A Critical Window? Longitudinal Changes in Plasticity in Motor Cortex following Ischaemic Stroke

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I, Duncan Austin, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

While spontaneous recovery occurs in most patients following stroke, it is often incomplete. Recovery seems to be mostly confined to the first 6 months. Data from animal models suggest there is a critical period of enhanced plasticity similar to that seen in early development. Evidence for such a critical period has not yet been established in humans.

Repetitive transcranial magnetic stimulation is a suitable tool for measuring changes in plasticity in human motor cortex. However, its long-term test-retest reliability has not been widely studied.

Experiment 1

19 younger (average 29.9 years) and 20 older (average 65.9 years) subjects had repeat sessions of spaced cTBS to motor cortex 6 months apart. Change in average MEPs over 30 minutes post spaced cTBS showed fair intraclass correlation across 6 months in young (0.458 CI [-0.406, 0.791]) and older (0.572 [95%CI -0.08, 0.83]) subjects. This is broadly equivalent to other forms of plasticity-modulating non-invasive brain stimulation.

Experiment 2

29 subjects (average 68.2 years) had repeat spaced cTBS to contralesional motor cortex at 2, 4, 6 and 26 weeks following ischaemic stroke. There was a significant decrease in LTD-like plasticity across sessions ($p<0.01$). There was no change in resting motor threshold in either hemisphere and no change in intracortical excitability. Small vessel disease measured on MRI did not influence response to spaced cTBS.

Experiment 3

To complement the expansion in clinical research examining the benefits of fluoxetine in enhancing post-stroke plasticity, 31 healthy volunteers (average age 26.3 years) received fluoxetine 20mg or placebo prior to undergoing spaced cTBS in a double-blind randomised cross-over trial. There was no effect of fluoxetine on response to cTBS ($p=0.472$).

Conclusions

There is a decrease in LTD-like plasticity in the 6 months following a stroke in humans. 20mg of fluoxetine had no effect on LTD-like plasticity in healthy subjects.

Impact Statement

Regeneration of dead brain tissue after stroke or other injuries in humans is negligible. What then underpins the recovery seen in the majority of stroke patients, why does it appear to be time-limited, and how might it be enhanced to achieve better recovery for stroke patients? Such questions are integral to the study of post-stroke plasticity. However, studies in humans are constrained by the need to use non-invasive methods, and much evidence has instead been extrapolated from animal studies, which suggest there is a brief period of increased plasticity lasting a few weeks (typically 10-14 days).

Repetitive Transcranial Magnetic Stimulation (rTMS) has proven to be a useful non-invasive tool for studying brain plasticity in humans, but can be inconsistent across different individuals. In order to study changes in plasticity after stroke, it was first necessary to establish that repetitive transcranial magnetic stimulation shows a consistent response within subjects across an appropriate time period. We studied the reproducibility of the response to a new form of rTMS called spaced continuous Theta-Burst Stimulation (spaced cTBS) in healthy people across 6 months. We showed that spaced cTBS has a reasonably consistent response in motor cortex over time, and was therefore suitable for the task of measuring changes over time after stroke. Importantly (given that stroke predominantly affects older people) we demonstrated that spaced cTBS has reasonable consistency in both younger and older age groups. Our data for spaced cTBS in healthy controls set a new benchmark for measuring and reporting the long-term test-retest reliability of rTMS that should be considered by all researchers using brain stimulation to investigate longitudinal changes in plasticity, particularly those studying chronic neurological disease.

Subsequently, we applied spaced cTBS to subjects who had recently suffered a stroke affecting their motor cortex and followed them up over 6 months. We were able to show that changes in plasticity can be observed in healthy tissue in the contralesional hemisphere, confirming previous indirect evidence from functional

brain imaging. We found that plasticity in motor cortex declined between the first six weeks and 6 months after stroke. This finding has profound implications for the design of stroke research and rehabilitation services, which often engage with patients far later than this time window, and are therefore currently unable to exploit this period of optimal brain plasticity.

Finally, we found that the drug fluoxetine had no effect on spaced cTBS in healthy subjects. Previous experiments had suggested it might have an effect, leading to several large clinical trials using the drug in acute stroke patients. The first of these studies has now reported that fluoxetine did not provide any benefit to stroke recovery, and our own study showing no effect on plasticity in healthy subjects supports the viewpoint that fluoxetine does not enhance plasticity. We have shown that spaced cTBS can however detect changes in plasticity post-stroke, and so it should be an important tool for identifying other medications that could be used to increase this in future clinical trials.

Table of Contents

List of Tables & Illustrations

Tables

Illustrations

Schedule of Work

Experiment 1 (Chapter Three) was conducted between November 2013 and March 2017 at University College London. The study protocol was designed by DKA with assistance from JCR and RJG and performed by DKA.

Experiment 2 (Chapter Four) was conducted between February 2015 and November 2017 at University College London. The study protocol was designed by DKA with input from JCR and RJG at UCL and Professor Mike Ridding at the University of Adelaide. Data were collected by DKA with assistance from clinical staff.

Experiment 3 (Chapter Five) was conducted between January and June of 2017 at University College London. The study protocol was designed by DKA with input from JCR and RJG at UCL and Dr Lucia Li at Imperial College London. Data were collected by DKA and Lourenco Amador.

Comments on the manuscript and on the MPhil Upgrade Report in 2016 were received from Prof Sven Bestmann, Dr Marco Davare and Dr Lorenzo Rocchi. The manuscript was prepared and authored by Dr Duncan Austin – all external contributions have been appropriately acknowledged in the text.

Acknowledgements

It takes a village to raise a child, as the proverb has it, and the same is surely true of our intellectual children. The seeds of this thesis were planted as far back as 2009, and will I trust continue to (exponentially!) bear fruit in the years to come, both for myself and my colleagues professionally, but also in some small way for the scientific community at large. Stroke is a devastating condition, one which many people quite reasonably fear above all others, and whilst progress is finally being made, a colossal effort is still required before such fears can be finally put aside. The challenge that even this small offering has presented in its creation must give us all pause at the scale of the work that still faces us, but also must spawn a deep indebtedness to the many people that in have, whatever way, helped to share the burden.

Firstly I would wish to acknowledge the many outstanding stroke physicians I have had the opportunity to work alongside, both before, during and after the performance of this research, in all honesty too many to name, but to single out individuals: Dr Isam Salih, Dr Peter Sleight, Dr Nick Losseff, Dr Robert Simister, Prof David Werring, Dr Ronak Patel and Dr Yash Gawarikar. *The art is long and life is short*; but it is greatly encouraging to work with people who embrace the challenge with enthusiasm and perseverance.

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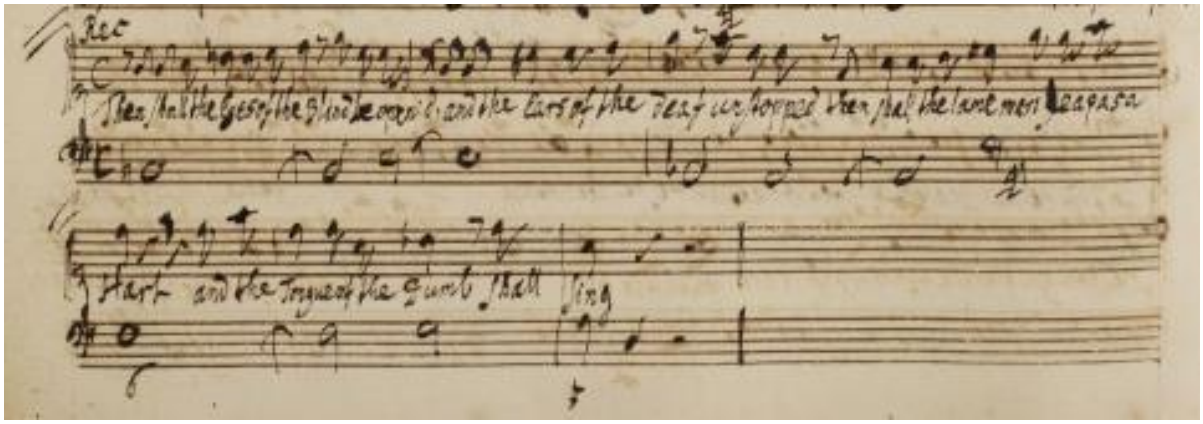


Fig P1: Movement Nineteen from Handel's *Messiah*, text from Isaiah Ch. 35 v 5 (image courtesy of the British Library)

'Then Shall the Lame Man Leap as an Hart' – a miraculous recovery from stroke?

On May 14th 1737, an announcement appeared in the London Evening Post: "*The ingenious Mr. Handell is very much indispos'd… with a Paraletick Disorder, having at present no Use of his Right Hand, which, if he don't regain, the Publick will be depriv'd of his fine Compositions*" (Bäzner, 2015). Georg Friedrich Händel, the celebrated composer, was at that point at the peak of his career as a musician and composer: as well as composing , was director of the Covent Garden Theatre and had premiered several new works in the previous twelve months, including *Israel in Egypt* and *Alexander's Feast*.

Then aged 52, grossly overweight and fond of tobacco, Handel was nonetheless not in good health, as attested by his sudden incapacitation in April 1737. His friend and collaborator Charles Jennens clearly describes the disorder as "*affect[ing] his hand and speech*": symptoms we would probably recognise today as typical for a cerebrovascular infarct – or stroke. What is known for certain is that, following some of the less hazardous advice of the physicians of the age, Handel took the bathing cure at Aix-la-Chappelle that summer, where after six weeks rehabilitation he surprised his fellow inmates by giving a spontaneous organ recital. 6 months later he returned to the London theatre and was able to finish his oratorio *Saul,* performed the following year.

When he next came to prepare a major oratorio, Jennens suggested a libretto comprising texts from the Old and New Testament that would come to form the famous *Messiah*, and included a text from the book of Isaiah which in this context must surely have been a subtle acknowledgement of his friend's impressive recovery:

"*Then shall the eyes of the blind be opened, and the ears of the deaf unstopped. Then shall the lame man leap as an hart, and the tongue of the dumb shall sing*."

Whilst the aetiology and even the territory of Händel's stroke could potentially be deduced by a modern reader of these descriptions, the impressive recovery he made still seems remarkable. How is it that the human nervous system, once

lesioned, is able to make such a substantial and rapid return to function? And can knowledge of this process guide us in our 21st Century search for treatments to augment the all-too-frequently incomplete recovery seen by patients?

Chapter One

Introduction: *Evidence for a Period of Enhanced Plasticity following Stroke*

The WHO ranks stroke as one of its leading priorities for global health, which currently sits in the top two leading causes of adult disability in most developed countries (Katan & Luft, 2018). Recent advances in acute stroke care have greatly improved the chances of survival, meaning that, on a global scale, stroke is now morphing from a killer disease into a chronic condition causing long term disability (O'Neill, Horgan, Hickey, & McGee, 2008). The financial implications of this shift are profound in terms of lost productivity and the ongoing costs of health and social care, and likely to escalate rapidly in the coming decades, particularly since the incidence of stroke amongst the young continues to rise (Saka, Mcguire, & Wolfe, 2009). Much of the pathophysiology that leads to stroke is increasingly well understood, and consequently a number of novel treatment strategies aimed at stroke prevention and hyper-acute reversal of cerebral ischaemia have been developed in recent years. However, advances in our understanding of the brain's own innate recovery process following cerebral infarction have proceeded much more slowly, and therapeutic targets for enhancing this process (or processes), which is typically prolonged and yet incomplete, are comparatively in their infancy. The stage is set for this next stage of development in stroke medicine, as continued advances in hyper-acute care throw into sharp relief the lack of treatments to improve long term function and decrease disability during the chronic stage of the disease. As stroke care improves and stroke survival rates increase, the need for therapeutic options for stroke survivors left with persistent deficit becomes ever more pressing. This thesis will explore what is known about this recovery process, what remains to be identified and how this might be manipulated to improve out comes for stroke survivors.

Stroke – a Neurodegenerative Disease?

It is perhaps remarkable, on consideration, that stroke patients like Georg Friedrich Händel should make any significant recovery whatsoever. Although sometimes classified alongside neurodegenerative diseases such as Alzheimer's or Parkinson's, and sharing the same cardinal feature of an irreversible loss of neuronal function, stroke, in common with spinal cord and traumatic brain injury, is marked out by the

prospect for some functional recovery. There is accumulating evidence that damage from stroke continues well beyond the first few hours of an infarct (Guadagno et al., 2008; Witte & Stoll, 1997) and in some patients appears to trigger continuing neurodegeneration and decline in cognitive function (Kraemer et al., 2004). Nonetheless, recovery of a significant proportion of neurological function remains the general rule after stroke, even when severe (Ward, 2017). The question is rather to what extent does the witnessed improvement in function after stroke represent true biological recovery, versus simply a learned adaptation to a persisting deficit? We will consider first the biological processes leading to short- and long-term tissue damage following cerebral infarction. We will then consider the known processes of neural repair (often overlapping with and not always readily distinguishable from the former category) and to what extent they may be contributing to functional recovery. As will be demonstrated, the mechanisms of neurological repair appear to have temporal limits and we will consider the attempts to first delineate and secondly extend this critical recovery period.

Infarcts and Haemorrhage

It should be noted that stroke is a something of an umbrella diagnosis, incorporating damage to the central nervous system from vascular causes, both thrombo-embolic infarction and also haemorrhage. Although haemorrhagic strokes represent more than 10 percent of new stroke diagnoses annually (An, Kim, & Yoon, 2017), they make a disproportionate contribution to the number of patients left with long-term disability. Furthermore, the incidence of haemorrhagic stroke continues to rise, reflecting the current paucity of effective preventative treatments and the health implications of a rapidly aging population. Nonetheless, haemorrhagic stroke/intracerebral haemorrhage is a comparatively neglected phenomenon in stroke research at both the clinical and experimental level, in part due to the lack of good animals models, as well as the inherently heterogeneous nature of the disorder clinically (Alharbi, Tso, & Macdonald, 2016). Both ischaemic and haemorrhagic stroke however represent an abrupt interruption in cerebral blood flow, and there is probably much crossover between the subsequent

pathophysiology of the two conditions, with damage primarily occurring through ischaemic/anaerobic processes in both cases. It is likely that the recovery from both these forms of stroke likewise share many common pathways (Ward, 2017). Most of the literature reviewed below comes from animal models of cerebral ischaemia or from human ischaemic stroke patients, but much of these data can also probably be extrapolated to haemorrhagic stroke. Unless otherwise stated, all data are derived from cerebral infarcts or from undifferentiated human stroke populations, but again unless explicitly stated to the contrary, it is assumed that results can be carried over at least in part to haemorrhagic populations.

Tissue Damage and Excitotoxicity

Following stroke, either ischaemic or haemorrhagic, there is a cascade of biochemical events as a region of brain loses its blood supply and undergoes metabolic failure, resulting in spreading infarction within ischaemic tissue. The metabolically-expensive task of maintaining neuronal membrane resting potential is among the first physiological processes to fail, resulting in depolarisation of ischaemic neurons and release of the excitatory neurotransmitter glutamate (Lai, Zhang, & Wang, 2014). Glutamate release triggers further depolarisation of neighbouring neurons, leading to spreading waves of depolarisation through the cerebral cortex, resulting in increasing levels of intracellular calcium, mitochondrial failure and accumulating oxidative stress, termed excitotoxicity (Carmichael, 2016). This process will ultimately result in necrotic cell death unless the tissue can be re-perfused and aerobic respiration restored. At the level of the individual neuron, excitotoxicity rapidly becomes irreversible, making it an unpromising target for medical intervention (O'Collins et al., 2006). As an infarct proceeds and expands, synaptic connections between neurons spread waves of depolarisation outwards through the ischaemic region, from the central core of the infarct through a neighbouring region of threatened ischaemic penumbra (Heiss, 2012). Accumulating calcium efflux and oxidative stress trigger an inflammatory response from local astrocytes, with subsequent invasion of neutrophils and later macrophages into the infarcted region over the following hours and days. This

inflammatory response, both cellular and humoral, results in the production of further toxic free radicals, promoting thrombosis and oedema in surround tissue and exacerbating the final scale of the ischaemic damage (Schofield, Woodruff, Halai, Wu, & Cooper, 2013). Reperfusion, if and when achieved, can also paradoxically causes a second wave of free radical generation as metabolic processes are restored, leading to additional cellular damage.

Neuroinflammation

There is thus broadly speaking a brief metabolic stage of tissue damage, lasting minutes to hours, followed by an inflammatory stage that continues for several days post infarct (fig 1.1). Abatement of this inflammatory response likely represents the underlying cause of the initial burst of spontaneous recovery frequently seen in patients in the first days following a stroke. Toxic metabolites are removed and local tissue oedema recedes, allowing electrically silent but anatomically intact circuits to return to activity (Lipsanen & Jolkkonen, 2011). Functional recovery however continues for (at least) several weeks following an infarct, and so it does not seem plausible that this process alone can account for its entire trajectory. Interestingly however, the inflammatory response that may have initially aggravated the ischaemic insult also appears to serve as a trigger for mechanisms of neural repair. As well as removing redundant necrotic tissue and toxic metabolic products, activated astrocytes promote angiogenesis and axonal sprouting (Gleichman & Carmichael, 2014), whilst cytokines from invading inflammatory cells also act as a trigger and a target for neuroneogenesis (Ohab & Carmichael, 2008). Thought for many years to be limited exclusively to foetal brain development, the process of neuroneogenesis (production of new neuronal cells from stem cell progenitors) has been consistently demonstrated in subventricular and subgranular zones of the brain during adult life, with migration of neuronal precursor cells from the subventricular zone towards the infarct zone observed following stroke (Arvidsson, Collin, Kirik, Kokaia, & Lindvall, 2002). It appears however that the majority of these new neurons do not survive for any length of time, particularly at sites that are some way distant from the subventricular zone,

and the contribution of neuroneogenesis to stroke recovery is probably fairly minor, particularly in larger mammals. Other mechanisms must surely play a larger role.

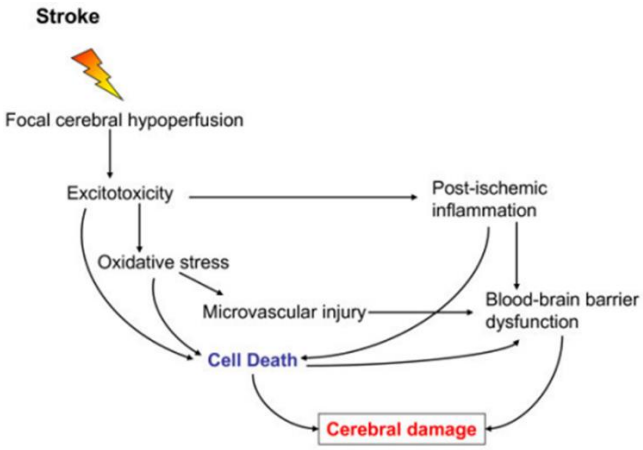


Fig. 1.1 – from Lakhan, Kirchgessner & Hofer (2009) *J Transl Med* 7:97

Diaschisis

Running in parallel to this process of local cellular damage and restitution is the phenomenon of diaschisis (von Monakow, 1914), whereby functionally connected regions of healthy brain tissue distant to the primary lesion demonstrate depression in activity and even subsequent regional atrophy (Witte, Bidmon, Schiene, Redecker, & Hagemann, 2000), mediated either through loss of afferent stimulation or for through unbalanced neuronal inhibition, with changes in distant regional activity evident as early as 3 days (Iizuka, Sakatani & Young, 1990) and peaking at around day 15 following stroke in animal models (Stefanis & Burke, 1996). This phenomenon seems to be particularly relevant to the clinical syndrome of cerebellar, thalamic and subcortical strokes, and can have a significant negative impact on recovery (Block, Dihné & Loos, 2005). Reversal of diaschisis, due to alleviation of or compensation for inhibitory drive to distant brain regions also probably contributes to some of the early spontaneous return of neurological function seen post-infarct. Intriguingly the phenomenon of diaschisis was not seen with pharmacological deactivation of mouse cortex using tetrodotoxin, and may be specific to ischaemic stroke (Mohajerani, Aminoltejari, & Murphy, 2011).

A particularly pertinent feature of diaschisis for strokes involving motor cortical regions is that of interhemispheric inhibition, which will be touched upon in more detail below. The precise mechanisms and importance of diaschisis remain debatable, partly due to a lack of understanding of the precise relationships between neurovascular changes as identified by functional neuroimaging, anatomical changes as demonstrated by serial structural imaging, and functional change as observed through patient behaviour and neurophysiology, and of the significance of each of these for ultimate clinical recovery (Carrera & Tononi, 2014). It is likewise debated whether diaschisis, once triggered, follows an independent course or is entirely secondary to changes taking place within the infarct and penumbra. Nonetheless the principle that a stroke can affect function in regions of cortex remote from the site of the original lesion, including the contralateral hemisphere, is well established, with its precise role in recovery yet to be determined fully.

Recovery versus Compensation

Whilst it is clear that entirely compensatory mechanisms, such as learning to perform a task with the non-paretic limb, may account for some of the recovery in performance after stroke (and may even impede recovery with the paretic limb through the phenomenon of learned non-use (Taub et al., 1993)), spontaneous biological recovery in the paretic limb is a genuine feature of most cases of stroke recovery (Zeiler & Krakauer, 2013). Whilst in rats the degree of neurological deficit is directly correlated with volume of infarction in the first few days following stroke, this association weakens over time post infarct, in keeping with a discrete (and variable) recovery process (Centonze et al., 2007). In the study of motor activity in recovering stroke patients, a stage of compensatory movements, whereby trajectories are achieved using alternative muscle groups and alternative degrees of freedom at less effected joints (van Kordelaar, van Wegen, Nijland, Daffertshofer, & Kwakkel, 2013), is first preceded by a distinct stage of recovery involving the

acquisition of new, skilled motor behaviour in the paretic limb to perform an action using impaired end effectors (Michaelsen, Jacobs, Roby-Brami, & Levin, 2004; Michaelsen, Dannenbaum, & Levin, 2006). Whilst motor learning, and cortical plasticity, is an ongoing process in motor cortex throughout the healthy lifespan, underpinning the acquisition of motor skill, evidence suggests that this process is heightened by mechanisms in action during the weeks following an infarct (Murphy & Corbett, 2009). This stage of recovery is traditionally broken down into 'restitution', whereby damaged but intact neurons return to their previous function, and 'substitution', whereby new neuronal circuits develop to restore the lost function (Hylin, Kerr, & Holden, 2017). As mentioned above, development of entirely new neurons is probably not a major factor in neuronal substitution: cortical plasticity is more likely taking place at the level of the dendrite and synapse.

