 18-month in terms of reduced SICI; (C) Contralesional subgroup changes in SICI: there were significant between-group effects (ANOVA) for both stroke location and baseline impairment with SICI most reduced in those with cortical strokes and more impairment at baseline. SICI also normalized over time in the groups with subcortical stroke and mild impairment at baseline. * denotes statistically significant difference compared to controls. Threshold change is the difference in test stimulus intensity required to evoke the target MEP calculated as: [conditioned test stimulus intensity – RMT]/RMT X 100.

Averaged ICF remained unchanged over the longitudinal assessment period over both the ipsilesional (ANOVA: F=1.16, P=0.40) and contralesional (ANOVA: F=0.94, P=0.49) cortices, consistent with previous studies (Carmichael, 2011), as well as animal studies showing no long-term alterations in NMDA-mediated receptors following a vascular lesion (Mittmann et al., 1994).

3.3 Clinical correlations

At baseline, there were correlations between RMT of the affected hemisphere and functional scores although these did not reach statistical significance (BI: r=-0.29, P=0.053; FM: r=-0.26, P=0.075; mRS: r=0.24, P=0.09), but suggested that reduced corticomotoneuronal excitability may be present in those with most disruption to the functional integrity of ipsilesional motor pathways and consequently more functional impairment.

When the change in clinical scores between baseline and 3-months (period during which most clinical improvements occurred) were assessed against SICI, there were significant correlations between SICI at baseline in both ipsilesional (BI change: r=-0.59, P=0.003) and contralesional (BI change: r=-0.34, P=0.05) hemispheres, and changes in clinical scores such that those with most improvements in function were associated with more suppressed SICI at baseline (Fig. 5.4).