In animal models, injured neurons within the ischaemic cortex demonstrate an increase in dendritic sprout and a loss of specificity post stroke that in some ways mirrors that seen during the early stages of neurological development (Cramer & Chopp, 2000). A variety of gene expressions and growth factors have now been identified that are responsible for this (Carmichael et al., 2005; Li & Carmichael, 2006). These changes in perilesional cortex include axonal sprouting, dendritic branching and synaptogenesis. Whereas it was previously thought that the physiology on the cerebral cortex was somewhat fixed and "hard-wired", at least in adult life, a body of evidence has now emerged that the role of cortical neurons is adaptable, or "plastic" and responsive to environmental change (Rauschecker, 1995). That this can lead to remodelling of cortical representations is now well established in both sensory (Jang & Lee, 2013) and motor cortex (Nudo & Milliken, 1996), and has also been demonstrated in the context of motor learning in healthy organisms (Kleim, Barbay, & Nudo, 1998).

Brain Plasticity

The concept that even in healthy individuals the nervous system's functional topography is not permanently fixed but capable of reorganisation in response to experience was first termed 'plasticity' by William James (1890). It was initially thought this malleability was limited exclusively to critical developmental periods in early life, and that thereafter the brain remained fixed and immutable (Fu & Zuo, 2011). Modern imaging and neurophysiological data have now shown that this phenomenon is in fact present throughout the lifespan. The concept of plasticity is an appealing one in which to term brain repair following stroke, but if it is not properly defined it can be in danger of being over utilised to the verge of becoming meaningless (Berlucchi & Buchtel, 2009). The mechanisms underlying cortical plasticity are often considered in terms of classical Hebbian plasticity (Hebb, 1949), through use-dependent changes in synaptic excitability (termed Long Term Potentiation and Long Term Depression). James' original concept predates this concept however, and reflects a broader principle of allowing temporary changes in cerebral function to become more permanent (but not irreversibly so). It is now appreciated that even in healthy adults changes in cortical topography are achieved through a combination of structural changes (production of new synapses (synaptogenesis), and the pruning of old ones) and functional ones (changes in the strength of existing synapses) (Cooke, Bliss, & Cooke, 2006). We will consider both of these processes in turn, their implication for the observed phenomenon of cortical plasticity and their relevance for stroke recovery. The concept of time-specific plasticity requires first that we consider plasticity in the developing brain. It was here that much of the original evidence for cortical plasticity was established, and has continuing relevance to our understanding of plasticity after stroke in adults.

Developmental Plasticity

Much of the pioneering work establishing the principle of cortical plasticity originates in the visual cortex (Hubel & Wiesel, 1998). Early work established that

there is a critical post-natal period (approximately day 21-28 in mammals) during which ocular dominance columns are established, and if exposure to visual stimulation is delayed beyond this time point such columns never develop (Mower & Christen, 1985). However, in animals that are permanently deprived of visual stimulation, neurons in the 'visual' cortex develop responsiveness to auditory and somatosensory stimuli (Kaas, 2002) that appears to be functionally relevant and is not found in normally-sighted animals. Structural and functional imaging in congenitally blind humans suggests something similar also takes place in the human brain (Kupers & Ptito, 2011). Evidence in rodents now suggests that this phenomenon is achieved through the production of novel neuronal afferents from auditory and sensory cortex to the lateral geniculate nucleus, a primary 'relay station' in the optic tract (Izraeli et al., 2002). Within the visual cortex itself, visual deprivation leads to an increase in dendritic spine formation (Hofer, Mrsic-Flogel, Bonhoeffer, & Hübener, 2009) that appears to be under the influence of both excitatory and inhibitory signalling, and stabilises only if visual stimulation is restored during the critical period (21-28 days; (Tropea, Majewska, Garcia, & Sur, 2010). Thus, in contrast to the former orthodoxy, the central nervous system is now known to be capable of producing novel neuronal pathways in response to environment changes into adult life, that this process is dynamic, and that these changes occur not merely through the alteration of existing synapses but also through the formation of new axonal projections.

Experience-Driven Plasticity and Motor Learning

Such experience-driven changes have also now been demonstrated in sensory (Trachtenberg et al., 2002) and motor cortex well past the developmental critical period, with increased dendritic sprouting in layer V pyramidal neurons within mouse motor cortex observed within 1 hour of a motor learning task (Xu et al., 2009). Dendritic spine formation was found to be proportional to the degree of motor learning, and ongoing training resulted eventually in the elimination of old spines, suggesting that new spines ultimately come to replace them. This pattern of spine formation was not however observed in animals that had previously been

trained on the same motor task in early life, but was observed when those animals were trained on a separate novel task. This suggests that this spine formation response is task-specific and can occur only once for a given learnt motor skill. These dendritic changes were likewise specific to skill acquisition and do not seem to be found just with motor activity *per se* (Kleim, Cooper, & VandenBerg, 2002).

At the macroscopic anatomical level, this production of novel dendritic spines corresponds to a re-mapping of cortical topography, so that somatotopic motor maps (or for that matter, sensory or visual maps - Kaas (1991)) are themselves re-organised in their cortical distribution (Nudo et al., 1996), with expansion of areas of cortical representation that show increased use and reduction in the representation of areas that are comparatively underused seen in healthy adult mammals (Navarro, Vivó, & Valero-Cabré, 2007) and also humans (Pascual-Leone et al., 1995). As with the changes witnessed at the histological level, these changes in cortical map representations are potentially reversible (Milliken, Plautz, & Nudo, 2013), and are associated with skill acquisition and not merely activity-dependent (Plautz, Milliken, & Nudo, 2000).

Plasticity in Response to Injury and Pathological Conditions

Such cortical somatotopic remodelling has also been demonstrated in response to pathological states. Following peripheral nerve lesioning in animals (Sanes, Suner, Lando, & Donoghue, 1988) and also with limb amputation (Schieber & Deuel, 1997) there is a pattern of almost instant (within minutes) silencing of the disconnected cortex, followed within days by a period when stimulation of the silenced cortex produced activity in muscles with adjacent cortical representations. In one animal study, remodelled motor cortical maps following botulinum-toxin paralysis of the vibrissal pad were already well established by day 6 (Franchi, 2002), with shrinkage of the cortical representation of the affect muscles accompanied by expansion of the cortical representation of neighbouring muscles, in keeping with the pattern of change seen following disuse of a limb or muscle group (Adachi, Lee, Hu, Yao, & Sessle, 2007) –nature seemingly abhorring the vacuum produced by a region of

"unused" cortex without a function output. This phenomenon has also been demonstrated in human patients with peripheral nerve lesions, using both functional neuroimaging and TMS (Rijntjes et al., 1997), and its correlate in somatosensory cortex has been speculated to underpin the phenomenon of phantom-limb pain amputees (Flor et al., 1995). This phenomenon of expansion of adjacent motor representations and inter-mingling of cortical projections may also underlie the disabling phenomenon of motor synergies in stroke patients, where paretic muscles and joints can only be activated in tandem with their neighbours, resulting in undesired co-contraction and a significant detrimental effect on motor control (McMorland, Runnalls, & Byblow, 2015).

Cortical Reorganisation following Stroke

As well as in response to a loss of afferents or effectors following peripheral injury, cortical reorganisation has been demonstrated following direct lesion of the cortex. This can be traced back to seminal work by Glees & Cole (1949), who demonstrated remapping of the thumb representation in motor cortex into adjacent cortical territory following focal lesioning in a macaque monkey. Nudo et al. (1996) demonstrated that this phenomenon of topographical remapping (fig. 1.2) was dependent on training post-lesioning, in a similar manner to the dendritic branching later observed on a microscopic level by Plautz, Milliken, & Nudo (2000) and others. Dijkhuizen et al (2001) likewise demonstrated changes with fMRI patterns of activation in the lesioned rat brain that corresponded to increased activation in perilesional cortex and contralesional primary motor cortex, a phenomenon that has also been demonstrated in humans (discussed below). Recovery was associated with a return to an ipsilesional pattern of activation, but still an extensively remapped topography persisted.

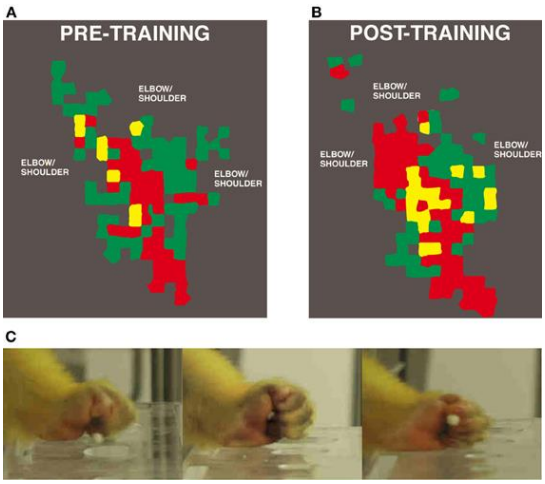


Fig. 1.2 *From* Nudo (2013) *Front. Hum. Neurosci* 7:887

Recovery Recapitulates Ontogeny?

Thus, we have seen that malleable changes in cortical functionality are observed both during early development and in response to peripheral and cortical injury, but to what extent do these processes overlap? Does "recovery recapitulate ontogeny"? During early brain development, axonal sprouting is also activity dependent, in response to axonal guidance molecules such as BDNF that are cued by cortical activity (Uesaka, Ruthazer, & Yamamoto, 2006). Hubel and Wiesel's work in cat visual cortex (Hubel, Wiesel, & LeVay, 1977) demonstrated horizontal branching of axons to establish intracortical connections (as well as with more distant targets) that are then either reinforced or 'pruned' in response to activity. In stroke model rat brains, such axonal spreading is also seen and can propagate as far as the contralateral striatum (Carmichael & Chesselet, 2002).

Similarities also exist in the patterns of electrical activity and their relationship to plasticity. Waves of spreading synchronised cortical activity seemed to be a potential trigger for axonal sprouting post stroke (Carmichael & Chesselet, 2002) and are capable of being propagated to the contralesional hemisphere. The polymorphic delta wave activity seen on EEG post stroke (Sharbrough, Messick, & Sundt, 1973) and perhaps also the increase in seizures and epileptiform activity that often follows, potentially therefore represent a pathophysiological component of

the neuroplastic response. A similar pattern of synchronous neural activity characterises axonal branching in the developmental period, but is currently less well understood (Feller, 1999).

However, when the gene transcription profile for neuronal sprouting after stroke is compared to that during embryological development, there is a substantial difference between the two molecular programs (Li et al., 2010). Whilst common mechanisms may well be at play, the fingerprint of genetic expression during developmental plasticity and that seen during post stroke recovery are distinct, albeit overlapping (Carmichael, 2006), and it is clearly an oversimplification to say that developmental plasticity has been simply 'reactivated'.

Developmental and pathophysiological plasticity seemingly also interact, with greater potential for plasticity following neonatal neural insults than in adult life (Reid, Rose, & Boyd, 2015). Recovery from motor cortex lesioning was also much more pronounced in infant monkeys than adults, with faster and fuller restoration of motor deficit (Kennard, 1942), matching observational data in human stroke populations. Interestingly, a lesion in infant macaques resulting in reorganisation of primary motor cortex (Rouiller et al., 1998), whereas a similar lesion in adults resulted in minimal reorganisation in M1 but pronounced plasticity in premotor cortex (Liu & Rouiller, 1999).

Evidence of Cortical Remapping in Human Stroke Patients

In adult human stroke patients, functional brain imaging confirms reorganisation of cortical maps in motor cortex (Ward, Brown, Thompson, & Frackowiak, 2004), with extensive reorganisation of patterns of neural recruitment demonstrated with functional MRI. With motor activation in the paretic limb following stroke, activity is seen in a variety of ipsilesional secondary motor areas not typically activated in healthy controls (Ward et al., 2006), with increased activity in dorsal and ventral

premotor cortex and the supplementary motor area, as well as a posterior shift in activation within primary motor cortex (M1) itself. Interestingly, recruitment was also observed in motor regions in the contralesional hemisphere. Restoration of a normal pattern of cortical activity in chronic stroke patients was associated with better recovery (Buma, Lindeman, Ramsey, & Kwakkel, 2010), similar to the change in cortical BOLD signal observed in rats (Abo, Chen, Lai, Reese, & Bjelke, 2001).

It could be argued that these changes in cortical topography are not necessarily the substrate of recovery, but may merely represent an epiphenomenon of changes taking place elsewhere within the nervous system, for example in subcortical white matter. In healthy subjects, changes in cortical topography associated with motor learning did not need to be sustained in order to maintain the acquired skill (Molina-Luna, Hertler, Buitrago, & Luft, 2008). The fact that disruption of activity in the contralesional motor cortex often results in recrudescence of deficit in the paretic limb in both humans and animal models, discussed in more detail below, would suggest however that the changes are functionally relevant to recovery.

Neurophysiological Evidence for Cortical Reorganisation

Likewise, the hypothesis that cortical remapping seen on fMRI following stroke represents true functional plasticity and not merely altered neurovascular response is supported by neurophysiological data, which consistently demonstrates re-organised cortical topography, with expansion of cortical maps and map migration - typically along the mediolateral axis - demonstrated with both MEG (Rossini et al., 1998) and TMS (Traversa, Cicinelli, Pasqualetti, Filippi, & Rossini, 1998). Magnetoencephalography (MEG) is a non-invasive method of measuring changes in magnetic field at the cortical surface, generated by the summed electrical activity over a region. This technique has been used to demonstrate cortical plasticity within the primary somatosensory cortex more than a year after stroke (Rossini et al., 2001). Likewise EEG (electroencephalography), whilst lacking the spatial definition of MEG, can demonstrate synchronisation in beta-band neural

oscillations over primary motor cortex in humans early post stroke (2 weeks) that correlates with recovery at 3 months (Nicolo et al., 2015), supporting the idea that electrophysiology can capture important changes that correspond to underlying changes in cortical plasticity.

Transcranial Magnetic Stimulation (TMS), discussed in more technical detail below, is an alternative mechanism of measuring changes in cortical activity through non-invasive induction of electrical current to stimulate cerebral cortex. TMS of motor cortex in human stroke patients showed expansion of cortical representations of the ADM muscle (Liepert, Uhde, Gräf, Leidner, & Weiller, 2001), a phenomenon that also seemed to be modulated through rehabilitative therapy and correlated with functional recovery.

Thus, in addition to neurovascular changes seen with imaging, functionally relevant changes in neurophysiology can equally give us insight in to the mechanism of cortical reorganisation, particularly its time course, and potentially to identify patients likely to make a good recovery.

Contralesional Hemisphere Plasticity

At this point, further consideration should be given to the role of plasticity in the contralesional hemisphere following stroke, and how this may influence the recovery period and possible its duration. Whilst recruitment of adjacent and functionally homologous ipsilesional regions such as pre-motor cortex and SMA during motor cortical remapping is perhaps not surprising, the involvement of the contralesional motor cortex is more unexpected, in what was traditionally referred to as the “unaffected” hemisphere. It is known that the two hemispheres exhibit inhibitory effects upon each other via transcallosal circuits, and in the healthy nervous system these two processes are finely counterbalanced (Ferbert et al., 1992). This interhemispheric balance can be disturbed by infarction of (for example)

motor cortex (Murase, Duque, Mazzocchio, & Cohen, 2004), with the now disinhibited contralesional hemisphere exerting excessive inhibition on the already impaired ipsilesional cortex, exacerbating the impairment resulting from the stroke and possibly even introducing an ipsilesional deficit. Brodal (1973), in a celebrated autobiographical case study, reported deterioration in the quality of his handwriting with his right hand following right hemisphere stroke, and a number of observational studies have also identified weakness in the ipsilesional limb compared to healthy controls (Colebatch & Gandevia, 1989). The bilateral pattern of cortical activation seen on fMRI (Ward et al., 2004) is likewise associated with "mirror" activation seen in the ipsilesional limb when attempting to move the paretic limb (Kim et al., 2003). This functional impairment in the "unaffected" limb seems to improve on a similar trajectory and time course as the contralateral deficit (Xu et al., 2017).

In rodent models there also appears to be the capacity for enhanced motor learning with the non-paretic limb following stroke, suggesting an enhanced meta-plastic state in the contralesional hemisphere (Allred & Jones, 2008). Skill acquisition in the non-paretic limb post stroke however disrupts spontaneous recovery in the paretic limb (Allred, Cappellini, & Jones, 2010), suggesting that such enhanced plasticity in the contralesional hemisphere is required for paretic limb recovery. Biernaskie et al. (2005) found that in a murine stroke model, significantly increased numbers of dendritic branches were found in the contralesional hemisphere that were proportional to the infarct volume, with dendritic branching also directly correlated with functional improvement. Injection of lidocaine into the contralesional motor cortex subsequently produced recrudescence of the deficit (Biernaskie et al., 2005), suggesting reorganisation in the contralesional hemisphere remains functionally relevant after recovery. However in a classic early study in primates, when Leyton & Sherrington (1917) produced a contralesional hemiplegia through ablation of 6-8mm of cortex from the pre-central gyrus (primary motor cortex) followed by what is described as a full recovery, subsequent ablation of the contralesional motor cortex produced no recrudescence of the initial hemiplegia. In human subjects,

Lotze et al (2006) found that disrupting activity in contralesional motor cortex using repetitive TMS in stroke patients resulted in temporary recrudescence of motor deficit, whilst a number of small studies have paradoxically found a benefit from inhibitory TMS to contralesional motor cortex during rehabilitation (Boddington & Reynolds, 2017). Again, such a benefit seems to be timing dependent, and was reduced or lost in patients outside the first 6 months (Rose, Patten, McGuirk, Lu, & Triggs, 2014). Such an improvement in motor learning through inhibition of contralateral motor cortex is however also seen in healthy volunteers (Kobayashi, Hutchinson, Théoret, Schlaug, & Pascual-Leone, 2004), calling into question to what extent this is harnessing a specific post-stroke physiology.

Ipsilateral Corticospinal Projections

Lindau et al (2014) found that in rodents, sprouting of axonal projections from contralesional cortico-spinal tract to the paretic limb, peaking around Day 21, was associated with remarkable recovery in those animals treated with anti-NoGo-A antibodies, but that these projections receded with time. Whilst fMRI data from healthy human controls that suggest a degree of bihemispheric control is indeed normal with upper limb movement, particularly with fine motor control (Diedrichsen, Wiestler, & Krakauer, 2013), and it is known a small proportion of corticospinal neurons do indeed innervate the ipsilesional upper limb, their contribution to upper limb function in both normal and post stroke physiology is thought to be minimal (Soteropoulos, Edgley, & Baker, 2011; Zaaimi, Edgley, Soteropoulos, & Baker, 2012).

Bihemispheric Activation in Severe Stroke

Thus we have shown that in animal models activity in the contralesional hemisphere with use of the paretic upper limb appears to be proportional to the severity of the deficit (Biernaskie et al, 2004), in keeping with bihemispheric activation seen in imaging studies of recovered human stroke patients (Ward et al.,

2004), where bihemispheric activation persisted in patients with poor recovery/severe residual deficit, but returned to a 'normal' pattern of activation in those who had completed a good recovery. It seems that patients with poor recovery may remain permanently dependent on the contralesional hemisphere, explaining the effects of disruptive TMS to contralesional M1 in stroke patients (Mohapatra et al., 2016). We will now consider what these changes in contralesional cortex (and elsewhere) may represent at the neuronal level, and how they might be studied non-invasively during stroke recovery.

Microanatomical Changes

Experience-dependent plasticity in both motor learning and post-stroke recovery has been shown to have an anatomic component, with formation of new dendrites and new synapses (Fu & Zuo, 2011). In peri-infarct cortex, there is initially a loss of dendritic branches (Brown, Boyd, & Murphy, 2010), even in non-ischaemic regions, implying the phenomenon is at least partially neuronally driven (Mostany et al., 2010). Neurons with 1mm of the infarct recover their baseline level of dendritic branching, whereas those within 2-3mm gain additional supernumerary branches. Neurons within 200µm of the infarct also develop new branches to existing dendrites (Brown et al., 2010).

The promotion of new dendritic formation is not limited to the peri-infarct zone but exhibited in distant but functionally connected regions of cortex, mirroring the anatomical diaschisis described by von Monakow. In addition to Wallerian degeneration of axons originating from neurons within the infarct zone, other neurons may degenerate through loss of inputs, whilst yet others may experience excessive inhibition or programmed cell death (Wei, Ying, Cui, Langsdorf, & Ping Yu, 2004). Axons undergoing Wallerian degeneration themselves trigger a local inflammatory response that may exacerbate injury in these more distant site (Block et al., 2005) and that may constitute part of the diaschisis phenomenon.

Non-neuronal cortical components may also contribute to enhanced neuroplasticity. Perineural nets, first described by Golgi in 1893, are extra-neuronal matrix proteins believed to be responsible for maintaining synaptic stability and known to be essential for the opening and closing of critical plasticity periods during development (Tsilibary et al., 2014), and are known to be degraded by compounds known as matrix metalloproteinases (MMPs), particularly MMP-9 (Park et al., 2009). MMPS are believed to play a critical role in migration of novel neuronal progenitor cells to infarct region, but their prolonged presence inhibit cortical remodelling and may underpin the "opening" and "closing" of such a critical period following stroke (Zhao, Tejima, & Lo, 2007). They are rapidly upregulated following ischaemic stroke in both humans and animals (Montaner et al., 2001; Park et al., 2009) and contribute to the inflammatory component of infarction through a variety of processes (Zhao et al., 2007). They also play a role in regulating the activity of the variety of growth factors and growth inhibitors that are transcribed post infarct.

Synaptic Mechanisms of Cortical Reorganisation

In addition to the anatomical plasticity of new dendritic branches and synapses, changes in neuronal connectivity in response to synaptic activation may also contribute to the electrical remapping of cerebral cortex, and to the restoration of function. The precise balance between synaptic and structural factors in cortical plasticity is uncertain, and probably varies at differing points on the recovery curve (Murphy & Corbett, 2009). Seminal work in the physiology of synaptic plasticity was performed by Donald Hebb, who observed that "*when one cell repeatedly assists in firing another, the axon of the first cell develops synaptic knobs (or enlarges them if they already exist) in contact with the soma of the second cell*" (Hebb, 1949). Bliss & Lømo demonstrated this phenomenon electrophysiologically in the perforant pathway of the hippocampus (Lømo, 1966), observing that high frequency stimulation at a synapse resulted in a persistent alteration of synaptic strength which they labelled Long Term Potentiation (LTP). Its corollary – Long Term Depression (LTD) was also soon demonstrated, and in combination they were

proposed as a likely mechanism for learning, particularly given the evidence from neuropsychology for the hippocampus's role in memory formation (Scoville & Milner, 1957). An accumulating body of evidence implies that these processes of LTP and LTD underpin learning in motor cortex, and since the molecular substrates of these two phenomena have now been largely elucidated, this makes them tempting targets for interventions to promote stroke recovery (Takeuchi & Izumi, 2015). The induction of LTP is dependent on activation of the NDMA glutamate receptor, with repetitive depolarisation of the post-synaptic membrane resulting in a voltage-dependent magnesium ion blockade of the NMDA receptor and influx of calcium ions, causing in turn rapid phosphorylation of AMPA receptors. LTP was dependent on activation of either the GluN2A- and GluN2B- subunits of the NMDA receptor, whereas LTD required only activation of GluN2A (Bartlett et al., 2007), confirming that they are indeed discrete processes and not simply the inverse of each other.

Ischaemic LTP (iLTP)

Of relevance to the role of LTP in stroke recovery, It was shown by Crepel, Epsztein, & Ben-Ari (2003) that ischaemic stress *in vitro* (deprivation of glucose and oxygen) could induce a long-lasting increase in excitatory synaptic strength in a similar manner to tetanic stimulation, and with similar molecular processes (Di Filippo et al., 2008). Such a phenomenon, termed ischaemic-LTP or iLTP, might explain the increased incidence of seizures following ischaemic stroke (Lenz, Vlachos, & Maggio, 2015), but also represents another potential target for therapeutic manipulation. However, the process of iLTP differs from canonical LTP in important respects, such as iLTP proving to be more GluN2B dependent (Picconi et al., 2006). Furthermore, as well as induced LTP itself, ischaemia seems to render the synapse unresponsive to tetanic LTP (Stein et al., 2015).

Metaplasticity

Such findings have given rise to the concept of metaplasticity, effectively the prospect that the plasticity of the nervous system is itself malleable or plastic, and can be increased or decreased (Müller-Dahlhaus & Ziemann, 2015). Of particular relevance is the concept of Homeostatic Plasticity (Bienenstock, Cooper, & Munro, 1982), whereby induction of plastic change at a synapse renders that synapse resistant to further plasticity, thereby stabilising synaptic weights within a neural network and sustaining and consolidating the changes (learning) that have been induced. Thus with increased LTP, further LTP becomes occluded and LTD facilitated, in a manner that appears to be NMDA-receptor dependent (Abraham, Mason-Parker, Bear, Webb, & Tate, 2001). Other factors appear to influence cortical metaplasticity, including voluntary muscle contraction (Iezzi et al., 2008), prior motor learning (Stöckel, Carroll, Summers, & Hinder, 2016; Ziemann et al., 2004) and genetic factors (Cheeran et al., 2008). The metaplastic change (occlusion of LTP) induced by motor learning in healthy human volunteers appears to subside after 5 days (Rosenkranz, Kacar, & Rothwell, 2007), sufficient time for new synapses to have formed following dendritic branching and suggesting a possible means whereby anatomical and synaptic factors interact to consolidate consecutive gains in motor learning (or stroke recovery). Of additional note is the possibility that metaplasticity could be influenced through pharmacological factors, which will be discussed in further depth shortly.

Altered Electrophysiology in the Post Stroke Cortex

Changes in the excitability of cortex following stroke likely contribute to, and are also to a degree a consequence of, both cortical infarction and repair (Lamola et al., 2016). Furthermore, such changes are potentially measurable and modifiable, creating both a marker for post-stroke plasticity and a target for intervention. The general trend, complementing the changes in activation seen on functional imaging, is for a decrease in cortical excitability in the perilesional stroke cortex, which returns to parity with the contralesional hemisphere over time in well recovery

patients (Swayne, Rothwell, Ward, & Greenwood, 2008). Changes in excitability in the contralesional hemisphere, corresponding to the contralesional activation on fMRI, has been less consistently demonstrated (McDonnell & Stinear, 2017).

Changes in cortical excitability may be mediated by the effect of GABAergic inhibitory interneurons (Talelli, Greenwood, & Rothwell, 2006), and there is a reduction in inhibitory interneurons in ischaemic cortex post stroke (Alia et al., 2016; Zeiler et al., 2013). In animal models suppression of GABAergic inhibition following stroke lead to improved outcomes (Clarkson, Huang, Macisaac, Mody, & Carmichael, 2010; Lake et al., 2015) and appears to have an effect also on experience-driven plasticity (Chen et al., 2011). Reduction in intracortical inhibition also seems to augment Hebbian plasticity at the synaptic level in the form of LTP (Hagemann, Redecker, Neumann-Haefelin, Freund, & Witte, 1998). In human stroke patients there appears to be a reduction in GABA-A receptor availability, particularly in the contralateral cortex, that was negatively correlated with stroke recovery in a study by Kim, Yang, Cho, Lim, & Paik (2014).

As well as decreases GABAergic transmission, enhanced glutamatergic activity may also underpin cortical plasticity and changes in cortical excitability post stroke. Glutamate activity at AMPA receptors drives BDNF-mediated neuroplasticity following stroke (Clarkson et al., 2011). Administration of an NMDA antagonist blocked expansion of the hand area following median nerve lesioning in primates when given within a month of the injury, but not at later time points (Myers, Churchill, Muja, & Garraghty, 2000). This implies a role for NMDA-mediated plasticity that becomes established, and seemingly irreversible, after a certain time point. Glutamatergic transmission at both NMDA- and non-NMDA receptors has been shown to be increased at the synaptic level for up to 4 weeks post stroke (Centonze et al., 2007). The balance between excitatory and inhibitory phenomena post stroke may could therefore potentially represent the opening and closing of a ‘critical period’ post stroke (Carmichael, 2012).

Genetic and Molecular Changes

As recovery following stroke proceeds, there is a transition from expression of proteins for axonal sprouting towards those for synaptogenesis (Biernaskie, Chernenko, & Corbett, 2004; Carmichael, 2006). The pattern of gene and protein expression following stroke give us some indication to the timetable that different processes run along, and goes some way to help delineate the critical period of stroke recovery in theoretical terms. The balance between excitatory and inhibitory electrophysiology is again seen, with an increased induction in post-stroke ischaemic cortex of proteins with a role in the induction of LTP such as GAP43, SCG10, and MARCKS within the first 2 weeks (Gregersen, Christensen, Lehrmann, Diemer, & Finsen, 2001). There is a concomitant reduction in expression of inhibitory calcium-binding proteins parvalbumin, calretinin, and calbindin within peri-infarct cortex within the in the first week after stroke (although interestingly this was once more only seen in those animals exposed to rehabilitative activities (Zeiler & Krakauer, 2013)).

Likewise there seems to also be a dissociation between gene expressions that enhance and inhibit neuronal growth – although many genes code for proteins that promoted both LTP and also favour axonal sprouting (Carmichael, 2003). Such growth factors are not exclusive to the post-stroke brain, but there nonetheless appears to be a unique signature of growth factors expressed (Li & Carmichael, 2006). After the initial bloom of growth promoting factors, there is a later surge of growth inhibiting factors such as chondroitin sulphate proteoglycans (CSPG; Sandvig, Berry, Barrett, Butt, & Logan, 2004) and myelin associated proteins like NoGo A that block axonal sprouting (Lee, Kim, Sivula, & Strittmatter, 2004). This biphasic pattern of the induction of growth-promoting and growth-inhibiting factors creates an additional mechanism for the opening and curtailment of the critical period of optimal stroke recovery (Carmichael et al., 2005). Days 1-3 post stroke there is an initial burst of factors promoting growth cone upregulation, triggered by the synchronised neuronal discharges seen during acute infarction (Carmichael & Chesselet, 2002). Days 7-14 there is a rise and then decline in the

expression of p21, Ta1 tubulin, and L1, which promote axonal sprouting. There is a later peak around Day 28 of factors SCG10 and SCLIP that are implicated in axonal sprouting (Iwata et al., 2002). From Day 14 there is an increase in levels of NG2, which synthesises a CSPG called versicans responsible for scar formation (Fawcett & Asher, 1999). A distinct separate pattern of gene expression, as well as a reduced rate of axonal sprouting, is seen in older animals (Li & Carmichael, 2006), with a different profile of genes expressed, a slower increase in growth promoting factors and an earlier introduction of growth inhibiting ones, possibly underpinning the reduced recovery in such animals. These periods of gene expression roughly coincide with the histological animal data that postulate a period of enhance neuroplasticity for 5-14 days post infarction.

Time Course of Recovery

Biernaskie, Szymanska, Windle, & Corbett (2005) found that in a mouse model, significantly increased number of dendritic branches were found in the contralesional hemisphere, proportional to the volume of infarct in ipsilesional cortex. These changes were only seen in those animals exposed to an enriched environment, considered to be the equivalent of physical therapy in human stroke patients. More specifically, the increase in dendritic branching was only found in those animals exposed to the enriched environment at 5 days post stroke, but not with delayed exposure at 14 or 30 days. These findings mirrored functional outcomes: animals exposed to the enriched environment at day 5 made significant functional gains, whilst those delayed to 14 days showed only intermediate effect and those delayed to 30 days no benefit, with dendritic branching directly correlated with functional improvement. In another rodent study, Jones & Schallert (1992) found a biphasic response in contralesional sensorimotor cortex following infarction, with proliferation of dendritic branches peaking at around day 14-18, and subsequently partial elimination of branches between day 30 and 120. Such proliferation was associated temporally (after a delay of a few days) with maximal disuse of the contralesional limb. Such dendritic arborisation was blocked however by restricting use of the ipsilateral (“good”) limb. Furthermore, unilateral limb use-

restriction in healthy (sham-operated) animals did not produce any significant increase in dendritic branching. Neither the infarct nor limb restriction could produce this dendritic proliferation in isolation, which seems to be reliant on a lesion-behaviour interaction (Jones & Schallert, 1994). The later, pruning phase (Day 30 to 120) did not seem to be blocked by unilateral limb use restriction, suggestion a different process to be in place at this stage.

Zeiler et al (2016) found that mice retrained on a reach-to-grasp task 7 days after a photocoagulation-induced stroke to the caudal forelimb area (equivalent to primary motor cortex in primates) failed to reach pre-infarction skill levels despite continued training for 19 days, but that if a second infarct was induced in the medial premotor area and training commenced after a further 48 hours, the animals recovered to skill levels consistent with performance prior to the first infarct. However, when the second infarct was induced in the visual cortex, no such reopening of the sensitive period was witnessed, with no benefit from training, suggesting that the later recovery observed was neither merely consequent on the first ischaemic stroke, nor a generic effect of cerebral ischaemia (or of the lesioning technique). Promisingly from the perspective of potential clinical applications, a study by the same research group (Ng et al., 2015) found that by administering fluoxetine to the mice, they appeared to be able to prolong this sensitive period beyond 7 days, even in the absence of training - but only if the fluoxetine was itself commenced during the first 7 days. Earlier work on rodent visual cortex had found that fluoxetine could also re-open critical period of developmental plasticity in ocular dominance columns in juvenile mice (Maya Vetencourt et al., 2008), another intriguing similarity between the two conditions.

Likewise in primates, Black, Markowitz, & Cianci (1975) observed that in a rhesus monkey, following a surgical lesion to motor cortex (precentral forelimb area), 85% recovery of hand grip was seen with both training of the paretic limb and bimanual training, but only if the training was instigated immediately post lesioning and not

after a 4 month delay. In squirrel monkeys (Barbay et al., 2006), delaying rehabilitative training for 30 days post infarct prevented the restoration of previous cortical maps seen in monkeys exposed to early training (Nudo & Milliken, 1996).

Sugiyama et al. (2013) trained Macaque monkeys in a finger dexterity task prior to corticospinal tract lesioning. Those that commenced retraining early (same day) did just as well on the task as those who started late (after a month delay), but kinematic analysis found that the late trained monkeys were heavily reliant on compensatory movements, whereas early trained monkeys demonstrated kinematics similar to their pre-lesioned performance. Thus, there is substantial data to suggest that the neuro-anatomical changes seen in the weeks post stroke are accompanied by a limited period of increased sensitivity to training.

Plasticity – A Critical Window?

Combining these findings with the profile of genetic expression in the peri-lesional cortex mentioned earlier (Carmichael et al., 2005) and, this would strongly imply there is a critical 'window' of plasticity when access to rehabilitative therapy and training is likely to be most effective at driving recovery. Based on the behavioural data from animal studies, this critical window is quite short, and may have already been 'missed' by day 30, since delaying rehabilitative training for 30 days post infarction (Barbay et al., 2006) prevented the restoration of previous cortical maps seen in squirrel monkeys exposed to early training (Nudo & Milliken, 1996). Most rehabilitation physicians would however propose a slightly longer timeframe for the period of maximal recovery in human patients, although scientific attempts to measure this have often been blurred by the failure to discriminate adequately between true functional recovery and compensatory behaviour.

Trajectory of Recovery of Motor Deficit in Humans

Motor recovery from hemiparetic stroke in humans appears to have its own fairly stereotyped sequence of events, following a fairly standard trajectory, first described systematically by Twitchell. Initially, improvement in the upper limb is seen in the proximal flexors, spreading more distally as part of motor synergies (involuntary co-contraction of a group of muscles), followed by a similar proximal-to-distal restoration of extensor muscles. Subsequently the independent recovery of flexors and extensors separate from motor synergies emerges, including the hand. Finally (in patients who approach full recovery) individual use of single digits returns, starting with the index finger and then the thumb, progressing to full or near full recovery of hand function (Twitchell, 1951).Those who recovered full hand function had shown signs of voluntary hand movements within two weeks, and had made a complete recovery within 3 months. In a larger series of patients reported by Duncan, Goldstein, Matchar, Divine, & Feussner (1992), using a specific measure of motor deficit rather than functional impairment (the Fugl-Meyer Motor Assessment), it was shown that the bulk of motor recovery took place over the first 30 days regardless of the severity of the initial deficit, but that some patients with more severe deficit continued to recover neurological function for up to 90 days. Jørgensen et al. (1999) in the Copenhagen Stroke Study found that 80 percent of the patients had reached maximal upper limb function within three weeks, and that those with mild upper limb deficits reached their peak performance by six weeks whereas those with severe paresis did not do so until eleven weeks.

More recent work by Kwakkel, Kollen, & Twisk (2006) has found that this is trajectory holds not just across a variety of measures of upper limb function, but in the lower limb and across a variety of other neurological domains. Longitudinal behavioural analysis shows a non-linear logarithmic relationship between recovery and the passage of time that was strongest in the first six weeks and appears to be independent of the dose of rehabilitative therapy during this period (Duncan et al., 1994; Kwakkel d et al., 2006). When comparing motor control with upper limb power, Cortes et al. (2017) identified separate windows of 5 weeks and 8 weeks

respectively. Likewise Xu et al. (2017) found that recovery in the hand continued longer than the rest of the upper limb for both power and motor control, proceeding up to 3 months. It has been proposed that recovery continues for longer in more severely impaired patients, up to six months (Semrau, Herter, Scott, & Dukelow, 2015), although this may represent a ceiling effect on measures of impairment in mildly affected patients.

Hence it would be more useful to consider the critical period as tailing off gradually rather than having discrete boundaries. Furthermore, the duration of any critical window appears to vary depending on stroke severity.

Proportional Recovery

Duncan et al. (1992) found that 82% of the variation in recovery achieved at 6 months could be predicted by the degree of recovery at 30 days, and more recently this finding has been developed in to the "proportional recovery rule" (Prabhakaran et al., 2008), challenging the suggestion that stroke recovery is highly dependent on activity during this early period. Prabhakaran and colleagues found that not only was recovery made in proportion to the initial deficit, but that this relationship was relative constant at around 70% (0.7). i.e. most stroke patients will recovery 70% of their motor deficit by 3 months. This relationship however broke down in patients at the more severe end of the deficit scale (Fugl Meyer score 10 or less). Around 50% of these patients with more severe deficit obeyed the proportional recovery rule, whilst the remained showed minimal improvement (Winters, van Wegen, Daffertshofer, & Kwakkel, 2015).

Estimates of the final level of disability can be made with reasonable reliability as early as 3 days post stroke using single-pulse TMS (Byblow, Stinear, Barber, Petoe, & Ackerley, 2015), and much of the subsequent recovery appears to be predictable by functional and structural measures of cortico-spinal tract integrity (Byblow et al.,

2015; Stinear et al., 2012). These measures predicted not only functional upper limb movements, but even fine finger dexterity at 6 months, which was also reliably predicted based on progress on the Fugl Meyers score of global arm function at 4 weeks (Kwakkel, Kollen, van der Grond, & Prevo, 2003). Progress beyond 4 weeks had no further benefit in terms of prognostic accuracy, which would suggest that a patient's recovery is set in train by this point and relatively impermeable to external influence. Only 56% of variance in recovery at 30 days is predicted by deficit at baseline however, suggesting that factors other than the sheer scale of the initial deficit are at play, but that critically these factors are active during and seemingly restricted to this initial 30-day period, after which recovery is far less malleable.

Therapy Dose Response

In another pioneering early study, Ogden & Franz noted that if an animal was allowed to favour their non-hemiparetic limb in functional tasks, recovery in the paretic limb was extremely limited. However, if the animal were forced to attempt to use the paretic limb by constraint of the non-paretic upper limb, recovery was substantial (Ogden & Franz, 1917). Likewise, cortical remapping in squirrel monkeys following stroke was only seen following training in skilled hand movements (Nudo & Milliken, 1996), but delaying rehabilitative training for 30 days post infarct (Barbay et al., 2006) prevented the restoration of previous cortical maps seen in monkeys exposed to early training.

Evidence for a dose-response or therapy-dependent effect in human stroke patients is weaker, in part due to the ethical obstacle of denying patients access to therapy following a stroke, but would seem to be at contradicted by the finding by Prabhakaran et al (2008) that the majority of patients follow a fixed proportional recovery rule, despite what are known to be substantial variations in actual therapy dose across patients and across centres. Meta-analysis of stroke studies shows only a weak correlation between therapy dose and final recovery (Lohse, Lang, & Boyd, 2014), regardless of whether therapy was instigated in the chronic or acute phase,

with no dose response able to be detected in a more recent trial of patients randomised to therapy during the chronic phase of recovery (Lang et al., 2016). Thus, whilst animal models imply lack of activity in the paretic limb during the critical period impedes recovery, patient studies paradoxically fail to show that increasing activity in this period improves it.

Fixed or Malleable Recovery Curves?

This divergence between humans and animal stroke models may simply represent the more heterogeneous nature of human stroke patients, but might equally be related to the vastly greater number of repetitive training movements used in animal models versus that seen in stroke physiotherapy (Lang, Lohse, & Birkenmeier, 2015). Thus, the relationship between therapy dose, cortical plasticity and ultimate recovery is poorly delineated, and potentially weak at best (at least within a population receiving standard clinical care post stroke, and even within those participating in large scale clinical trials). Furthermore, it may again be dependent on successfully aligning therapy with the period of peak cortical plasticity, the precise boundaries of which have not been clearly demonstrated in humans, a concept with significant implication for the planning and delivery of clinical rehabilitation services.

A Target for Intervention – Human Evidence for Optimal Timing of Therapy

The interaction between physiotherapy and plasticity, and the temporal limits of recovery observed across different paradigms has stimulated recent clinical trials to identify the optimal time window for commencing therapy in acute stroke patients, but evidence for a definite time period is so far lacking. A pilot study (Poletto et al., 2015) had examined early mobilisation within 48hrs of infarction, but had to be terminated early due to slow recruitment. A further study by Herisson et al., (2016) looked at the impact of sitting-balance exercise in a Very Early (<24hrs) stroke window versus after Day 3, and was likewise negative, although the power of the

study was again compromised by slow recruitment and a suboptimal sample size. The AVERT trial (Bernhardt et al, 2015) randomised acute stroke patients to either early (>24hrs post stroke) or Very Early (<24hrs) physiotherapy intervention, aiming to capture the peak of the therapeutic window, which they postulated to begin almost immediately after stroke. This trial disappointingly found evidence of worse outcomes in the Very Early mobilisation group measured at 3 months. This was perhaps not surprising given the animal data indicating the peak of the neuroplasticity window occurs later than 24 hours post infarct, and the fact that excitotoxicity may contribute to further tissue damage in the first hours following an infarct. Two further studies have employed the AVERT trial protocol with Very Early mobilisation in the first 24 hours. Chippala & Sharma (2016) found a dramatic effect of Very Early mobilisation (85% independent at 3 months vs 45% with usual care, $p<0.001$) in a sample of 86 Indian patients. A larger study of 340 Italian patients (Morreale et al., 2016) however found no difference between groups: the difference in outcome between these studies may possibly be attributable to the difference in sample size, and also perhaps in the variation in the therapy received by the "usual care" arm between developed and developing world settings.

Kwakkel et al (2016) found that promoting using of the paretic limb through Constraint-Induced Movement Therapy (CIMT) was more effective than usual care during the early post stroke period (commenced within 14 days, on average day 8), but only in patients with voluntary finger extension present in the paretic limb at randomisation. No concomitant improvement in neurological deficit was demonstrated, leader the authors to observe "*functional improvements of the mCIMT group were based on adaptation strategies to use intact end-effectors in a more optimal way*."

Later in the recovery stage, the EXCITE trial (Wolf et al., 2010) found that patients given CIMT between 3 - 9 months post stroke responded better than those delayed to 12 months later –although the average time post stroke for the "early" group was 178 days at randomisation, far later than when the postulated critical period is thought to be most active. In the VECTORS trial (Dromerick et al., 2009), patients

who received CIMT starting 9 days post stroke showed a dose-response to therapy at 90 days, but that those who received the lower therapy dose later still 'caught up' with the others at 1 year, suggesting that the critical window may never truly "shut" – provided perhaps that at least some activity was introduced during the critical stage.

Thus, the data from clinical trials leaves it unclear as to the precise timings of optimal rehabilitative intervention, as well as the appropriate frequency and intensity of therapy, other than that this can comfortably be delayed for 24 hours after the onset of stroke. To study the interaction between optimal therapy dose and timing would require a large clinical trial with multiple arms and well-balanced patient groups. The use of non-invasive techniques to first map the time course of the critical period in humans would be vital, in order to help formulate the hypotheses for such clinical trials to test.