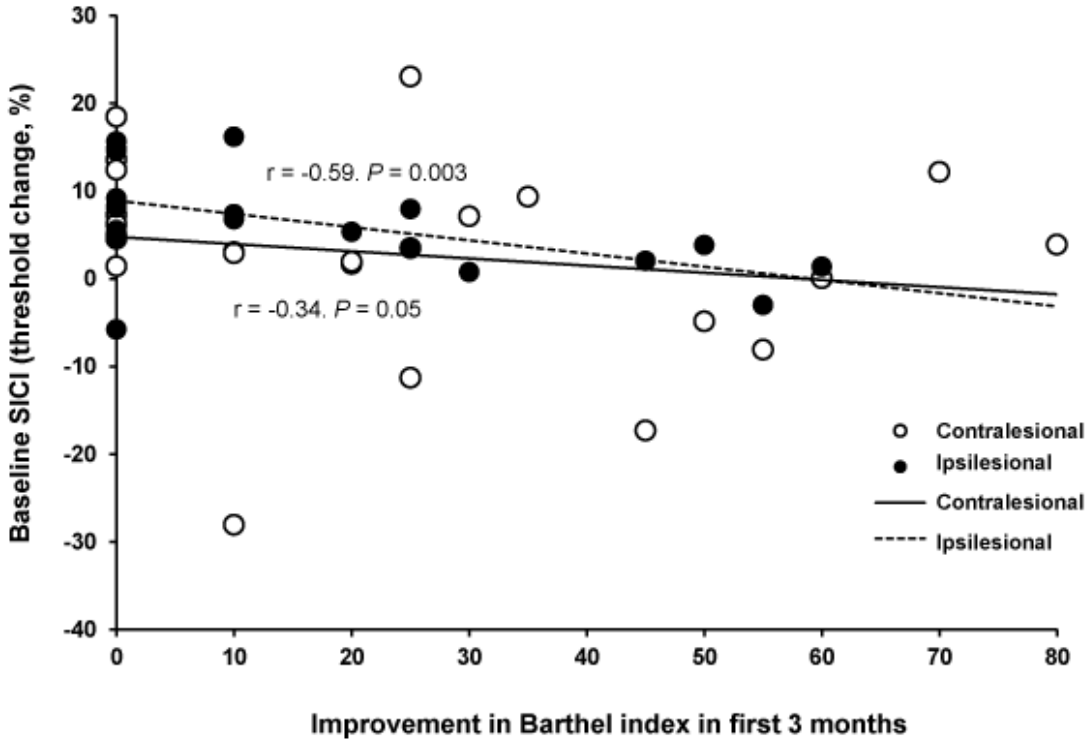


Figure 5.4. Correlations between baseline short-interval intracortical inhibition (SICI) and improvement in function as measured by the change in Barthel index, with data points from ipsilesional (closed circles) and contralesional (opened) hemispheres together with their linear regression lines. More reductions in SICI at baseline in both ipsilesional and contralesional motor cortices were associated with more improvements in function during the initial 3 months after stroke.

4 DISCUSSION

The present study has established the evolution of intracortical excitability changes that occurred over both motor cortices, in a longitudinal prospective design involving a cohort of well recovered stroke patients that were studied immediately after the acute event and for the first time, systematically reassessed at multiple time points up to 18-months. In addition, the current study also provided further insight into the nature of these changes over an extended follow-up period, and how the evolution of these potentially functionally relevant changes differed depending on baseline characteristics of the stroke.

4.1 Corticomotoneuronal excitability

Measures of corticomotoneuronal excitability including RMT, MEP amplitudes and CSP duration were not altered following acute stroke in both the ipsilesional and contralesional hemispheres consistent with previous reports (Butefisch et al., 2003; Cicinelli et al., 2003; Delvaux et al., 2003; Liepert et al., 2000d; Manganotti et al., 2002; Shimizu et al., 2002). Other studies have suggested reduced ipsilesional corticospinal excitability (namely elevated RMT and reduced MEP amplitudes) in the acute stroke period that tend to normalize over time (Caramia et al., 1996; Cicinelli et al., 2003; Liepert et al., 2000e; Murase et al., 2004). One possible explanation for this discrepancy is the widely variable RMT and MEP amplitudes even across control populations (Pascual-Leone et al., 1995; Wassermann, 2002). The absence of changes in CSP duration is consistent with animal and human studies in stroke that

have shown no alteration in GABA-B neurotransmission (Neumann-Haefelin et al., 1998; Sacco et al., 2009).

The present series has also demonstrated correlations at baseline between functional impairment and RMT that reflect the functional integrity of ipsilesional corticospinal pathways following stroke (Stinear, 2010; Swayne et al., 2008), with greater impairment in those patients having the most structural disruption to these tracts.

4.2 Intracortical excitability

4.2.1 Ipsilesional intracortical excitability

The results of the current study have revealed that over the ipsilesional motor cortex, intracortical hyperexcitability, as reflected by a reduction in SICI, persisted over the 18-month follow-up period and were associated with clinical improvements in function. This suggested that the ipsilesional changes likely represent adaptive neuroplastic processes mediating stroke recovery, and is consistent with results of previous reports (Butefisch et al., 2008; Liepert et al., 2000e; Manganotti et al., 2008; Wittenberg et al., 2007). Neuroimaging studies have also demonstrated regional over-activations in the ipsilesional hemisphere that persisted during the recovery process (Calautti and Baron, 2003; Carmichael, 2011; Cramer et al., 2002; Grefkes and Fink, 2011; Nair et al., 2007; Rehme et al., 2012; Ward et al., 2006; Werhahn et al., 2003), which may in fact represent the

imaging correlate of the electrophysiological changes observed using TMS (Logothetis et al., 2001; Ward and Frackowiak, 2006).

In addition, our results have also shown that the ipsilesionally suppressed SICI persisted regardless of lesion location or baseline functional impairment. There was also a correlation between baseline SICI over the ipsilesional hemisphere and the degree of improvement in function over the first 3 months, the period in which the most significant recovery occurred over. This correlation indicates that more intracortical disinhibition in the ipsilesional motor cortex facilitates more cortical reorganization and thereby driving recovery in the early critical stages following stroke. Similar correlations have been described previously, but have been between SICI and functional status at cross-sectional time-points (Swayne et al., 2008; Takeuchi et al., 2010). Our correlations were with longitudinal changes in function which provides a better reflection of the neuroplasticity process (Calautti and Baron, 2003).

4.2.2 Contralesional intracortical excitability

The results of the present study have demonstrated reductions in intracortical inhibition over the contralesional hemisphere during the 18-month follow-up period, in association with clinical improvements in function. During this period, there were significant between-group effects of stroke location and baseline stroke impairment, such that most reductions in SICI over the contralesional motor cortex occurred in those with cortical location of stroke and those with moderate to severe

baseline functional impairment. Furthermore, SICI in subcortical and mild baseline impairment groups normalized towards control values over the longitudinal assessment period, suggesting that the evolution of contralesional excitability differs between groups of stroke patients with differing lesion and clinical characteristics.

Controversy continues concerning the role and evolution of contralesional hemispheric changes observed in functional imaging and electrophysiological studies following acute stroke, particularly in regards to their functional relevance to the recovery process. In general, previous results using these techniques have revealed over-activations in multiple regions (Calautti and Baron, 2003; Chollet et al., 1991; Cramer et al., 1997; Foltys et al., 2003; Marshall et al., 2009; Rehme et al., 2011a; Ward et al., 2003a; Weiller et al., 1993) as well as reductions in intracortical inhibition (Butefisch et al., 2003; Butefisch et al., 2008; Liepert et al., 2000d; Liepert et al., 2000e; Manganotti et al., 2008; Shimizu et al., 2002) over the contralesional hemisphere immediately after the event, consistent with the current study.

Similar to other studies, contralesional hyperexcitability occurred across all subgroups according to location (Butefisch et al., 2003; Butefisch et al., 2008; Cicinelli et al., 2003; Manganotti et al., 2002; Manganotti et al., 2008). In addition, the present study also showed such intracortical disinhibition in both mild and moderate-severe impairment groups at baseline, although those with most reductions in SICI at baseline correlated with greatest improvements in function

during the first 3-months after ictus, suggesting SICI reductions being functionally relevant electrophysiological changes supporting stroke recovery in those patients who need it most.

Following the acute event, observed changes over the intact hemisphere as a function of time vary between previously reported studies. There are cross-sectional studies that have shown increased cortical excitability in patients with good recovery (Butefisch et al., 2008), whilst those without such changes in the contralesional hemisphere have poorer outcome (Butefisch et al., 2003; Shimizu et al., 2002). Findings from longitudinal studies however, have suggested that patient's with good motor recovery tend to demonstrate normalization of these parameters with time, whilst persistently reduced intracortical inhibition in the contralesional motor cortex were observed in those with poorer recovery (Manganotti et al., 2008). However, there are also neuroimaging studies that have shown persistent over-activation in the contralesional cortices in well recovered patients (Butefisch, 2004; Butefisch et al., 2005; Cramer et al., 1997; Gerloff et al., 2006; Marshall et al., 2009).

The results of the present study suggested that in this cohort of well-recovered stroke patients, contralesional intracortical disinhibition was most prominent during the follow-up period in patients with cortical strokes as well as those with more severe baseline impairment compared to their counterpart subgroups, and that the hyperexcitability persisted in these patients whilst those with subcortical strokes

and milder baseline impairment normalized with time. Our results also suggested that the more impaired patients at baseline were likely to have had significant disruption to the functional integrity of ipsilesional motor pathways, given the correlation between ipsilesional RMT and functional impairment. The balance of brain activation after stroke may in fact be governed by integrity of ipsilesional sensorimotor cortex and its corticospinal tract (Newton et al., 2006; Ward et al., 2006). Hence, those in whom the cortex is spared and the ipsilesional motor pathway integrity sufficiently intact (in the milder impairment group), good recovery may be mediated by normalization of activity back to the ipsilesional hemisphere. On the contrary, in patients with lesions involving the primary motor cortex and/or damaged ipsilesional corticospinal tract, persistent over-recruitment of contralesional networks may be required to achieve functional recovery (Cao et al., 1998; Chollet et al., 1991; Cramer et al., 1997; Feydy et al., 2002; Foltys et al., 2003; Murase et al., 2004; Schaechter, 2004; Ward et al., 2003b; Ward, 2006; Ward et al., 2006; Weiller et al., 1993).