Techniques for Studying Changes in Post Stroke Plasticity

Transcranial magnetic stimulation (TMS – Rothwell, 2010), is a non-invasive technique that allows induction of an electrical current within cerebral cortex to be achieved whilst by-passing the high impedance of the skull and associated unpleasant side effects of transcranial electrical stimulation (Barker, Jalinous, & Freeston, 1985). Since a magnetic field can penetrate the skull without impedance, a rapidly changing magnetic field can be used to generate an electrical current within a perpendicular conductor such as a neuron under Faraday's law (Polson, Barker, & Freeston, 1982). When performed over motor cortex this induced current can depolarise cortical synapses and generate an action potential which is propagated along the corticospinal tract and manifests as a Motor Evoked Potential (MEP) in contralateral skeletal muscle than can be recorded through electromyography to produce a measure of cortical excitability. Since its discovery, TMS has been identified as a uniquely useful tool for studying cortical excitability and investigating cortical plasticity.

Tracking Changes in Cortical Excitability following Stroke with TMS

The ability to induce a motor evoked potential with TMS to ipsilesional hemisphere have been associated with good (or at least proportional) motor recovery following stroke (positive predictive value 0.94 – Zarahn et al., 2011). The negative predictive value of an absent MEP is less clear cut (negative predictive value 0.83), possibly due to the technical limitations of TMS meaning that a population of supramaximal-threshold corticospinal neurons can survive in some MEP-negative patients, or perhaps suggesting a role for non-CST or non-M1 neuronal pathways in post-stroke recovery in some patients. Nevertheless, a gradual reduction in excitation threshold following stroke has been seen with TMS over ipsilesional motor cortex, correlating with recovery (Traversa et al., 2000). Perhaps surprisingly in light of the postulated role of interhemispheric inhibition following stroke, motor excitation thresholds in the contralesional hemisphere nevertheless appear to be stable across the period of stroke recovery (Stinear, Petoe, & Byblow, 2015). Precisely such a contralateral disinhibition/increased excitability is nonetheless demonstrated in animal models in the first hours following an infarct (Mohajerani, Aminoltejari, & Murphy, 2011). This phenomenon continues well beyond the period where improvement might be attributed to the resolution of cerebral oedema, lasting up to 4 months. This would thus seem to provide a potential surrogate marker for the re-organisation of cortical circuitry described in experimental models, and for the possible restoration of balanced interhemispheric interactions.

Intracortical Inhibition

Administration of paired pulses of TMS at short interstimulus intervals have been shown to capture the effect of inhibitory interneurons within motor cortex, thought to be mediated through inhibitory (GABAergic) interneurons within motor cortex. In particular, short interstimulus intervals (2-3ms) can elicited the phenomenon known as short interval cortical inhibition (SICI), thought to be mediated by $GABA_A$ receptors. SICI is reduced in motor cortex in the initial weeks following stroke, and

it is possible that this intracortical 'disinhibition' may leaded to enhanced synaptic plasticity (Di Pino et al., 2014). Swayne, Rothwell, Ward, & Greenwood (2008) examined TMS parameters in recovering patients during the acute post-stroke period (up to 6 months) and found a difference in motor cortical excitement in the stroke hemisphere that resolved over 3 months, whilst reduced intra-cortical inhibition in the stroke hemisphere at 3 months was significantly correlated with recovery. Manganotti, Palermo, Patuzzo, Zanette, & Fiaschi (2001) observed a significant decrease in intracortical inhibition in both hemispheres during a timeframe spanning 5 – 7 days to 30 days post-stroke, but only changes in the contralesional hemisphere were associated with motor recovery. Thus, it seems that TMS, and specifically paired-pulse TMS, can probe cortical circuits in both hemispheres that are relevant to the process of motor recovery during the acute phase.

Repetitive TMS

Repetitive stimulation with TMS (a rapid train of several hundred pulses over several seconds) has been shown to be capable of producing temporary changes in cortical excitability that persist beyond the stimulation period (Hoogendam, Ramakers, & Di Lazzaro, 2010), and that resemble the synaptic Hebbian phenomena LTD and LTP. The nature of the change produced in cortical excitability depend on the pattern and frequency of the repetitive stimulation, with low-frequency protocols generally producing LTD-like suppression of excitability and high frequency stimulation producing LTP-like facilitation (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). All repetitive TMS protocols have been well characterised in motor cortex, which is well suited to the technique since the ability to elicit MEP provides a ready measure of cortical excitability through direct and trans-synaptic depolarisation of corticospinal neurons. The precise mechanism by which rTMS induces changes in synaptic plasticity is still debated, but there is accumulating evidence that its effects are manifested through changes in synaptic transmission that strongly resemble Hebbian plasticity. The effects of both inhibitory and excitatory rTMS can be blocked by NMDA-antagonists, suggesting

rTMS works via glutamatergic circuits which are both known to be behaviourally relevant for motor learning (Ziemann et al., 2004) and known to exhibit LTP and LTD *in vitro* (Huang, Chen, Rothwell, & Wen, 2007).

Theta Burst Stimulation

Whilst traditional rTMS protocols utilise supra-threshold stimuli (i.e. stimuli capable of eliciting an MEP) in long trains, Theta Burst Stimulation (TBS - (Huang et al., 2005)) allows repetitive stimulation to be delivered over much shorter time frames via a pattern of 5Hz 'bursts' of 3 50Hz pulses, imitating the theta burst rhythm used successfully to induce LTP *in vitro* (Staubli & Lynch, 1987). *In vivo,* TMS theta burst stimulation induces (LTD-like) cortical inhibition or (LTP-like) facilitation depending on whether it is delivered as a continuous or intermittent train. An additional advantage of theta burst stimulation is the use of a subthreshold stimulus, which carries a better safety profile than repetitive stimulation at suprathreshold intensities (Rossi et al., 2009). This was initial set as 80% of Active Motor Threshold (AMT), but subsequent studies have shown that 70% of Resting Motor Threshold (RMT) is equally valid, and may indeed be preferable, as muscle pre-contraction has been found to interfere with the effect of theta-burst stimulation (Gentner, Wankerl, Reinsberger, Zeller, & Classen, 2008).

Longitudinal rTMS in Stroke

Clinical trials using TMS protocols to try and promote stroke recovery through manipulation of cortical plasticity have so far proven disappointing (Hsu, Cheng, Liao, Lee, & Lin, 2012), and research is now focusing on the potential use of plasticity protocols as a biomarker for post-stroke plasticity to guide treatment and inform prognosis. Surprisingly few of the clinical trials of rTMS to date have even recorded post rTMS MEPs to provide any neurophysiological measure of the efficacy of the intervention, perhaps due to the perceived challenges of recording a reliable MEP from a stroke affected hemisphere, and there is at present very

limited data as to the neurophysiological response to repetitive TMS in the acute stroke cortex. Di Lazzaro et al., (2010) found that the increase in MEP amplitude in the affected hemisphere following facilitatory rTMS within the first 10 days of stroke was significantly correlated with future recovery, showing for the first time that rTMS can detect clinically meaningful changes in plasticity during the acute stroke period. However, no study to date has examined longitudinal changes in response to rTMS post stroke (McDonnell & Stinear, 2017). Thus, it is timely to see whether rTMS can demonstrate changes in cortical plasticity over the weeks and months following stroke that might help elaborate the duration of the critical window.

Pharmacological Manipulation of Plasticity

There is currently an urgent search for medication which might boost plasticity to enhance recovery after brain injury. A variety of drugs have been tried in small scale clinical trials, including levodopa, reboxetine and citalopram (Liepert, 2008). Noradrenergic agents have shown particular promise in rat models (Boyeson & Feeney, 1990) but only limited results in humans (Gladstone et al., 2006). As mentioned above, and of potentially great clinical relevance, Ng et al (2015) found that they could extend the critical window in rats post stroke using fluoxetine, with emerging data that such an effect may exist in humans also (Chollet et al., 2011). In visual cortex, SSRIs increase plasticity through reduced GABAergic inhibition (Maya Vetencourt et al., 2008), probably though 5-HT 1A receptors on interneurons (Puig, Watakabe, Ushimaru, Yamamori, & Kawaguchi, 2010). A single dose of a citalopram (which inhibits reuptake of serotonin from the synapse) has been shown to affect cortical excitability in healthy controls (Robol, Fiaschi, & Manganotti, 2004) and was found to modulate the response to cortical plasticity induced with Trans-Cranial Direct Current Stimulation (TDCS) in human volunteers (Nitsche et al., 2009). Tantalising evidence of an effect of fluoxetine on recovery in the first three months of a stroke was produced by a recent small clinical trial, which appears to be independent of its effect as an antidepressant (Chollet et al., 2011). This concept is now being expanded in to large Phase III Clinical trials to test the rehabilitative

effect of 6 months of fluoxetine in acute stroke (Mead et al., 2015). Thus, as well as the known anti-depressant and neuroprotective effects of fluoxetine, it may be promoting stroke recovery through direct synaptic mechanisms. This hypothesis is yet to be tested in humans, but may provide important insight in to the mechanism of the drug in stroke recovery.

Conclusions

There is an accumulating body of evidence that there is a period of enhanced plasticity following a cortical infarct, in keeping with all that we know about the mechanisms of cortical injury and repair. This period probably lasts between 10-15 days in rodents, possibly longer in humans, with recovery predominantly confined to the first six weeks and largely plateauing after 6 months. Training during this enhanced period appears essential to maximise recovery, although a proportion of patients with severely damaged corticospinal tract will probably make no recovery despite intense training, and once recovery in the upper limb has commenced it appears to follow a fairly established trajectory. This recovery is probably mediated through recruitment of motor representations in supplementary motor regions (SMA, PMC) and contralesional M1, with the best recovery associated with ultimate 'release' of these recruited regions and a return to a unilateral pattern of activation. At present there is no convincing evidence that this critical period can be manipulated in humans to facilitate recovery, although tantalising evidence suggest fluoxetine and/or serotonin may have a role in this regard. Advances in stroke imaging and in neurophysiological techniques may soon allow the critical window to be more precisely defined in human cohorts and ultimately in individual stroke patients, and may also serve to identify the most promising pharmacological means of prolonging and augmenting cortical plasticity during the recovery period. This thesis will explore the use of transcranial magnetic stimulation to examine the changes in cortical neurophysiology following stroke, and whether the drug fluoxetine can manipulate cortical plasticity in a way that might benefit recovery.

Chapter Two

Methodology

Experimental Methods

Participants

All research was prospectively approved by the appropriate local Research Ethics Committee, with written informed consent of participants in accordance with the Declaration of Helsinki. Methodology was designed and reported in accordance with the International Consensus Guidelines for using TMS to study the motor system (Chipchase et al., 2012). Subjects were screened for any contra-indications to TMS using the screening questionnaire developed by the Safety of TMS Consensus Group guidelines (Rossi et al., 2009). People with a history of chronic neurological disorders were excluded from experiments involving healthy controls. Subjects with a historical diagnosis of migraine were permitted to take part provided they had not had a recent attack and were not currently taking migraine preventative medication. Subjects with a historic diagnosis of anxiety/depression were included provided their clinical condition was stable and they were not currently taking antidepressant medication. Subjects on other prescribed medications were permitted provided these were not CNS active.

Stroke patients in Experiment 2 taking CNS active medications were permitted to take part provided these did not constitute an unacceptable safety risk (Rossi et al., 2009), they were stable on their present dose and took the medication consistently throughout the course of the study. Left handers were not excluded from any experiment (numbers reported in each chapter) given the lack of any consistently demonstrated differential TMS physiology between left handers and right handers (Daligadu, Murphy, Brown, Rae, & Yielder, 2013). All subjects were stimulated in their dominant hemisphere, except for stroke patients who were stimulated in their contra-lesional hemisphere. Handedness was taken as self-reported, with the Edinburgh Handedness Index (Oldfield, 1971) utilised where handedness was ambiguous or self-reported as ambidextrous.

Clinical Scales

Patients in Experiment 2 had clinical scales recorded at each session using pen and paper proformas (Appendix B). Post stroke disability was assessed using the 6 point modified Rankin Scale (van Swieten, Koudstaal, Visser, Schouten, & van Gijn, 1988) rated from 0-6 based on degree of independence (0 – fully independent with all activities of daily living; 6 – dead (see Appendix B)). Subjects were scored at their worst level of performance in the preceding 2 weeks as a global measure of function. Consequently, subjects who are independent in most domains of their daily life but need assistance with a single task e.g. transport were scored as needing assistance.

Post-stroke depression was screened for using the MHI-5 screening questionnaire (Berwick et al., 1991) based on self-reported frequency of symptoms of depression (see appendix B). Clinical examination was performed by a trained physician immediately prior to each stimulation session to produce a score on the NIH Stroke Score (Wityk, Pessin, Kaplan, & Caplan, 1994). Subjects are scored from 0 – 42 across a series of domains based on clinical examination findings, with 0 indicating no clinical signs of stroke and 42 indicating signs of a severe stroke.

The Fugl Meyer Upper Limb Score, a subset of the Fugl Meyer Assessment of Sensorimotor Function (Fugl-Meyer, Jääskö, Leyman, Olsson, & Steglind, 1975) was used to assess upper limb function. Subjects are scored from 0-66 based on motor performance, with 66 indicating perfect performance. Grip strength was assessed at different joints using a tennis ball, a cylinder, a piece of A4 paper and a pencil. Deep tendon reflexes were elicited using a standard Queen Square tendon hammer.

Imaging

All participants in Experiment 3 had brain imaging as part of their medical care prior to enrolment in the study. These data were used to confirm the location of their infarct but were not otherwise used in the delivery of TMS. At a later stages subjects who had clinical MRI (GE Genesis Signa or Siemens Avanto) available and who consented to the use of their images had their T2 sequence axial plane images reviewed and graded (Fazekas, Chawluk, Alavi, Hurtig, & Zimmerman, 1987) from 0-3 for periventricular white matter and 0-3 for deep white matter according to the size and confluence of white matter lesions in comparison to prepared templates. Ultimately only deep white matter scores were used as a covariate due to their superior correlation with ischaemic small vessel disease (Kim, MacFall, & Payne, 2008).

Neuronavigation

All subjects in all sessions were testing using Brainsight™ (Rogue Resolutions Inc., Cardiff, UK) neuronavigation. Neuronavigation was guided using surface landmarks and electrophysiological feedback in the form of EMG and without the incorporation of neuroanatomical imaging. Subjects wore custom glasses with a reflective Subject Tracker attached on the side opposite to the hemisphere received cTBS. Subjects were asked to keep the glasses in position for the duration of TMS testing. A second reflector was attached to the TMS coil using a Brainsight™ TMS Coil Tracker Fixation Adaptor. TMS Coil Tracker, Subject Tracker (reflective glasses) and anatomical landmarks were calibrated in 3D space using an NDI Polaris Vicra™ optical infrared position sensor (NDI Medical, Waterloo, Ontario, Canada) fixed to the ceiling. Brainsight™ software then provides continuous real-time 3-dimensional feedback on coil position and orientated. MEPs collected whilst the coil was more than 1mm or 3° off target in any plane were discarded and repeated.

Test Conditions

All testing took place in the TMS Laboratories in the Sobell Department at the National Hospital for Neurology and Neurosurgery, London, UK. To control for physiological diurnal variation (Sale, Ridding, & Nordstrom, 2007) experimental sessions were scheduled to be as close as possible to the timing of previous sessions for the same subject in that experiment. Subjects were asked to ensure they were well rested, but explicit levels of sleep quality and quantity were not recorded. Subjects sat in a comfortable armchair and were asked to keep their eyes open, their hands relaxed and their legs uncrossed. All subjects were offered ear plugs for hearing protection. An EMG was recorded from the first dorsal interosseous (FDI) muscle of the stimulated upper limb, using Ag/AgCL surface electrodes in a belly-tendon montage. Electrode signal was amplified and filtered with a band-pass filter of 30Hz to 1 kHz (D360^{TM}, Digitmer Ltd, Welwyn Garden City, UK) and digitized at 2kHz via a 1401 acquisition interface (Cambridge Electronic Design Ltd, Cambridge, UK). EMG reading was displayed on a computer screen via Signal^{TM} software (Cambridge Electronic Design Ltd., Cambridge, UK). Recording did not proceed until EMG showed no baseline activity, or until this had fallen to an acceptable minimum in patients with abnormal muscle tone.

Stimulation

Single-pulse TMS and paired pulse stimuli were delivered using a Magstim 200^2 stimulator (Magstim Co., Whitland, Dyfeld, UK) connected to an Alpha coil (Magstim Co.) in a figure-of-eight configuration with a 70mm internal diameter. This delivers a rapidly-changing magnetic field in a near-monophasic waveform over 82µs, inducing current in the cortex in a posterior-anterior direction. EMG was monitored online for voluntary activity and frames showing excessive activation were discarded.

The coil was held tangentially to the scalp with the handle pointing posteriorly at an angle of approximately 45° to the sagittal plane, whilst simultaneously monitoring for response with both visible muscle contraction and EMG activity. Sessions began

with location of the FDI 'hotspot' in the hemisphere of interest by systematically searching the scalp contralateral to FDI. The hotspot was defined as the location which yielded the largest and most consistent MEP on FDI EMG, and the location was fixed using Brainsight™ neuronavigation system (Rogue Resolutions Inc., Cardiff, UK). The coil was then maintained in position and orientation using real-time visual feedback to the experimenter. At the hotspot, test pulses were administered at 0.2 Hz to avoid any unintended plasticity-inducing effect (Hoogendam et al., 2010). First, resting motor threshold (RMT) was established as the lowest stimulator output intensity to evoke an MEP in the relaxed muscle with a peak-to-peak amplitude larger than 50 µV in 5 of 10 trials. The test pulse intensity for baseline and post theta-burst MEPs was then established as the intensity (as a percentage of maximum stimulator output) producing an average MEP closest to 1mV in amplitude. Following the recording of 2 x 20 baseline 1mV intensity MEPs, RMT measurement at the FDI hotspot was repeated using a biphasic 300µs TMS pulse using the same coil attached to a Magstim Rapid™ stimulator (Magstim Co., Whitland, Dyfeld, UK). cTBS comprising 200 bursts at a rate of 5Hz, each burst consisting of 3 pulses delivered at 50 Hz (Huang et al., 2005) was then administered at an intensity of 70% of biphasic RMT (Goldsworthy, Pitcher, & Ridding, 2012) The subject then rested quietly and avoided sustained contraction of FDI for 10 minutes, following which a second train of cTBS (600 pulses over 40 seconds, as described above) was delivery. Immediately after the end of the second train, the first sample of post cTBS test pulse MEPs were collected in a similar manner to the baseline recording. Post-spaced cTBS MEPs were collected in batches of 20 (Experiment 1 + 2) or 30 (Experiment 3) as described in the text, with MEPs collected either every 10 minutes for 60 minutes (Experiment 1), every 10 minutes for 30 minutes (Experiment 2) or every 5 minutes for 30 minutes (Experiment 3). Subject and investigator were blinded as to the amplitude of the post spaced-cTBS MEPs by increasing the scale of the y-axis on the computer monitor so that the peaks of the MEP were not visible.

Paired-Pulse Stimulation

Prior to spaced cTBS, paired-pulse stimulation was delivered to probe intracortical circuits. Short Intracortical Inhibition (SICI) and Facilitation (ICF) were assessed in the protocol by randomly cycling between a condition where a single monophasic test pulse was delivered at 1mV intensity and conditions where a 1mV intensity pulse was preceded by a subthreshold (70% RMT) monophasic conditioning pulse at an interval of 2ms, 3ms, 10ms or 15ms (Kujirai et al., 1993). SICI, felt to represent the action of $GABA_A$ergic cortical interneurons (Ziemann, Rothwell, & Ridding, 1996) and ICF, felt to represent glutamatergic excitatory cortical interneurons, were assessed as respective increase or decrease in the conditioned pulse MEP compared to the test pulse MEP. A total of 50 trials were performed in each session with 10 trials of each condition (10 x test pulse alone, 10 test pulse conditioned at 2ms, 10 x test pulse conditioned at 3ms, 10 x test pulse conditioned at 10ms, 10 x test pulse conditioned at 15ms) in computer-generated random order at a frequency of 0.2Hz.

In a separate protocol, Long Intercortical Inhibition (LICI) was assessed. LICI is felt to represent $GABA_B$ergic activity (Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999) in more distant or recurrent neurons (Valls-Solé, Pascual-Leone, Wassermann, & Hallett, 1992). We utilised using a 1mV monophasic test stimulus, delivered alone in 15 trials out of 30 trials and preceded at an interval of 150ms by another 1mV intensity conditioning stimulus in the other 15 trials. 30 MEPs were collected in a computer-generated random order at a frequency of 0.2Hz. As with SICI, LICI is defined as a decrease in the conditioned MEP amplitude compared to the test stimulus MEP.

Data-Analysis and Statistics

For both single and paired-pulse MEPs, Data were saved and analysed offline using Signal™ software (Cambridge Electronic Design Ltd., Cambridge, UK). Peak-to-peak

amplitude was measured using an in-house developed script and averaged across all MEPs from that time point or condition. All statistical analysis was performed using SPSS (Version 22.0., 2013, Armonk, NY: IBM Corp). Where stated, raw data were normalised to baseline average to facilitate comparison between subjects. MEP amplitudes frequently display a positive skew and data were inspected visually and then statistically using a Kolmogorov-Smirnov test of non-normality. Where appropriate a log or square root transformation was applied to averaged MEP data prior to further analysis, as outlined in the results of each experiment. Data that did not meet the assumptions of the parametric model, such as those using ordinal rather than continuous scales, were analysed using non-parametric models as described in the respective chapters. Violations of sphericity were assessed using Mauchly's test, with Greenhouse-Geisser correction applied as necessary. Correlations were tested using linear regression analysis. Values were not corrected for multiple comparisons where the comparison formed part of the *a priori* hypotheses, and all correlation coefficients are supplied with uncorrected P values. Intraclass correlation coefficients were calculated to assess the test-retest reliability of measures, using a 2-way random model (fixed observer, subjects allocated at random) on average MEPs. Other statistical tests will be outlined in each chapter - all appropriate statistical tests were two-tailed analyses.