4.2.3 Cellular mechanisms mediating cortical hyperexcitability and neuroplasticity

The widespread cortical hyperexcitability resulting from reductions in SICI following stroke is mediated by a down-regulation in processes involving GABA-A neurotransmission (Bashir et al., 2010; Neumann-Haefelin et al., 1998) that result in functional reorganization of cerebral networks, ultimately leading to structural reorganization conducive to motor and functional recovery. Greater plasticity is

associated with greater degrees of excitability, whilst enhanced inhibition is associated with impaired plasticity (Benali et al., 2008). These changes occur in regions structurally connected to the lesion in both hemispheres.

Animal and human studies in stroke have demonstrated reductions in both GABA-A receptors as well as GABA-A neurotransmitter levels following stroke in both hemispheres (Buchkremer-Ratzmann et al., 1996; Glodzik-Sobanska et al., 2004; Hagemann et al., 1998; Imbrosci and Mittmann, 2011; Neumann-Haefelin et al., 1998; Neumann-Haefelin and Witte, 2000; Sacco et al., 2009; Zeiler et al., 2013), that were a result of recovery rather than the infarct itself (Zeiler et al., 2013). Other studies that have supported the role GABA-ergic down-regulation in stroke recovery, have shown that introducing a selective GABA receptor antagonist in the chronic stages of stroke in rats resulted in motor recovery (Clarkson et al., 2010), whilst another study that involved administration of a GABA-ergic drug (midazolam) in chronic stroke patients led to re-emergence of stroke deficits (Lazar et al., 2002).

The resultant reduction in GABA-mediated inhibition facilitates the process of LTP (Castro-Alamancos et al., 1995; Hagemann et al., 1998), a mechanism fundamental to synaptic plasticity and hence cortical reorganization governing recovery after stroke. Associated with this, terminal axonal sprouting, dendritic branching and synaptic numbers are enhanced in both the ipsi- and contralesional hemispheres in animal models of stroke (Hermann and Chopp, 2012; Jones and Schallert, 1992; Jones and Schallert, 1994; Jones et al., 1996; Stroemer et al., 1995). Subsequently,

these cellular changes allows for the unmasking of previously silent networks and by converting these to functional networks is per se a mechanism of functional reorganization and cortical map plasticity (Imbrosci and Mittmann, 2011). This process also permits surviving neurons remote but connected to the damaged area to acquire information previously processed by the damaged tissue. The entire process culminates in the final structural modification to stabilize the new connectivity patterns (Imbrosci and Mittmann, 2011) in a way that drives functional recovery after stroke.

4.2.4 Clinical Implications

The use of novel non-invasive brain stimulation (NIBS) techniques such as repetitive TMS (rTMS) and transcranial direct current stimulation (tDCS) are becoming useful tools to modulate the plastic processes in order promote stroke recovery. Based on the concept of interhemispheric inhibition (Murase et al., 2004), a suggested model to improve function is the up-regulation of cortical excitability in the motor cortex (M1) of the lesioned hemisphere and/or down-regulation of excitability in M1 of the intact hemisphere (Hummel and Cohen, 2006). However, the prevailing view of needing to suppress contralesional excitability to redress this interhemispheric imbalance, has not considered effects on output pathways other than transcallosal projections (Bradnam et al., 2013), as well as potential deleterious effects by suppressing alternate ipsilateral pathways from the contralesional M1 that may be important for functional recovery in those patients with more damaged ipsilesional corticospinal tracts.