Chapter Three

Experiment 1

Long term variability of continuous theta-burst effect in healthy individuals

Background

Whilst much is now known, or at least inferred, about the mechanisms whereby repetitive TMS and other forms of non-invasive brain stimulation induces plastic-like changes in cerebral cortex, variability in an individual subject's response has been a major source of criticism of Non-Invasive Brain Stimulation (NIBS) studies of plasticity and an obstacle to effective experimental design. Across all NIBS methodologies, approximately 55-60% of subjects show a standard "canonical" response, whilst 40-45% of subjects show no response or even a paradoxical response (López-Alonso, Cheeran, Río-Rodríguez, & Fernández-Del-Olmo, 2014). However, whilst variability between subjects is wide, response using the same NIBS protocol within a given subject tend to be more consistent. It has been proposed that genetic single-nucleotide polymorphisms (SNPs) in the BDNF receptor may underpin this bimodal response pattern (Jannati, Block, Oberman, Rotenberg, & Pascual-Leone, 2017), but the data to support such a claim remain limited. Furthermore, it has been rare for intra-individual variability to be explored over lengths of time comparable to the expected recovery period of stroke patients (3-6 months), whereas we know from imaging data that quite substantial cortical reorganisation is possible in both stroke patients and healthy subjects over such a time frame (Dang et al., 2013; Sampaio-Baptista et al., 2014). Moreover, these inter-individual correlations have primarily been observed in healthy controls who are significantly younger than our anticipated stroke population. Older subjects tend to show less corticospinal excitability than younger subjects (Pitcher, Ogston, & Miles, 2003) and to exhibit greater interhemispheric asymmetry (Chagas et al., 2018). It is also proposed that older adults show reduced response to some NIBS protocols (Müller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008; Todd, Kimber, Ridding, & Semmler, 2010) and at the anatomical level older subjects have smaller cortical volumes (Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003), and so it cannot be assumed that a stroke age population will show the same response to theta burst stimulation, nor that that the reasonable test-retest reliability demonstrated in young controls can be extrapolated to this age group. Pitcher et al (2003) found that trial-by-trial variation in MEP amplitude increased with age, which may either

interfere with induction of NIBS-induced plasticity (since stimulation parameters are usually set relative to TMS excitation thresholds), or make its effects harder to detect statistically. It is therefore necessary to first establish the test-retest reliability of the current measure (spaced continuous theta-burst stimulation) in younger and older healthy adults before exploring any variation post-stroke. These data will also allow us the first cross-sectional comparison of response to theta-burst stimulation between these populations.

Theta burst stimulation (TBS) (Huang et al., 2005) appears to demonstrate reduced variability compared to other plasticity-inducing protocols whilst, simultaneously employing a much shorter stimulation protocol. TBS was originally conceived based on the 4–7 Hz frequency of discharges (theta range in EEG) recorded from the rat hippocampus (Diamond, Dunwiddie, & Rose, 1988) and used to study synaptic plasticity *in vitro* in animal brain slices (Capocchi, Zampolini, & Larson, 1992; Larson & Lynch, 1986; Larson & Lynch, 1989). TBS consists of 50Hz bursts of three pulses repeated every 200ms, for a total of 600 pulses. Depending on the protocol, either an inhibitory or facilitatory response is seen: (1) a 2-sec on, 8-sec off intermittent TBS (iTBS) pattern for 190 sec, which in most individuals increases MEP amplitude by approximately 35% for up to 60 min, or (2) a continuous TBS (cTBS) pattern for 40 sec, which can reduce MEP amplitude by approximately 25% for up to 50 min (Wischnewski & Schutter, 2015). Suppression of MEPs by cTBS and their enhancement by iTBS are considered indices of long-term depression- (LTD-) and long-term potentiation- (LTP-) like mechanisms, respectively (Huang et al., 2005; Huerta & Volpe, 2009).

Given that the modulation of MEPs seen with theta-burst stimulation with TMS is typically far briefer than the changes induced *in vitro* (Wischnewski & Schutter, 2015), and building on experience using transcranial direct-current stimulation (Monte-Silva, Kuo, Liebetanz, Paulus, & Nitsche, 2010), recent work has suggested that delivery of theta-burst stimulation in a ‘spaced’ protocol, with back-to-back

stimulations separated by a 10 minute interval, results in a prolonged suppression of MEPs (up to 120 minutes in Goldsworthy et al. (2012)) and a greater proportion of canonical responders. As well as a prolonged cortical suppression being potentially more clinically useful, this tool shows promise as a means of further reducing the variability in response to TMS that has limited its usefulness for comparing populations.

Additionally, whilst most studies of TBS have used a stimulus intensity set at 80% of active motor threshold, it has been argued (Gentner et al., 2008) that the prolonged muscle contraction required to ascertain this interferes with subsequent plasticity-induction through cortical priming (i.e. pre-activation of motor cortex), and other studies have suggested that using a stimulus intensity set to 70% of resting motor threshold is of comparable efficacy whilst doing away with the need for preceding muscle contraction (Goldsworthy et al., 2012). Furthermore, the measurement of RMT is more objective, and therefore more easily reproducible between different experimenters. Consequently, in this study the intensive of the conditioning stimulus for cTBS will be set at 70% RMT, without measurement of or reference to AMT, and avoiding any contraction in the target muscle prior to delivery of the protocol.

Hypotheses:

- Response to spaced cTBS is relatively consistent across 6 months within young volunteers (Experiment 1A)
- This relationship is preserved in older subjects (>55 years) of an age more relevant to the stroke population (Experiment 1B)

Experiment 1A

Long term variability of continous theta-burst effect in healthy young individuals (<35)

Subjects

19 subjects (12F 7M, 16 RH 3LH) aged 23-34 (mean age 29.9 years, s.d. 4.2), were recruited from the staff and students of the Institute of Neurology or by word of mouth. Subjects with contraindications to TMS (Rossi et al., 2009) or with a history of chronic neurological illness were excluded. Subjects concurrently taking neurotropic medications were also excluded. All readings were taken from the dominant cerebral hemisphere/upper limb, as outlined in Chapter Two. Handedness was self-reported, or where ambiguity existed determined by formal scoring with the Edinburgh Handedness Index (Oldfield, 1971). The study was approved by the Joint Ethics Committee of the Institute of Neurology, University College London, and the National Hospital for Neurology and Neurosurgery, UCL Hospitals NHS Foundation Trust, London, and it was conducted in accordance with the principles expressed in the Declaration of Helsinki. Subjects were asked to attend 2 sessions six months apart (average 226 days, range 160-393). In accordance with previous recommendations, subjects were tested at the same diurnal stage (AM/PM) in both sessions (Sale et al., 2007).

Data Analysis

As described above, MEP amplitudes were averaged at each time point and also pre- and post cTBS. Data were analysed using the Kolmogorov-Smirnov test of normality and transformed where appropriate, as outlined in the text, to conform with the assumptions of the parametric model. Likewise, a Levene test of homogeneity of variance was performed and where necessary data transformations applied, as outlined in the text. Where data violated the assumption of sphericity (significant Mauchly's test) a Greenhouse-Geisler correction was applied to p-

values. Efficacy of spaced cTBS was assessed using a paired-sampled two-way t-test, comparing baseline average MEPs with post-cTBS MEPs averaged across multiple time points. Differences in change over time between each session were analysed in a two-way repeat measures ANOVA with factors SESSION (S1, S2) and TIME (baseline, 0m, 10m, 20m, 30m, 40m, 50m, 60m).

Intraclass correlation between testing sessions was examined using a two-way random model (single investigator, randomly allocated subjects), for single measures when comparing thresholds, and for averaged measures when comparing MEPs. In keeping with the recommendations from the Beaulieu, Flamand, Massé-Alarie, & Schneider (2017) review paper, we have published alongside the ICCs for our data the Standard Error of Measurement (SE_{Meas}) and Minimal Detectable Change (MDC), using the following formulae:

$SE_{Meas} = SDp \times \sqrt{(1-ICC)}$

$MDC = SE_{Meas} \times 1.96 \times \sqrt{2}$

Where SDp is the pooled standard deviation across sessions.

Results

Baseline Measures

There were no reported adverse effects and all subjects completed the study. Resting motor thresholds did not differ significantly between the two sessions in a paired sample t-test ($t_{(18)} = -.238$, $p = 0.81$) and were closely correlated across sessions with an intraclass correlation coefficient of 0.876 (CI[0.706, 0.950] $p<0.01$). Baseline MEP amplitude also did not differ significantly in a paired sample t-test

(Session 1: mean 1.16mV sd 0.51; Session 2: mean 1.04mV sd 0.32; $t_{(18)} = 1.07$ p = 0.31).

Effect of spaced cTBS

Because the raw data values were non-normally distributed, the statistics were performed on square-root transformed data. In a two-way repeat measures ANOVA (fig. 3.1), there was a significant effect of TIME ($F_{(7,126)} = 3.88$, $p < 0.01$) but no effect of SESSION ($F_{(1,18)} = 1.08$, $p = 0.31$) and no significant TIME*SESSION interaction ($F_{(4.03,72.59)} = 0.50$, p= 0.737). After normalising to baseline average (fig. 3.2), a two-way repeat measures ANOVA (excluding baseline data) showed no significant effect of TIME ($F_{(6,108)} = 1.06$ p = 0.388), suggesting that the amount of inhibition did not vary significantly with time in the post-cTBS period. There was also no effect of SESSION ($F_{(1,18)} = 0.05$, $p = 0.827$) or TIME*SESSION interaction ($F_{(6,108)} = 0.549$, p = 0.77).

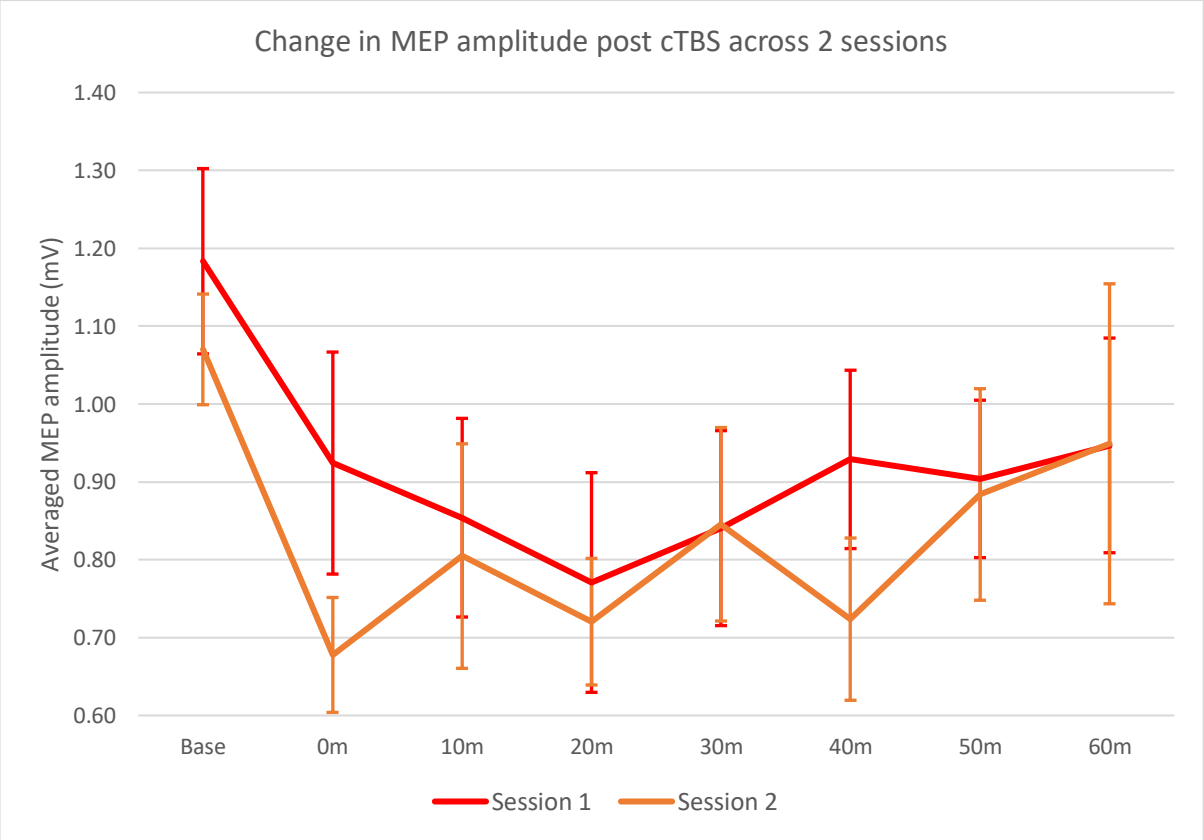


Fig. 3.1 Change in absolute MEP amplitude post cTBS across 2 sessions (young healthy volunteers)

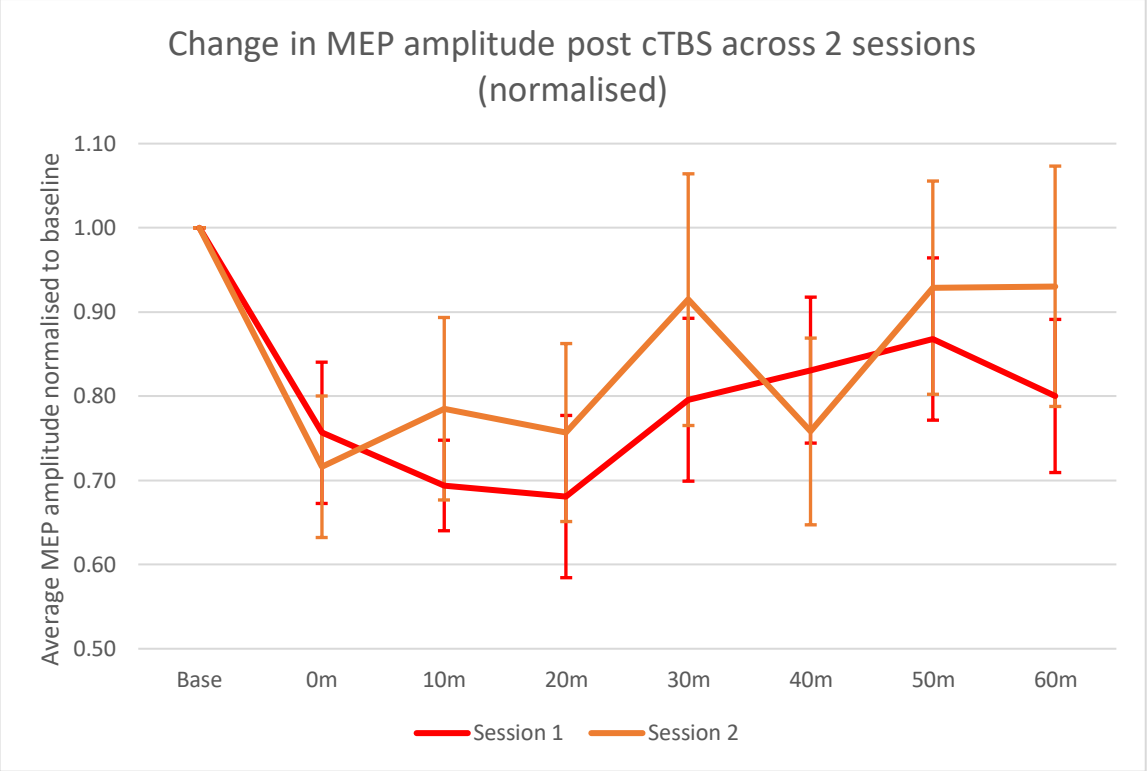


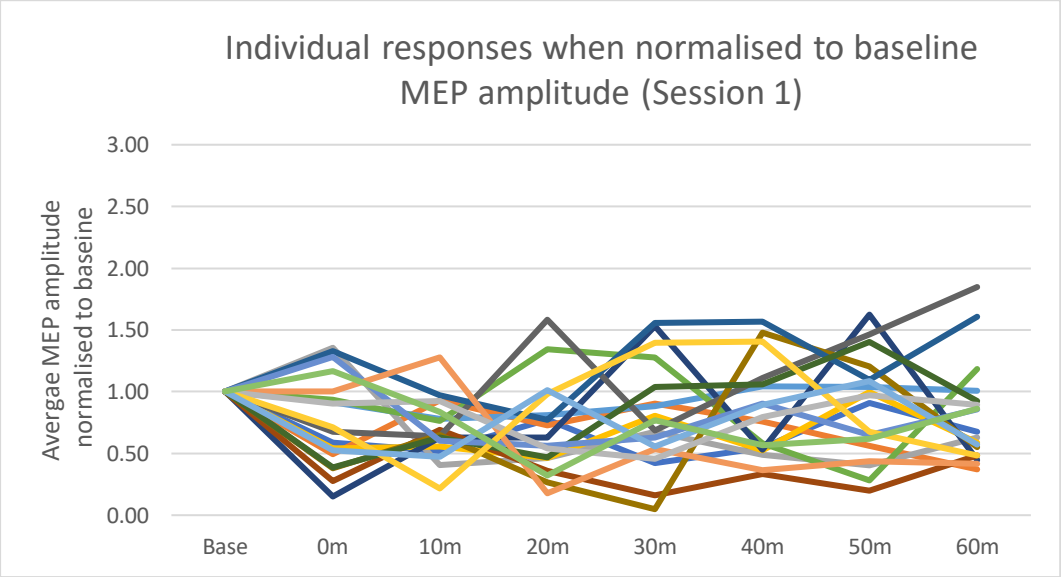
Fig. 3.1 Change in normalised MEP amplitude post cTBS across 2 sessions (young healthy volunteers)

Intraclass Correlation

Individual response over the 7 follow-up time points was highly variable (fig.3.3). Mean suppression in MEP (average change between pre- and post-cTBS) across the whole experiment (0-60 minutes) was 21.0% in session 1 and 18.2% in session 2. To compare the consistency of this response within subjects across both sessions, a 2-way random (single observer, randomly allocated subjects) interclass correlation was performed. Averaging suppression across all post stimulation time points (0-60m), ICC co-efficient was 0.758 (CI[-0.04,0.73], p= 0.03; SEMeas = 0.195, MDC 0.54mV), corresponding to good agreement. This correlation was lost however when the data were normalised to baseline MEP amplitude (ICC 0.128 CI[-0.387, 0.497], p = 0.387; SEMeas 0.291, MDC 0.806), despite the fact that 1mV MEP amplitudes did not significantly differ between sessions.

Previous studies (Vernet et al., 2014) have found that within-subject reproducibility is greatest in the period immediately after cTBS, whilst others (Vallence et al., 2015) have only studied MEPs up to 30 minutes post cTBS. Consequently, we conducted

an exploratory analysis to see if session to session correlation was greater at these early time points. When ICC was calculated using suppression in average MEPs over the first 30 minutes, it remained in good agreement on the raw data (ICC 0.739 (CI [0.323, 0.900]; SEMeas 0.201, MDC 0.557mV), p = 0.003; with improved agreement on the normalised data with ICC of 0.458 CI [-0.406, 0.791], p = 0.102; SEMeas 0.208, MDC 0.577) corresponding to fair agreement.



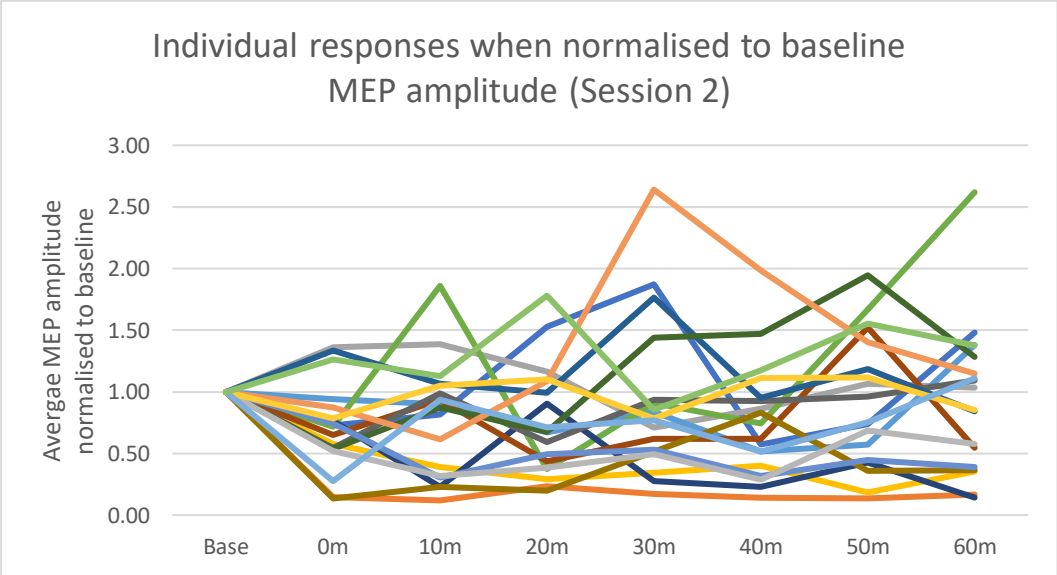


Fig. 3.3 – individual response for all 19 participants across both sessions, displayed in proportion to baseline MEP amplitude.

Wanting to identify the most consistent measure, we examined alternative time bins in turn: values are outlined in Table 1. Correlation between the two sessions was strongest in the earliest time points, with the strongest agreement at 0 minutes i.e. immediately after the second train of cTBS (ICC = 0.751 CI [0.353, 0.904], $p < 0.01$). Fair reproducibility was also observed in the 0-10min and 0-30min periods. Individual data are shown in fig. 3.4.

Time window	ICC	95% CI_{upper}	95% CI_{lower}	SE_{Meas}	MDC	P	Agreement
0-60 minutes	0.128	-0.387	0.497	0.291	0.806	0.387	Poor
0-30 minutes	0.458	-0.406	0.791	0.208	0.577	0.102	Fair
0-20 minutes	0.395	-0.57	0.767	0.211	0.586	0.148	Poor
0-10 minutes	0.54	-0.194	0.823	0.200	0.554	0.054	Fair
10 minutes	-0.313	-2.407	0.494	0.396	1.098	0.715	Poor
0 minutes	0.751	0.353	0.904	0.182	0.505	0.003	Good

Table 1 – Intraclass Correlation for change in MEP amplitude, averaged across different time windows.

The proportion of subjects who responded to the protocol (defined as a net reduction in mean MEP amplitude averaged over 60 minutes post cTBS – Table 2) was 84.2% in the first session and 63.2% in the second session, with concordant responses (same response in each session) in 10 out of 19 (52.6%). Over the early period (0-10 minutes) these response rates improved to 94.7% (session 1) and 73.7% (session 2) with 13 out of 18 concordant (68.4%). Response rate immediately post cTBS (0 minutes) was 68.4% and 84.2% respectively, with 68.4% concordance across the two sessions.

Time Window	Responders (S1)	Responders (S2)	Concordance
0-60 minutes	16 (84.2%)	12 (63.2%)	10 (52.6%)
0-30 minutes	16 (84.2%)	14 (73.7%)	12 (63.2%)
0-20 minutes	16 (84.2%)	15 (78.9%)	13 (68.4%)
0-10 minutes	16 (84.2%)	15 (78.9%)	13 (68.4%)
10 minutes	18 (94.7%)	14 (73.7%)	12 (63.2%)
0 minutes	13 (68.4%)	16 (84.2%)	13 (68.4%)

Table 2 - response rates per session for each specified time window (proportion of concordant responses across sessions in right hand column)

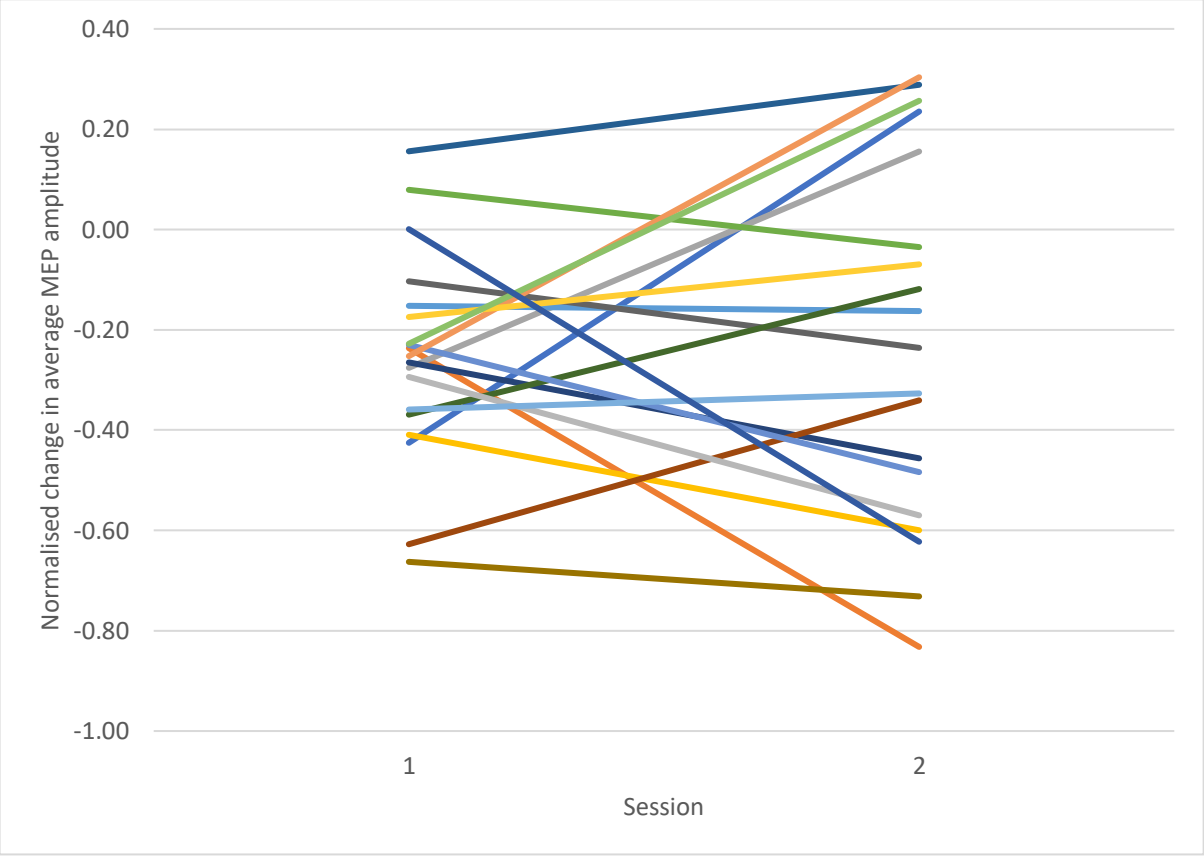


Fig. 3.4.1 plasticity (absolute change in MEP amplitude averaged over 30 minutes) across 2 sessions – each subject plotted as a separate line.

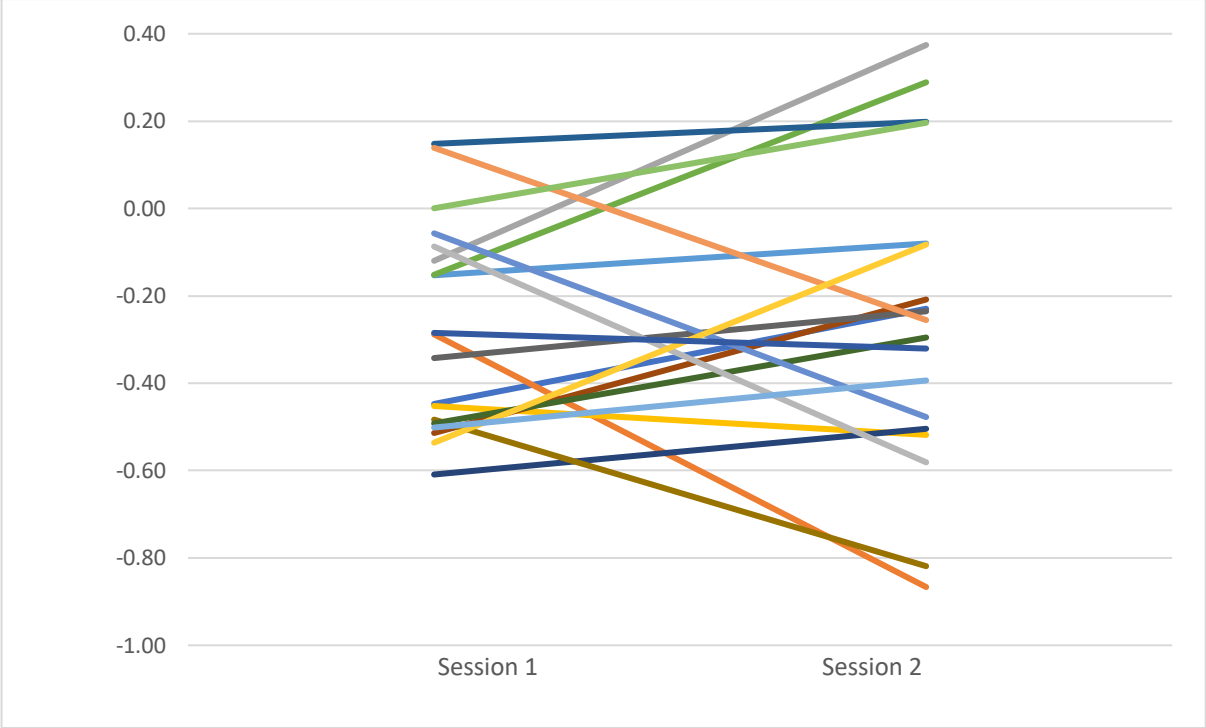


Fig. 3.4.2 plasticity (change in MEP amplitude) averaged over the first 10 minutes post cTBS across 2 sessions

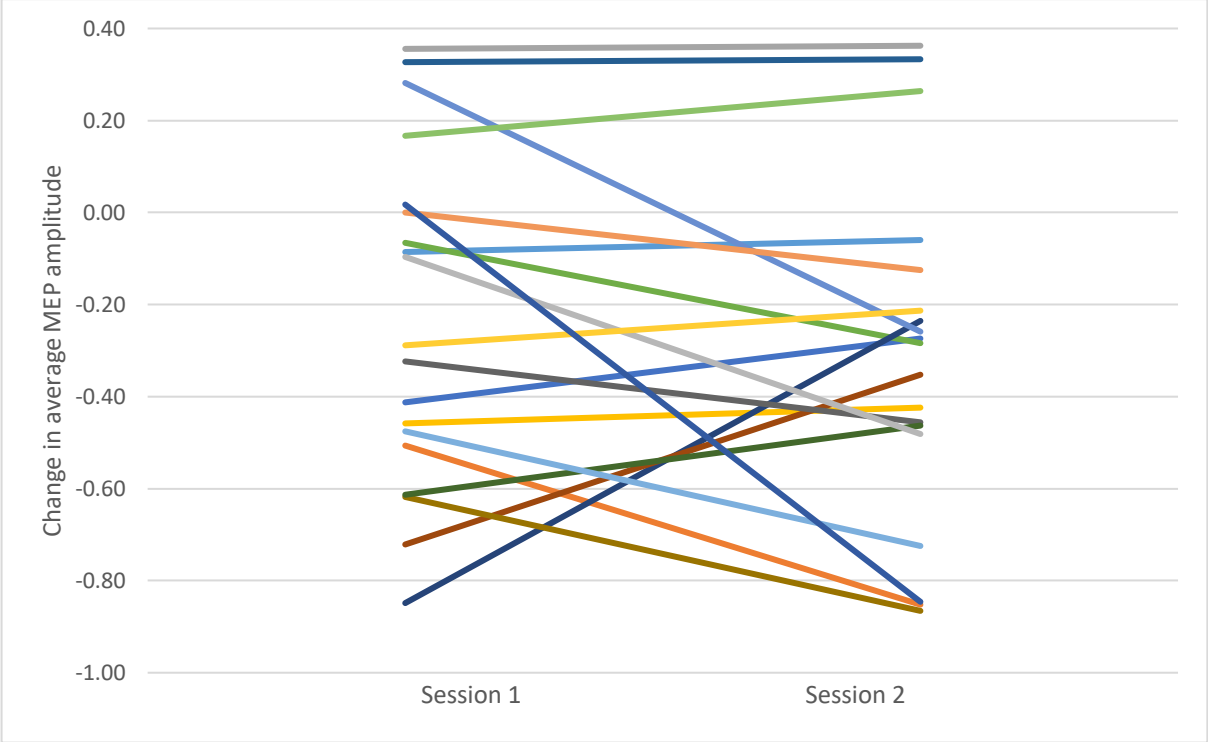


Fig. 3.4.3 plasticity (change in MEP amplitude) at 0 minutes (immediately post cTBS) across 2 sessions

Thus, whilst spaced cTBS shows poor agreement across 2 sessions over 6 months when MEPs are compared for 60 minutes post stimulation, the agreement between response compared within the first 30 minutes post stimulation (corresponding to the period of maximal average suppression of MEPs) ranged from fair to good.

For the tool to have utility in the population of patients suffering from first ever ischaemic stroke, its reliability should be validated in an older population. In the second part of the experiment the protocol was repeated in a population of healthy over-55-year-olds.

Experiment 1B

Comparison of continuous theta-burst effect between healthy young (<35) and older (>55) individuals.

Subjects

20 older (over 55 years) subjects were recruited from the staff of the Institute of Neurology or by word of mouth (9F 11M; 19RH 1LH; age range 56-76, mean age 65.9 yrs, s.d. 6.8). All were in good general health and were not taking neurotropic medications. Two subjects (both male) had a history of minor head injury/concussion not requiring hospital admission.

Methodology

Experimental conditions and methodology were otherwise the same as the younger control group. Average interval between sessions was 240 days (range 190-364 days).

Statistical Analysis

Change over time with cTBS was again examined in a two-way repeat measures ANOVA. Data from the younger and older groups were compared in a three-way repeat measures ANOVA, with factors GROUP (young, old), SESSION (S1, S2) and TIME (baseline, 0m, 10m, 20m, 30m, 40m, 50m, 60m) on MEP values normalised to individual baseline average.

Results

Again, there were no reported adverse effects and all subjects completed the study. Resting motor thresholds did not differ significantly between the two sessions in a paired sample t-test ($t_{(19)} = 0.742$, $p = 0.467$) and were closely correlated across

sessions with an intraclass correlation coefficient of 0.702 (CI[0.387, 0.871] p<0.01). Baseline MEP amplitude again did not differ significantly in a paired sample t-test (Session 1: mean 0.981mV sd 0.55; Session 2: mean 1.12mV sd 0.55; $t_{(19)}$ = -1.12 p = 0.274).

Effect of cTBS in the Older Population Group

Net suppression of MEPs (fig. 3.5) across 60 minutes was consistent across the two sessions (11.2% in Session 1, 11.9% in Session 2). A two-way repeat measures ANOVA on square-root transformed raw data over the whole 60 min post-cTBS period did not find any significant effect of TIME ($F_{(7,266)}$ = 1.89, p = 0.07) nor any effect of SESSION ($F_{(1,38)}$ = 1.51, p= 0.226) or TIME*SESSION interaction ($F_{(7)}$=0.696, p=0.675). This relationship remained non-significant when data were normalised to pre-stimulation baseline, with no effect of TIME ($F_{(4.58,174.1)}$ = 0.509, p=0.754), SESSION ($F_{(1,38)}$=0.012, p = 0.914) or TIME*SESSION interaction ($F_{(4.58)}$=0.410, p = 0.872).

Given that intersession repeatability in Expt 1A was greatest in the earliest time windows, the analysis was repeated looking for a TIME*SESSION interaction using only MEPs from within the first 30, 20, and 10 minutes, none of which were significant.

Time Window	F statistic	p value
0-30 minutes	0.631	0.597
0-20 minutes	1.044	0.342
0 -10 minutes	1.64	0.687

Table 3 - Effect of cTBS in older subjects between 2 sessions over different time windows – F statistic and p value for rm ANOVA of average MEP amplitudes

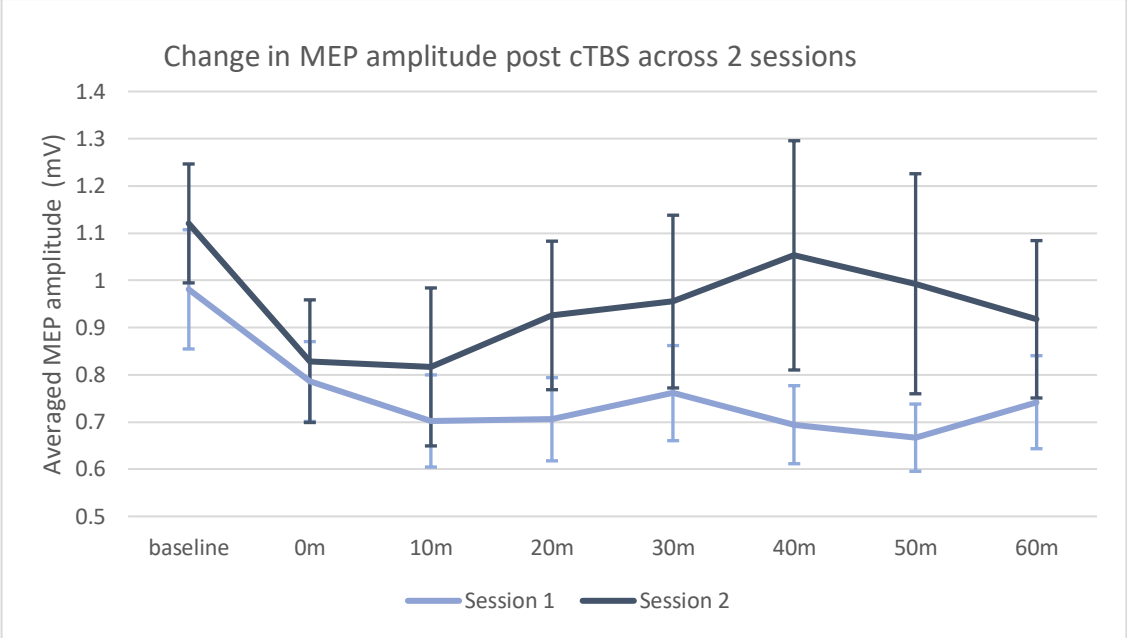


Fig 3.5.1 – effect of cTBS in older control subjects across 2 sessions

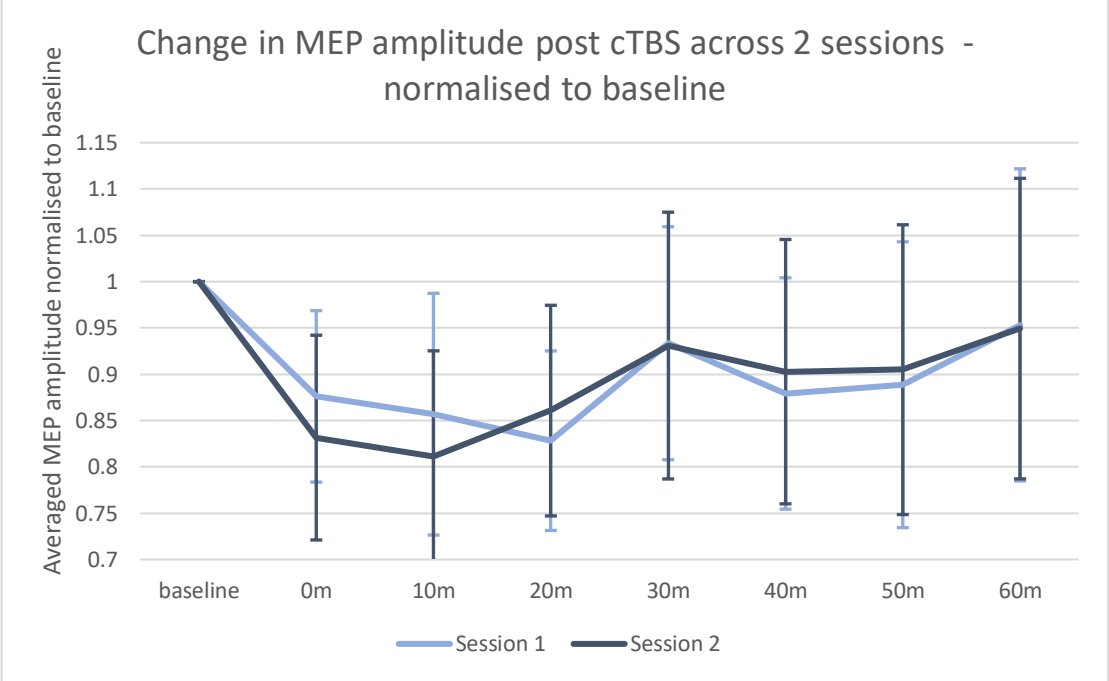


Fig 3.5.2 – effect of cTBS in older control subjects across 2 sessions (normalised to baseline)

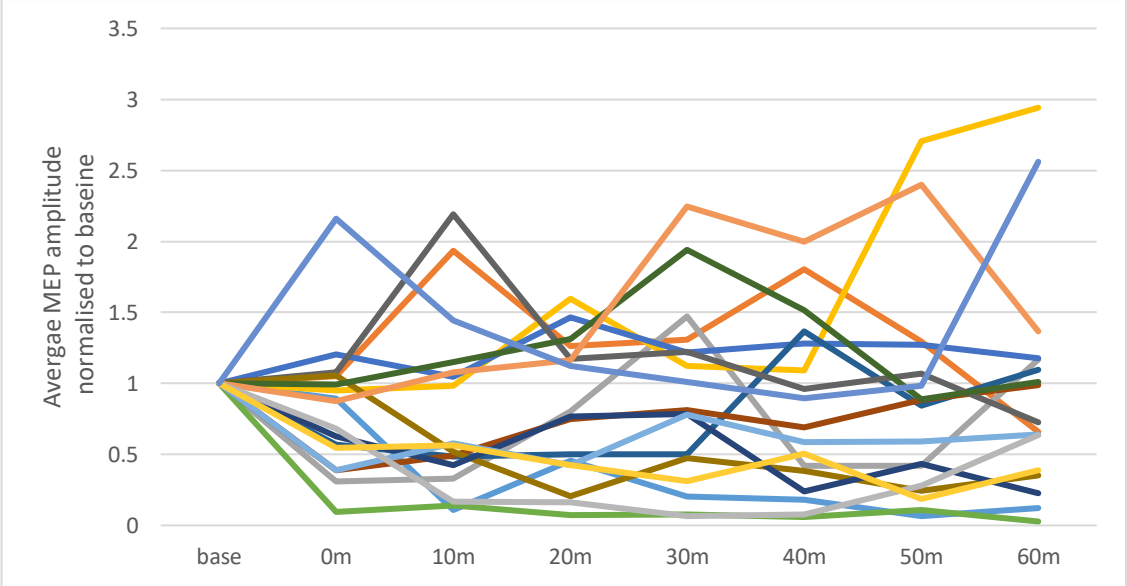


Fig. 3.6. Individual response to cTBS amongst older healthy volunteers –normalised to baseline

Reliability Measures

Reliability measures were again calculated for the various time windows. Reliability was best in the 0-30 minutes window, with a significant correlation with fair agreement (ICC = 0.572).

Post-cTBS MEPs							
Time window	ICC	95% CI_{upper}	95%CI_{lower}	SE_{Meas}	MDC	p	Agreement
0-60 minutes	0.568	-0.91	0.829	0.312	0.865	0.037	Fair
0-30 minutes	0.572	-0.081	0.831	0.279	0.774	0.036	Fair
0-20 minutes	0.398	-0.521	0.762	0.323	0.895	0.139	Poor
0-10 minutes	0.258	-0.875	0.706	0.382	1.060	0.261	Poor
10 minutes	0.353	-0.636	0.744	0.357	0.991	0.176	Poor
0 minutes	0.105	-1.26	0.646	0.505	1.401	0.405	Poor

Table 4 – Intraclass Correlation for change in MEP amplitude, averaged across different time windows (older controls).

Using our previous definition of 'responders' as those demonstrating a net depression in MEP amplitude, 12 out of 20 (60.0%) subjects in Session 1 showed a canonical response to cTBS over 60 minutes, with the same number of responders over 30 minutes and 13 out of 20 (65.0%) at 0 minutes. In Session 2 the number of responders were 13 (65.5%) over 30 and 60 minutes, and 16 (80.0%) at 0 minutes. Individual responses are shown in fig. 3.6.

Comparison with Younger Subjects

Effect of cTBS in the older cohort was compared to data from the first session in younger volunteers (experiment 1A). Averaged Baseline 1mV MEP amplitude did not differ significantly between groups in a repeat measures ANOVA (no significant effect of AGE ($F_{(1,37)}$=1.056, p=0.311) or AGE*SESSION interaction ($F_{(1)}$ = 1.027, p= 0.317; data natural logarithm transformed to meet assumption of normality). Likewise RMT also did not differ significantly across the two groups with no effect of AGE ($F_{(1,37)}$=0.671, p=0.418) or AGE*SESSION interaction ($F_{(1)}$=0.572, p=0.454).

	Mean RMT – S1	Mean RMT - S2	1mv:RMT – S1	1mv:RMT – S2
Young	40.8 (36.9, 44.8)	41.1 (37.6, 44.5)	1.26 (1.20,1.31)	1.17 (1.13,1.22)
Older	43.8 (39.8, 47.7)	42.5 (37.4, 47.6)	1.23 (1.19,1.26)	1.34 (1.05,1.62)

Table 5 – RMT and 1mV:RMT across session (Session 1 (S1), Session 2 (S2)) in younger and older controls (95% confidence intervals in brackets)

Formal Input/Output recruitment curves were not recorded in either sample – however 1mV intensity as a percentage of RMT did not differ significantly between groups in an repeat measures ANOVA (effect of AGE $F_{(1)}$ = 0.447 p = 0.508, GROUP * SESSION borderline interaction ($F_{(1)}$=3.86,p=0.057); group data in Table 5), suggesting there should have been no differential ceiling/floor effect for MEPs in either population.