Results of the current study suggested that in patients with cortical stroke locations and more severe baseline impairment (reflecting significantly disrupted ipsilesional motor pathways), a reliance on alternate circuits orchestrated by contralesional networks may be functionally relevant for the recovery process and that interfering with this activity may in fact be detrimental. This is in keeping with previous studies that have demonstrated deterioration in function of the paretic hand following down-regulatory stimulation of contralesional M1 (Ackerley et al., 2010; Bradnam et al., 2013; Talelli et al., 2012; Theilig et al., 2011), and highlights the fact that a "one-size-fits-all" protocol is not appropriate and the importance for careful selection of patients for such interventions.

5 CONCLUSION

The present study investigated the nature and evolution of intracortical excitability in a cohort of well-recovered stroke patients over a longitudinal study period of 18-months since stroke onset. The results have demonstrated that intracortical inhibition over the ipsilesional hemisphere remained suppressed in all patients throughout the duration of the study, whilst contralesional intracortical inhibition remained suppressed only in those with cortical strokes and those with more severe baseline functional impairment. This suggests that in patients with lesions involving the primary motor cortex as well as significant disruptions to ipsilesional motor pathways, persistent hyperexcitability and thereby recruitment of contralesional networks may serve as functionally relevant substrates mediating recovery. This information has important clinical implications for novel interventional strategies

for stroke recovery and may guide the development of stimulation parameters and protocols when selecting patients for non-invasive brain stimulation.

SUMMARY, CONCLUSIONS AND FUTURE STRATEGIES

The studies comprising the present thesis have explored the physiological mechanisms of neuroplasticity underlying functional recovery in ischemic stroke patients. The studies utilized a combination of novel threshold-tracking transcranial magnetic stimulation (TMS) and peripheral nerve excitability techniques together with comprehensive clinical assessments with a view to identifying electrophysiological biomarkers of adaptive and maladaptive forms of neuroplasticity in stroke. The knowledge acquired from these studies may facilitate with the development of novel neuromodulatory interventions to promote the process of neuroplasticity and thereby recovery, above and beyond conventional means of neurorehabilitation which at present have a number of limitations.

1 PERIPHERAL NERVE EXCITABILITY CHANGES DEMONSTRATE NA+/K+ PUMP DYSFUNCTION IN LIMB ISCHEMIA

In Chapter 1, axonal excitability studies were undertaken on the affected upper limb in a patient with limb ischemia secondary to an occluding thrombus within the brachiocephalic artery. The studies were performed prior to, and after revascularization surgery, and the results were compared to ten control participants whom each had nerve excitability studies undertaken on 2 separate occasions to assess the reproducibility and intra-individual variability of excitability parameters. Results from the control cohort demonstrated reproducibility with minimal intra-individual variability amongst all axonal excitability parameters between the

separate study occasions. Changes in excitability parameters pre- and postsurgical revascularization were significant, with pre-vascularization indices demonstrating membrane depolarization compared to post-surgical values, reflecting impairment of the energy-dependent electrogenic Na^+/K^+ pump as a consequence of the ischemia. Furthermore, depolarization of the axonal membrane may serve as the pathophysiological mechanism for symptoms present in patients with limb ischemia.

The results in chapter 1 have allowed for the comparison of peripheral nerve excitability changes between ischemia directly affecting the limb in peripheral vascular disease, and that affecting the brain from ischemic stroke.

2 CHANGES IN CORTICAL AND PERIPHERAL NERVE EXCITABILITY IN ACUTE STROKE PATIENTS

2.1 Intracortical disinhibition is present in both hemispheres and may represent functionally relevant neuroplastic changes.

In chapter 2, threshold-tracking TMS demonstrated that cortical hyperexcitability with intracortical disinhibition was a prominent neurophysiological feature present in both the lesioned and contralesional hemispheres immediately after acute stroke, and that these changes persisted over the first 3 months after ictus. This reduction in short-interval intracortical inhibition (SICI) bilaterally likely represented neuroplastic processes that involve the down-regulation of GABA-ergic

neurotransmitters systems. The suppression in SICI was also accompanied by significant improvements in clinical function, suggesting that cortical hyperexcitability may represent a neurophysiological biomarker for acute stroke recovery.

2.2 Ipsilesional inexcitable motor cortex acutely does not always correlate with poor stroke outcome

Previous studies have reported that patients with inexcitable motor cortices acutely after stroke tend to have poor functional recovery. The results in chapter 2 of this thesis however, have demonstrated a cohort of acute stroke patients with an initially inexcitable motor cortex of the affected hemisphere, all of whom subsequently went on to show significant clinical recovery at 3 months follow-up. This was associated with resumption of cortical excitability. Although poorer function correlated with higher resting motor thresholds (RMT) and hence more corticomotoneuronal dysfunction at baseline, the ultimate outcome and recovery may depend on an interplay between resolution of initial perilesional changes such as oedema and diaschisis, as well as functional and structural changes occurring at the intracortical level of both the lesioned and contralesional hemisphere that contribute to the process of neuroplasticity in stroke recovery.

2.3 Peripheral nerve excitability changes may represent down-stream transynaptic neuroplasticity

Peripheral nerve excitability studies in chapter 2 have demonstrated acute alterations in excitability parameters of the affected upper limb of stroke patients that persisted over the 3 month follow-up period. The impaired accommodation to hyperpolarizing currents as reflected by changes in TEh(90-100ms) and hyperpolarizing I/V slope, were suggestive of reductions in I_H conductances, but there were also changes in TEd(90-100ms) suggesting additional biophysical alteration in motor axons (reduction in slow K^+ conductance). There was also a trend for similar changes observed in the unaffected limb although these were not statistically significant.

The alterations in peripheral nerve properties were different to those demonstrated in chapter 1 following peripheral ischemia, and may represent a down-stream transynaptic plastic process that reflect more central processes occurring in the brain after stroke.

3 CORTICAL MALADAPTIVE FORMS OF NEUROPLASTICITY IN POST-STROKE SPASTICITY

Chapter 3 investigated the neurophysiological mechanisms for post-stroke spasticity and the effects of treatment with botulinum toxin, by utilizing a combination of paired-pulse TMS and peripheral nerve excitability techniques. The results demonstrated that in chronic stroke patients with disabling spasticity, there was considerable disruption to ipsilesional corticospinal tract integrity and consequently, an inexcitable cortex on that side. In addition, contralesional motor cortices revealed significant reductions in SICI.

Following peripheral intramuscular administration of botulinum toxin, there were significant improvements in clinical scores for spasticity which were accompanied by an increase in SICI in the contralesional motor cortex normalizing to control values. The results suggested that pre-existing maladaptive plastic responses, present in the contralesional hemisphere, may contribute to the development of spasticity in the chronic stages of stroke, and that this may be modulated by the effects of botulinum toxin administration. The study also demonstrated that intramuscular botulinum toxin injection had no effect on the excitability properties of peripheral motor axons apart from the expected reduction in CMAP amplitude post-injection.

4 MOTOR CORTEX HYPEREXCITABILITY IN CEREBELLAR STROKES

Given the intricate connection between motor cortex and cerebellar structures particularly in regards to their role in motor control, threshold-tracking TMS studies were utilized in chapter 4 in patients with acute unilateral cerebellar infarct to investigate changes in motor cortex excitability and their potential relevance to functional recovery.

The results of the chapter have established significant reductions in intracortical inhibition (SICI) over the contralateral (to the cerebellar stroke) motor cortex whilst SICI was reduced over the ipsilateral motor cortex in patients with most functional impairment. The cortical changes persisted over the follow-up period in association with significant functional improvements, suggesting that cortical reorganization, particularly over the ipsilateral motor cortex, may represent adaptive changes facilitating the process of recovery in cerebellar stroke patients. This may have clinical implications for the development of novel neuromodulatory strategies over the motor cortices for strokes of this phenotype.