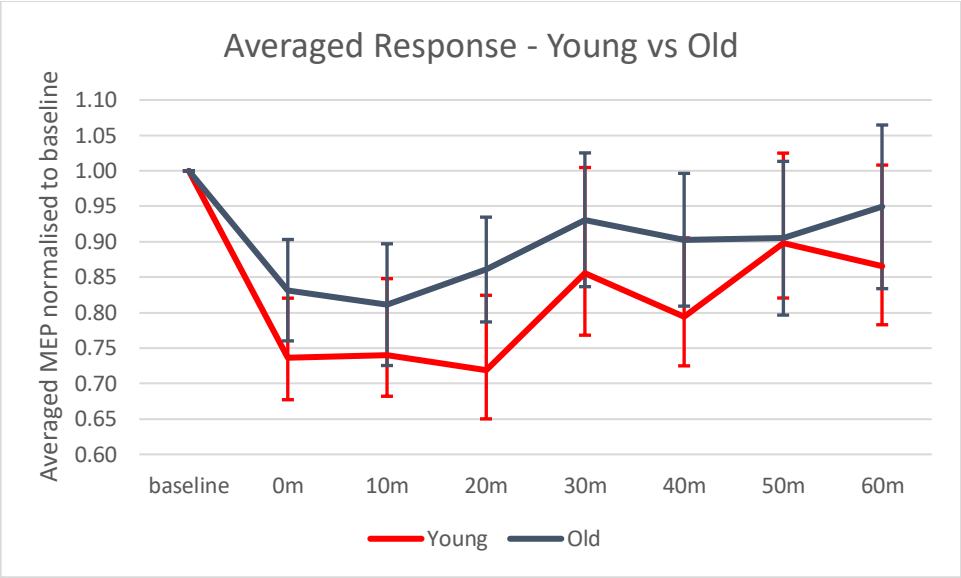


Fig. 3.7 Younger vs Older Controls normalised to baseline and averaged over both sessions

Pre- and post-cTBS MEPs were compared between groups in a multifactorial repeat-measures ANOVA with within-subjects factors TIME and SESSION and between-subjects factor GROUP (Fig 3.8). Raw data were transformed using a natural logarithmic function to meet the assumption of normality. Analysis of transformed data showed a significant effect of TIME ($F_{(7,259)}$= 6.757, p <.001) but no effect of GROUP ($F_{(1,37)}$ = 0.277, p = 0.602) or GROUP*TIME ($F_{(7,259)}$ = 0.534, p = 0.809) or GROUP*SESSION*TIME ($F_{(5.166)}$=0.454, P=0.816).

To aid comparison between different groups post cTBS MEPs were once again normalised to baseline for each session (Fig. 3.8). Baseline data were excluded from analysis following normalisation. Statistical analysis revealed no significant effect of GROUP ($F_{(1,37)}$ = 0.064, p = 0.802). There was also no significant effect of TIME ($F_{(6,222)}$ = 0.515, p = 0.797) or TIME*SESSION interaction ($F_{(6,222)}$ = 0.733 p = 0.623), although this would be expected since baseline data have been excluded.

Finally both the two-way interaction AGE*TIME ($F_{(6,222)}$ = 0.620, p 0.714) and the three-way interaction AGE*SESSION*TIME ($F_{(6)}$=0.508, p=0.802) were non-significant.

Additionally, the proportion of concordant responders did not differ between the two age groups in a Chi-Square proportional analysis (concordant response in 65% of older controls, 52.6% of younger controls, χ^2=0.603, p=0.437 over 60 minutes; 65% and 63.2% over 30 minutes, χ^2=0.013, p=0.9079).

Thus, the effect of cTBS in older adults in repeat sessions does not differ significantly from that in younger subjects.

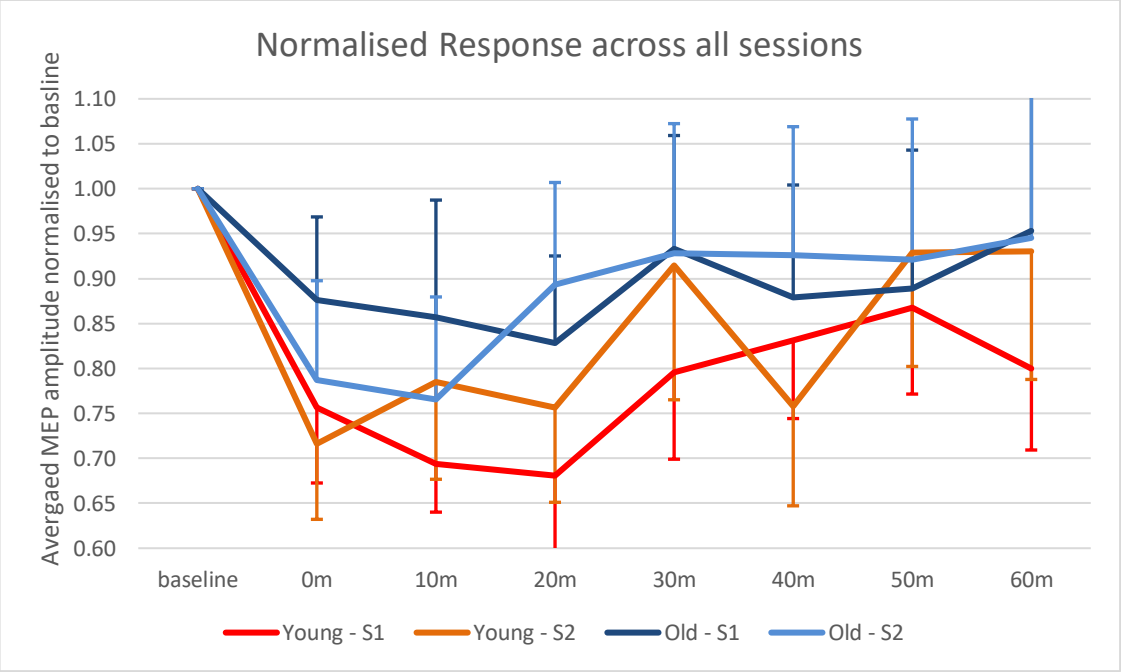


Fig. 3.8 Normalised MEP data in younger vs older healthy volunteers follow spaced cTBS

Discussion

Variability of effect of NIBS remains a major obstacle to the development of TMS research, and has both led some to question the validity of TMS-invoked MEPs as a neurophysiological measure, and also driven the search for increased standardisation and methodological refinements that might improve the reproducibility of TMS-based research. These present data confirm the current

broad consensus that whilst inter-individual variability can be large, intra-individual responses are reasonably consistent.

Spaced continuous theta burst stimulation demonstrated the anticipated inhibitory effect on MEPs, in keeping with the published literature. In their original paper, Goldsworthy et al. (2012) reported 100% of their 12 subjects showing suppression in average MEPs over 60 minutes, which is broadly comparable with our results of 16 out of 19 young subjects and 28 of all 39 subjects across both groups. It remains to be seen whether the lower response rate and shallower plasticity curve in older subjects represents a reduced effect of spaced cTBS in this age group, as this experiment found no significant difference in response and was not powered to assess this question.

Whereas the intra-class correlation of the measure over 60 minutes was poor (0.128), the relationship was stronger in measures over the first 30 minutes and particularly the first 10 minutes (ICC 0.548 and 0.450), in keeping with findings reported previously for other rTMS protocols (Vernet et al., 2014). Response was highly correlated at 0 minutes (ICC 0.751) and one is forced to consider in hindsight the wisdom of collecting MEPs for a full 60 minutes post stimulation in every session! It is worth noting that this reading at “0 minutes” is of course 10 minutes after the first (priming) run of cTBS in the spaced protocol, corresponding with a window of strong suppression of MEPs and higher test-retest reliability in other cTBS studies (Vallence et al., 2015; Vernet et al., 2014). This peak reliability at 0 minutes and to a lesser extent in the first 20 minutes corresponds to the period of peak suppression in MEPs in both age groups (fig. 3.7), and as outlined below (table 6), matches a trend found with other plasticity protocols and may be a generalised feature of non-invasively induced neuroplasticity. However, the temporal response of maximal MEP suppression may be more variable than the level of suppression – time to peak excitability with iTBS was very poorly correlated (ICC 0.025) in Hinder et al. (2014), despite good correlation between averaged change in MEPs (ICC 0.534).

As mentioned, intra-individual variability with cTBS has been studied in two previous papers, Vallence et al, (2015) and Vernet et al (2014), showing fair reproducibility (ICC 0.539 over 37 minutes (Vallence et al, 2015): Vernet et al (2014) did not calculate ICCs). However, this is the first study to investigate reproducibility of spaced cTBS, and also the first time that the effects of cTBS has been shown to be consistent over longer time periods (6 months). Vernet et al (2014) examined the reproducibility of single cTBS over 3 months, and found that the least variability between sessions occurred in MEPS at just 5 minutes post cTBS, consistent with our finding that early MEPs were the most consistent measure of plasticity across sessions.

Recent studies have explored the long term reproducibility of other related NIBS protocols, with our results comparing favourably. Hinder et al. (2014) explored intra-individual variability over 1-3 weeks in response to facilitatory iTBS and found similar ICC (0.534) when comparing averaged change in MEP amplitude over 36 minutes. López-Alonso et al (2015), Madhavan, Sriraman, & Freels (2016), and Thapa & Schabrun (2018) all found fair correlation with response to an inhibitory protocol with Anodal tDCS, including over long time intervals of 6-12 months in López-Alonso et al. (2015). All found a similar phenomenon to our current data, with a trend towards greater test-retest reliability with earlier post-protocol MEPs. However, Horvath, Vogrin, Carter, Cook, & Forte (2016) found very poor reproducibility with both anodal and cathodal tDCS over 3 months, whilst Dyke, Kim, Jackson, & Jackson (2016) likewise found poor reproducibility with both protocols, possibly hampered in this study by their choice of a single post-stimulation recording, when the time course of peak response to TDCS has been shown to be quite variable (Li, Uehara, & Hanakawa, 2015). The existing data on PAS (Fratello et al., 2006) show very poor reliability (ICC = 0.05), again possibly hindered by their choice of a single time point post stimulation

Authors	Year	Average Age	n	Methodology	Stimulus	Probe	Time Interval (days)	Follow Up MEPs	Neuronavigation	ICC
Thapa et al.	2018	23 +/- 5	10	spaced anodal TDCS	1mA	15 x1mV	2-7	0-20 minutes	N	0.43–0.67
Fried et al.	2017	58 +/- 9	12	iTBS	80% AMT	30 x 120% RMT	14	0-50 minutes	Y	0.29–0.48 (Cronbach's α)
Schilberg et al.	2017	24 +/- 3	27	iTBS	80% AMT	30 x 120% RMT	7.8	0-60 minutes	Y	<0.173
Madhavan et al.	2016	27	15	anodal TDCS	1mA	8 x 80% - 140%AMT	7	0-30 minutes	Y	0.73-0.80
Dyke et al.	2016	24 +/- 4	10	anodal + cathodal TDCS	2mA	10 x 100 - 150%RMT	3.5	0 minutes	N	0.276 (an) 0.038 (cath)
Horvath et al.	2016	23 +/- 4	18	anodal + cathodal TDCS	1mA	15 x 130% RMT	135	0-30 minutes	Y	0.06 (an) 0.055 (cath)
Chew et al.	2015	22 +/- 3	29	anodal TDCS	0.5mA	20 x 1mV	7	0-30 minutes	N	-0.500
Vallence et al.	2015	23 +/- 4	18	single cTBS	70% RMT	8 x 90% - 180%AMT	12	0 - 37 minutes	N	0.539
López-Alonso et al.	2015	21+/- 2	45	anodal TDCS	1mA	20 x 1mV	287	0-60 minutes	N	0.565 (0-30) 0.242 (0-60)
Vernet et al.	2014	33 +/- 18	10	cTBS	80% AMT	30 x 120% RMT	107	5-90 minutes	Y	Not calculated
Hinder et al.	2014	25 +/-9	30	iTBS	80% AMT	15 x 130%RMT	14	0-36 minutes	Y	0.534 (0-36) 0.486 (0-21)
Fratello et al.	2006	26+/- 1	18	PAS 25	132% RMT	20x 1mV	7	0 minutes	N	0.05

Table 6 - Existing Literature on test-retest reliability in plasticity protocols

One potential criticism of the present data is the use of 1mV MEP as the baseline measure, particularly when comparing older and young subjects. Vallence et al. (2015) speculated that the use of 1mV MEP as an arbitrary test probe is itself a significant contributor to NIBS variability, since it represents a variable position within each individual's recruitment curve, and therefore produces potential differential susceptibility to ceiling or floor effects and also differential probing of intracortical circuits, due to increased late I-wave recruitment at higher intensities near the top of the I/O curve (Di Lazzaro et al., 2005). Vallence et al. (2015) found both greater plasticity and greater test-retest reliability with a probe of 150% RMT, significantly larger than our own 1mV probe that average around 125% RMT. The significant suppression following cTBS to the 1mV intensity probe in our subjects in both young and older subjects suggests this widely used measure remains a valid probe for studying the effect of NIBS in vivo. It is likely that different NIBS differentially affect different cortical circuits (Dayan, Censor, Buch, Sandrini, & Cohen, 2013) and that consequently the optimal probe for measuring the effect of different NIBS may also vary, and is yet to be defined.

The alternative of instead using a IO recruitment curve as a probe carries significant disadvantages as well, firstly that the number of MEPs at a given intensity tends to be less (10 MEPs in Vallence et al (2015) vs 20 MEPs in our own data), rendering results more vulnerable to pulse-to-pulse variability, and secondly the increased amount of time needed to perform a full recruitment curve. More than just an inconvenience for researchers, this carries an additional possibility of subject fatigue and TMS response attenuation due to prolonged testing, as well as the theoretical possibility that the performance of a recruitment curve may itself alter cortical plasticity due to the large number of total MEPs delivered. Finally, unless corrected statistically the collection and comparison of multiple different MEPs post NIBS carries the risk of an inflated family-wise error rate and false-positive findings.

These current data, in combination with the results of Vallence et al (2015) and Vernet et al (2014), suggest that cTBS, either single or spaced, is an appropriate measure with which to study cortical responses over a six-month window in stroke patients. Earlier MEPs post cTBS show the most reproducibility across sessions and support the use of a shorten protocol in our patient population (Experiment 2), with additional practical benefits in this group. cTBS produced a significant response in this older cohort over the early time period, with fair reproducibility (ICC 0.57) averaged over 30 minutes, again supporting the use of this shortened time window in our stroke patients. This 20-30 minute window also appears to be supported by non-neurophysiological markers of the effect of NIBS, such as functional near-infrared spectroscopy (Tian et al., 2012) and fMRI (Bestmann, Baudewig, Siebner, Rothwell, & Frahm, 2005), both of which found that haemodynamic changes in M1 following rTMS were limited to this time window.

Also of interest is the finding that our older subjects did not differ significantly in response from the younger group despite the large average age gap (34 years). The lack of difference between the two samples may be attributable to the relative young age of the 'older controls', drawn from a working age or recently retired population rather than a truly 'elderly' sample. Freitas, Farzan, & Pascual-Leone, (2013) found a significant negative correlation between age and cTBS induced plasticity in a sample of 36 subjects aged between 18 and 81, although they did not perform an equivalent direct group comparison. RMT has also been correlated with increasing age (Bhandari et al., 2016), but this likewise did not significantly differ between our two groups ($p = 0.418$) suggesting that our older sample, which included a number of senior UCL academics, are both biological and chronologically young! However, Our older volunteers were nonetheless older than the 'elderly' sample recruited for Bashir et al., (2014), who found that inhibitory (1Hz) rTMS had a significant effect in younger but not older subjects, although they did not directly compare the two samples. Todd, Kimber, Ridding, & Semmler (2010) found a significant interaction between age, muscle and stimulation (real vs sham rTMS – young subjects exhibited inhibition in the target muscle but not at a distant site –

older subjects showed no response in either) but no direct effect of age. Müller-Dahlhaus et al. (2008), and Tecchio et al., (2008) both found a trend towards reduced plasticity in response to facilitatory PAS_{25} in older subjects, although even when combined in meta-analysis (Bhandari et al., 2016) this was non-significant, and a PAS study by Dickins, Kamke, & Sale (2017) failed to reproduce the effect. Likewise, neither Young-Bernier, Tanguay, Davidson, & Tremblay (2014) nor Dickins et al. (2015) found any significant difference between young and older volunteers in response to iTBS, despite an older set of "old" subjects (average age 70.1 years +/- 5.6 (Young-Bernier et al.), 70 +/- 3 (Dickins et al)), and likewise found no significant difference in RMT. Most recently, a study by Opie, Cirillo, & Semmler (2018) found delayed and reduced facilitation in older versus younger subjects when using I-wave periodicity repetitive TMS (iTMS) with a repeated paired-pulse protocol with intrapair interval set to that of late i-wave recruitment (and so consequently a longer ISI was used in the older subjects).

There is more consistent data (Di Lorenzo et al., 2016; Koch et al., 2014, 2012; Trebbastoni et al., 2016) to support a reduction in measures of plasticity in age-related cognitive decline. Evidence for reduced response to iTBS with preserved response to cTBS in patients with Alzheimer's Dementia when compared to aged match controls. Intriguingly, Fried, Jannati, Davila-Pérez, & Pascual-Leone (2017) found high reproducibility of the effects of iTBS in the first 20 minutes in the Alzheimer's group, whilst only moderate reproducibility in healthy older controls, a phenomenon which was mirrored in their measurements of RMT and baseline MEPs, potentially a consequence of underlying cortical pathology.

The present study was neither designed nor powered to detect such a difference, although the data suggest that if there is any difference it is likely to be small. Whilst it is often postulated that plasticity declines with advancing age, as it certainly does in the first years of life as modality-specific critical periods pass (Hubel & Wiesel, 1998), this is primarily based on experimental data from animals

that have very different life spans from *homo sapiens* (Hoppenrath, Härtig, & Funke, 2016). At present there is no evidence using cTBS, and limited evidence for any other NIBS protocols, that neuroplasticity declines significantly with age, and thus it remains an appropriate measure in older and by extension in clinical populations.

Conclusions

Spaced cTBS shows fair agreement when repeated across 6 months in the same subjects, in both younger and older subjects, broadly comparable to the known data for other forms of plasticity-modulating NIBS. Reproducibility tended to be best in the early time points post cTBS, supporting the use of a shortened post-stimulation recording window. There was no significant difference in response between young (<35 years old) and older (>65) subjects, suggestive cTBS is an appropriate measure for studying changes in plasticity in the clinical population of recovering stroke patients.

Chapter Four

Experiment 2

Longitudinal Measures of Plasticity across 6 months following Ischaemic Stroke

Background

Having determined spaced cTBS to be a reasonably consistent measure of plasticity in healthy controls, it is a suitable tool with which to attempt to delineate the 'critical window' of plasticity post stroke, a finite period lasting from a few weeks to a few months following the initial insult. Whilst there is consistent data in animals confining this to approximately 14-21 days (Murphy & Corbett, 2009), it has yet to be defined in humans. Whilst behavioural data imply recovery is fastest in the first 6 weeks (Cortes et al., 2017), and can perhaps continue as long as 6 months in the most severely impaired patients (Semrau et al., 2015), this is much longer than the plasticity window seen in experimental animals. The relationship between neuroplasticity and functional recovery will necessarily be indirect, with the latter likely to lag behind the former, and so it would seem probable that there is a shorter period of period of heightened synaptic plasticity which is then consolidated into improved motor function in the following weeks. Di Lazzaro et al. (2010), in a study of 17 patients with first ever acute ischaemic stroke, found that increase in MEP amplitude in response to facilitatory iTBS in the affected hemisphere within the first 10 days of an ischaemic stroke was significantly correlated with recovery at 6 months, showing for the first time that rTMS can detect clinically meaningful changes in plasticity during the acute stroke period. This study did not however probe any temporal relationship with repeat measures, nor did it explore the significance of LTD-like plasticity such as might be demonstrated with cTBS. Thus, it is timely to see whether rTMS can demonstrate changes in cortical plasticity over the weeks and months following stroke that might help elaborate the duration of the critical window. Intriguing data from a recent behavioural study found that (excitatory) 10Hz rTMS to the contralesional M1 improved performance on a finger tapping exercise at 2 weeks post stroke but not at 6 months – the first evidence that repetitive TMS can capture a biologically relevant process in the contralesional hemisphere during stroke recovery (Volz et al., 2017), replicating similar findings in a rodent model (Barry et al., 2014).

In addition to the acute impact of an ischaemic stroke, it is increasingly recognised that chronic global ischaemia in the form of small vessel disease affects many older patients, and is believe to both underpin vascular cognitive impairment (Bordet et al., 2017) and be an independent risk factor for poor recovery following stroke (Kim & Lee, 2015). Small vessel disease is associated with substantial disruption of cortical architecture (Bordet et al., 2017), which is thought to lead to impaired synaptic plasticity ultimately manifesting as cognitive impairment. However, the limited TMS evidence to date has found both reduced and enhanced response to TMS-induced plasticity in subjects with cerebral small vessel disease and vascular cognitive impairment (Lanza et al., 2017). Nevertheless, there seem to be good biological reasons to suppose that the plastic response of the critical period in stroke patients might be reduced in the presence of chronic cerebral ischaemia, since this negatively impacts on functional prognosis, and that therefore electrophysiological response will covary with radiological measures of small vessel disease on MRI.

As discussed in Chapter One, a number of TMS experiments have studied intra-cortical excitability in the acute stroke period, using paired-pulse protocols as such as the one outlined in Chapter Two. Swayne, Rothwell, Ward, & Greenwood (2008) found reduced intracortical inhibition in the stroke hemisphere that persisted at 6 months, but response in the contralesional hemisphere did not differ significantly from healthy controls. However, Manganotti, Palermo, Patuzzo, Zanette, & Fiaschi (2001) found reduced intracortical inhibition bilaterally, which persisted in those who made a poor recovery but improved in the contralesional hemisphere of those who made a good recovery. Thus, it is possible that any change in inhibitory plasticity in the contralesional hemisphere may be mirrored by changes in intracortical inhibition.