5 THE EVOLUTION OF CONTRALESIONAL CORTICAL EXCITABILITY IN STROKE MAY BE RELATED TO THE INTEGRITY OF THE CORTICOSPINAL TRACT.

In chapter 5, threshold-tracking TMS studies were utilized to clarify the evolution of intracortical excitability changes following stroke and their relevance to functional recovery, particularly pertaining to changes over the contralesional hemisphere. The results of this chapter have reaffirmed that SICI immediately after stroke is reduced in both the ipsi- and contralesional motor cortices that correlated with the extent of functional improvement over the first 3 months.

Over the subsequent 18 months longitudinal assessment period, SICI in the contralesional hemisphere remained suppressed in the groups with cortical location of stroke and those with more severe functional impairment at baseline, suggesting that ongoing contralesional network recruitment may be necessary for those patients who have significant disruptions to the integrity of ipsilesional motor pathways. This chapter has demonstrated that evolution of cortical excitability, particularly over the contralesional hemisphere, may vary between patients with differing baseline stroke and clinical characteristics, and that a “one-size-fits-all” approach when using novel neuromodulatory brain stimulation strategies to facilitate stroke recovery may be inappropriate.

6 PERSPECTIVES FOR FUTURE RESEARCH

Stroke and other neurological diseases typically affect multiple brain networks as demonstrated by the current thesis (Chapters 2, 3, 4 and 5). For this reason a network approach utilizing a combination of imaging and electrophysiological techniques, is likely to be appropriate in investigating the pathophysiological mechanisms underlying stroke recovery in other stroke phenotypes such as those involving the visual cortex or language centres, and the information gained from this can be utilized to develop novel interventional strategies to improve outcome (Naeser et al., 2012; Weiduschat et al., 2011). Furthermore, such information may help to decide whether interventional strategies targeting the motor network is a feasible approach to enhance recovery in these more remote stroke locations.

In chapter 4, it was demonstrated that cortical hyperexcitability in the motor cortices, particularly over the ipsilateral M1, may be relevant in the recovery process of unilateral cerebellar strokes. Future studies will investigate the potential role of non-invasive brain stimulation techniques over the motor cortices to promote cerebellar stroke recovery, which would offer a technical advantage over direct stimulation of the cerebellum, the latter of which is associated with difficulties in defining the actual site of stimulation because of the lack of focality and spatial resolution.

The role of the contralesional primary motor cortex in motor recovery remains controversial and needs to be further clarified before brain stimulation

interventions over this hemisphere can be successfully incorporated into novel neurorehabilitative strategies. It is likely that the ultimate reorganized structural and functional architecture will depend on the integrity of ipsilesional motor pathways. Hence, a combination of functional as well as structural imaging such as diffusion tensor imaging (DTI) together with TMS in future studies, will further our understanding of the functional relevance of contralesional network changes. Moreover, the evolution of maladaptive forms of plasticity requires defining. Chapter 3 of this thesis demonstrated maladaptive forms of plasticity in the contralesional motor cortex that may have contributed to the development of post-stroke spasticity in chronic stroke patients. Clarifying when these cortical changes transition from being adaptive to maladaptive, will aid in rationalising strategies to neuromodulate the changes at an appropriate stage and prevent post-stroke complications such as spasticity. Further studies can also identify whether similar electrophysiological mechanisms underlie spasticity from other neurological disorders such as multiple sclerosis.

In addition to anatomical-functional considerations, interventional studies need to address other potential factors that may influence response to neuromodulatory treatment such as the timing of treatment delivery, stimulation protocols, age of patients as well as comorbidities such as diabetes.

Furthermore, the electrophysiological and molecular mechanisms underlying improvement in stroke function following non-invasive brain stimulation and other

novel forms of intervention such as pharmacotherapy will be clarified in future studies. The knowledge acquired from studying plasticity of the central nervous system, may also be applied in future studies to interventional strategies used for the neurorehabilitation of disorders affecting the peripheral nervous system.

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