Study Design

Based on calculations from our collaborators (McDonnell et al., 2015), it was estimated that a target sample size of 43 patients would be needed in order to detect a 5% increase response to cTBS between the critical window and the chronic phase with 80% power. Subjects were recruited with a diagnosis of first monohemispheric ischaemic stroke. Exclusion criteria were as follows: recent craniotomy or other acute neurosurgical intervention; history of epileptic or provoked seizures; major chronic neurological disorder e.g. Parkinson's Disease; any concurrent medication likely to create a strong potential hazard of reduced seizure threshold (Rossi et al., 2009). Subjects received their first testing session between 7 and 14 days post stroke, and subsequent sessions at 4 and 6 weeks post stroke. Subjects were tested where possible at the same diurnal stage (AM vs PM) for each session. A final session was recorded approximately 6-7 months post stroke. These 4 time points (2, 4, and 6 weeks, and 6 months) were chosen in order to allow the identification of both a difference in plasticity between early and late stages of recovery, and also to define this more precisely if, as is suggested by some studies, the window is as short as 2-3 weeks (Nudo & Milliken, 1996).

Subjects

29 subjects (21M, 8F; Appendix A) with first ischaemic stroke (9 R hemisphere, 15 L hemisphere) were recruited via the Hyper-Acute Stroke Unit at University College Hospital and the Acute Brain Injury Unit at the National Hospital for Neurology & Neurosurgery. Average age was 68.2 +/- 9.8 years (range 46-82). All subjects had reported paretic symptoms in their stroke-affected upper limb at the time of presentation to hospital. Average NIHSS score at presentation was 4.2. The study was approved by the Joint Ethics Committee of the Institute of Neurology, University College London, and UCL Hospitals NHS Foundation Trust, London: written informed consent was obtained from each patient at the first session and reviewed at each subsequent session. An additional 3 subjects withdrew from the study during or after their first TMS session and do not form part of the data set presented here: 1 of these

reported intolerable side effects from cTBS (light-headedness), and 2 others withdrew due to concerns about the time commitment involved. Of the 29 recruited, 3 subjects attended the first three sessions but were uncontactable at the time of their final (6 month) recording. A fourth attended their final session but was unable to complete it. 2 subjects were taking neurotropic medication not felt to create a strong seizure hazard (citalopram 20mg and fluoxetine 20mg) – both subjects commenced the medication prior to enrolling in the study and were still taking it at follow up six months later. 8 subjects had received thrombolysis with alteplase within 4.5hrs of the onset of their stroke symptoms, with no evidence of haemorrhagic transformation on follow up imaging.

Methodology

Neurophysiology

MEPs were collected in a manner identical to Experiment 1 from the FDI hotspot in the hemisphere contralateral to the infarct based on clinical imaging or physical signs, and guided by Brainsight™ neuronavigation (without anatomical imaging data). In addition, RMT was recorded from FDI hotspot in both hemispheres and paired-pulse protocols in the contralesional hemisphere. These were followed by application of spaced cTBS at 70% RMT, or at the maximal stimulator output compatible with a theta burst protocol (51%) if this was exceeded (4 subjects in at least one session). To minimise subject fatigue and in keeping with our data from experiment 1, MEPs were collected at 4 time points post cTBS (0, 10, 20, and 30 minutes) and normalised to individual baseline for that session to control for baseline variation. 4 subjects missed their final session: missing data points were replaced with the group-average for that time point and session for MEP data, or with the value from the patient's previous session for clinical scores or stimulation thresholds.

Clinical

Post stroke disability was assessed using the 6 point modified Rankin Scale (van Swieten et al., 1988). Post-stroke depression was screened for using the MHI-5 screening questionnaire (Berwick et al., 1991). Clinical examination and motor tests were performed by a trained physician to produce a score on the NIH Stroke Score (Wityk et al., 1994)and the Fugl Meyer Upper Limb Score (Fugl-Meyer et al., 1975).

Imaging

Of the 29 patients presented here, 24 had clinical MRI using 1.5 T scanner (GE Genesis Signa or Siemens Avanto) performed with T1-weighted, axial T2-weight, fluid-attenuated inversion recovery, and diffusion-weighted sequences as part of their stroke work-up. Ethical permission to use this data for research was obtained and the scans examined by a trained clinician who scored them 0-3 on the Fazekas scale for deep white matter Iesions, a marker of chronic ischaemic small vessel disease (Fazekas et al., 1987) scored across both hemispheres, and believe to underpin both vascular cognitive impairment (Bordet et al., 2017) and poor recovery following stroke (Kim & Lee, 2015).

Statistics

Statistical analysis was performed on SPSS (Version 22.0., 2013, Armonk, NY: IBM Corp) using averaged MEPs for each time point. Both raw and normalised MEP data violated the assumption of normality with a significant Kolmogorov-Smirnov test at several time points. No transformation obtained perfectly normally distributed data and so a square-root transformation was utilised as the transformation that gave the best approximation to the assumption of normality (non-significant Kolmogorov-Smirnov test at all but 3 time points). Data were analysed using repeat-measure ANOVA for interval data and Friedman's ANOVA for ordinal data, as outlined in the text. A Greenhouse-Geisser correction was applied where the assumption of

sphericity was violated (significant Mauchly's test $p<0.05$). P values are not corrected for multiple comparisons.

Hypotheses

Primary Hypothesis:

- Plasticity (measured as change in average MEP amplitude post cTBS) will show a significant effect of SESSION (week 2, week 4, week 6, 6 months) or a SESSION x TIME interaction in a two-way repeat measures ANOVA.

Secondary Hypotheses:

- Plasticity induced by spaced cTBS in the contralesional hemisphere will be correlated with measures of underlying small vessel disease when inputed as a covariate
- RMT in the stroke affected hemisphere will decrease over time
- Difference between RMT in the two hemispheres will also decrease over time
- Measures of intracortical excitability in the contralesional hemisphere will decrease over the course of six months as transcallosal inhibition of the ipsilesional hemisphere on the contralesional hemisphere is restored
- Improvement on clinical scores of upper limb function (FMUL) over six months will be correlated with plastic response to TBS at week 2 (Di Lazzaro et al., 2010)
- Improvements in clinical scores of upper limb function (FMUL) over six months will be correlated with measures of corticospinal excitability at week 2

Results

As reported above, 1 subject reported intolerable side effects from cTBS (light-headedness), and withdrew from the study. A further subject was unable to complete their final session due to excessive somnolence. No other adverse effects were reported.

Clinical Measures

A full break down of patient demographics and clinical data is given in Appendix A. There was a significant improvement on the NIHSS, FMUL and the mRS over the 6 month period on a Friedman's ANOVA (Table 7). Average scores on the FMUL improved from 62.1 to 63.8 (range 56-66), close to a ceiling of 66, whilst NIHSS improved from 3.5 to 1.5 (range 0 – 5, a score of zero indicating no deficit), approaching floor, representing an overall mildly affected stroke population. There was no significant change in MHI-5 across sessions.

	FMUL	NIHSS	MHI	mRS
Week 2	62.1 (+/- 2.9)	3.5 (+/- 1.4)	25.1 (+/-4.3)	2.0 (+/-0.6)
Week 4	62.5 (+/- 2.8)	2.8 (+/- 1.5)	26.7 (+/-8.3)	1.9 (+/-0.6)
Week 6	63.1 (+/- 2.8)	2.2 (+/- 1.3)	25.6 (+/- 3.4)	1.4 (+/- 0.9)
Week 26	63.8 (+/-2.2)	1.5 (+/- 0.9)	25.2 (+/- 2.6)	1.1 (+/- 0.9)
$X^2_{(3)}$	18.9	51.8	3.65	27.17
Sig	<0.01	<0.01	0.304	<0.01

Table 7. clinical scores at each testing session (mean values, standard deviation in brackets)

Baseline Neurophysiology

Comparing motor threshold in the two hemispheres (Table 8), with a repeat measures multifactorial ANOVA (factors TIME (week 2, week 4, week 6, 6 months) and HEMISPHERE (Unaffected Hemisphere, Affected Hemisphere) there was no

effect of HEMISPHERE ($F_{(1, 109)}$ = 0.557, p = 0.462), TIME ($F_{(1.9,75)}$= 0.09, p = 0.908) or TIME*HEMISPHERE interaction ($F_{(1.9,75)}$ = 0.294, p = 0.712).

	RMT	
	UH	AH
Week 2	45.6 (+/- 8.3)	46.5 (+/- 11.0)
Week 4	45.8 (+/- 6.8)	46.8 (+/- 9.8)
Week 6	45.1 (+/- 7.8)	46.0 (+/- 9.6)
Week 26	45.2 (+/- 8.9)	46.3 (+/- 9.9)

Table 8: Resting Motor Threshold (RMT) in the Stroke Hemisphere (AH – Affected Hemisphere) and the Contralesional Hemisphere (UH – Unaffected Hemisphere) (mean values, standard deviation in brackets)

Stimulation Parameters

Baseline 1mV MEPs were not significantly different across the 4 sessions in a one-way repeat measures ANOVA on square root transformed values ($F_{(3,84)}$ = 0.622, p= 0.602), and 1mV intensity was not significantly different, both as absolute intensity (expressed as percentage of maximum stimulator output) on a repeat measures one-way ANOVA ($F_{(1.35,37.9)}$ = 1.57, p=0.23) or relative to RMT ($F_{(3,78)}$ = 0.326, p=0.805). Stimulation intensity used for cTBS, set as the lower out of 70% of RMT or 51% of maximum stimulator output (the highest intensity compatible with theta burst stimulation with a Magstim Rapid^{TM} stimulator), was not significantly different across session ($F_{(1.5,39.8)}$ = 1.605, p=0.216). Mean stimulation parameters across the 3 sessions are detailed in table 9.

	Week2	Week4	Week6	Week26	Sig.
1mV	55.9 (+/- 10.7)	55.6 (+/- 10.0)	56.7 (+/- 8.7)	51.9 (+/- 10.1)	0.230
1mV (%RMT)	123.3%	122.0%	125.0%	124.1%	0.805
cTBS pulse	44.9 (+/- 4.8)	42.9 (+/-5.6)	43.8 (+/- 6.1)	41.1 (+/- 7.0)	0.216

Table 9 - Respective stimulator settings across the 4 testing sessions (mean values, standard deviation in brackets)

Changes in Response to cTBS across Time

A two-way repeat measures ANOVA was performed on MEPs with factors with TIME (0m, 10m, 20m and 30m) and SESSION (week 2, week 4, week 6 and 6 months). MEPs for each reading were normalised to pre-CTBS baseline average and natural logarithm-transformed to meet the assumptions of the parametric model. There was a significant effect of TIME ($F_{(3,84)}$ = 3.372, p =0.022) suggesting that cTBS was inducing a plastic change in MEP amplitude. There was also a significant effect of SESSION*TIME ($F_{(9,252)}$ = 2.544, p = 0.008) but no significant effect of SESSION ($F_{(2.14,60.0)}$ = 1.76, p = 0.179) interaction (fig. 4.1).

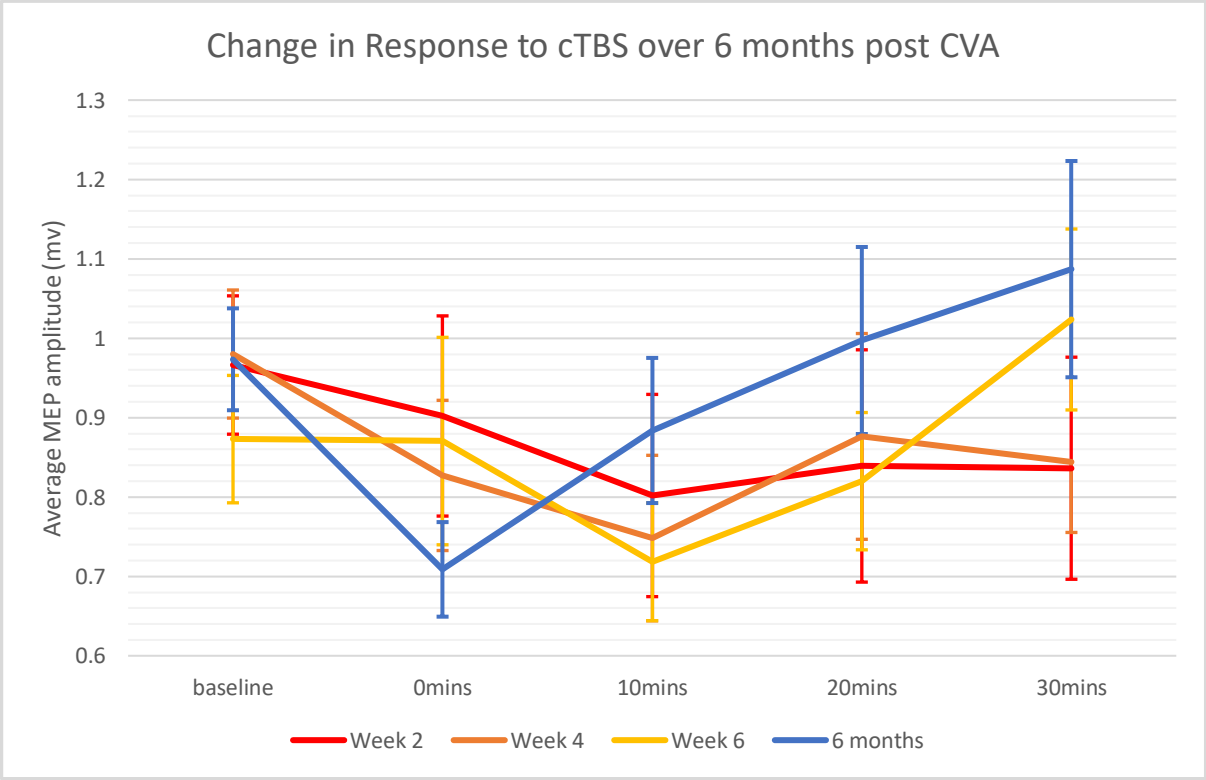


Fig. 4.1 – Change in average MEP across 4 sessions between pre cTBS baseline and 4 post cTBS readings

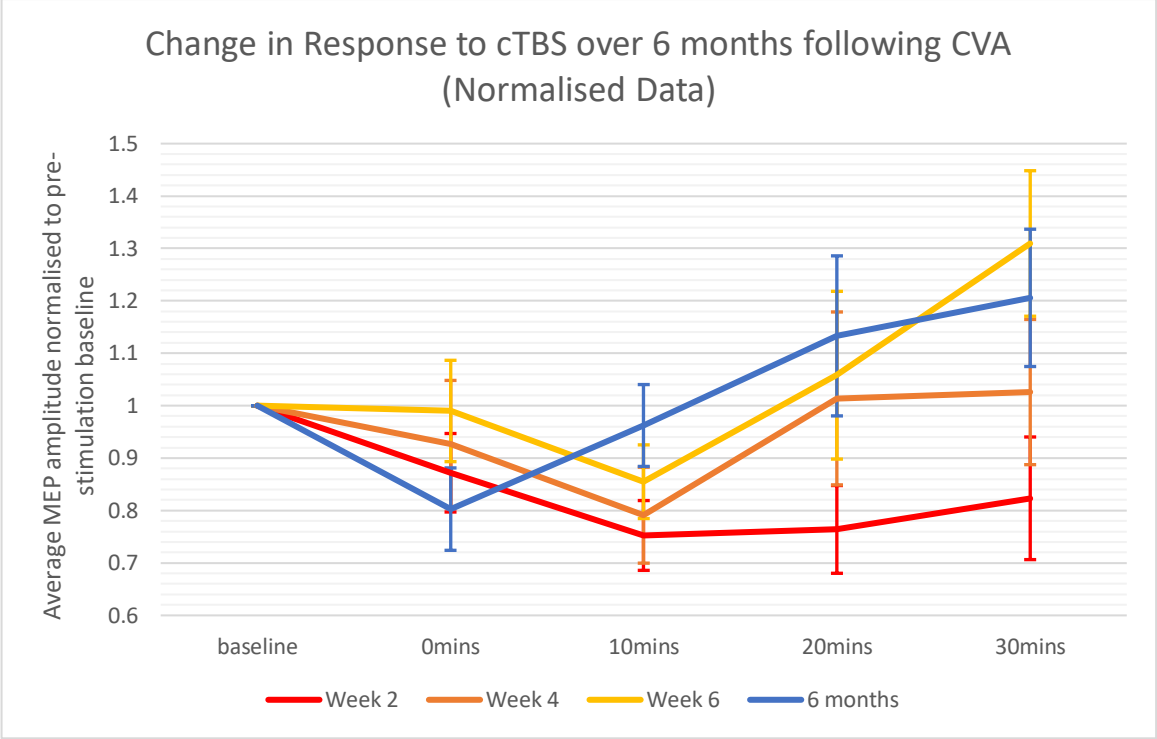


Fig. 4.2 Change in average MEP across 4 sessions between pre cTBS baseline and 4 post cTBS readings, normalised to pre-stimulation baseline

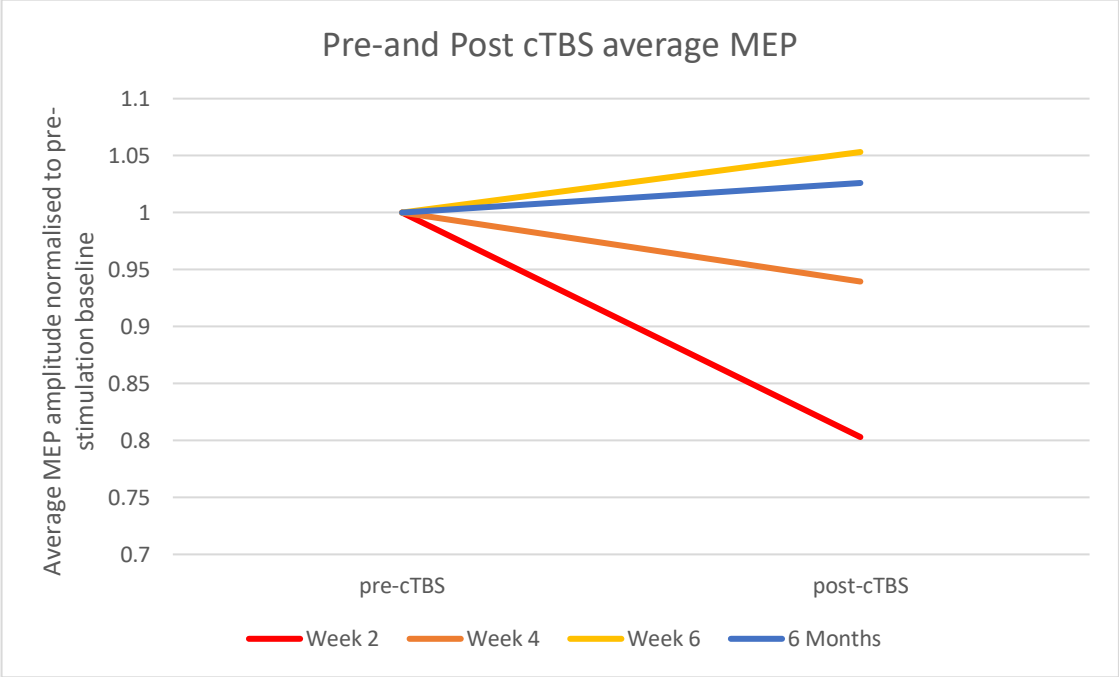


Fig. 4.3 – changes in post stimulation average MEP amplitude over 30 minutes (normalised to pre-stimulation baseline)

Contrasts

To investigate our hypothesis that response to cTBS differed specifically between the acute phase (weeks 2-6) and the chronic phase (6 months) a within-subjects (Difference) contrast was performed comparing the 6 Month session with the previous three sessions. This found a non-significant effect of SESSION ($F_{(1,28)}$=2.073, p=0.161), but the SESSION*TIME contrast for Session 4 versus the previous sessions was significant for both a linear ($F_{(1,28)}$=5.402, p=0.043) and quadratic ($F_{(1,28)}$=6.414, p=0.017) trend suggesting that the effect of SESSION on TIME was different at 6 months than in the first 3 sessions. Thus not only is does the response post cTBS significantly alter with time post-stroke, but this difference was significant when specifically comparing the chronic state (week 26) with the acute phase (weeks 2 - 6).

Defining the Window

An exploratory analysis was run using only data for first 6 weeks, to attempt to delineate further the duration of the post-stroke window. A two-way repeat measures ANOVA with factors SESSION and TIME revealed a SESSION*TIME interaction that remained borderline-significant ($F_{(6,186)}$=2.103, p=0.055). Comparing individual sessions to the mean overall effect using a Deviation contrast, the first session (week 2) was significant for a SESSION*TIME interaction (F(1,28)=6.12, p=0.02) for a linear trend. Thus within the first six weeks the first session (week 2) differed significantly from the others in terms of its SESSION*TIME interaction.

Deep White Matter Small Vessel Disease as a Covariate

The two-way repeat measures analysis was re-run as with SVD as a covariate (represented by the subjects' Deep White Matter Fazekas score; 5 subjects did not have available MRI and were excluded from this part of the analysis). The covariate

SVD did not significantly impact on patients' response to cTBS ($F_{(1,22)}$ = 0.575, p=0.457). This remained the case when only data for the acute period (weeks 2-6) were considered ($F_{(3,25)}$=0.508, p=0.68).

Paired Pulse Data

Paired-pulse conditioned MEPs were collected from the contralesional cerebral hemisphere as outlined in the methods chapter, using 1mV intensity TMS test pulse stimulus and a 70% RMT conditioning stimulus (SICI and ICF) or a 1mV intensity conditioning stimulus (LICI). Inhibition or facilitation were calculated as the ratio of the conditioned MEP to the unconditioned MEP, with a value less than 1 indicating inhibition and a value greater than 1 indicating facilitation. Data were analysed on natural-logarithm transformed data using one-way repeat measures ANOVAs.

There was no significant difference in the average amplitude of the test pulses across sessions ($F_{(4.2,92.8)}$=0.192, p=0.948). There was no change in intra-cortical inhibition over the study time period (Fig.4.4, Table 10), with no significant change using a 2ms-ISI conditioned pulse ($F_{(2.3,65.5)}$=0.167, p=0.877), 3ms conditioned pulse ($F_{(2.2,60.3)}$=0.587, p=0.571), average SICI ($F_{(2.2,62.2)}$=0.423, p=0.678) or LICI ($F_{(3,84)}$=0.371, p=0.774). There was significant change in intracortical facilitation at 10ms ($F_{(3,84)}$=3.98, p=0.01) but not at 15ms ($F_{(3,84)}$=0.378, p=0.769), nor when averaged across both conditions ($F_{(2.4,67.0)}$=2.02,p=0.132).

	Week2	Week4	Week6	Week26	Sig.
2ms	0.67	0.67	0.58	0.58	0.877
3ms	0.70	0.64	0.54	0.51	0.571
10ms	1.20	1.20	1.30	1.67	0.01
15ms	1.14	1.25	1.23	1.46	0.769
Average SICI	0.69	0.65	0.56	0.54	0.678
Average ICF	1.17	1.22	1.27	1.57	0.132
LICI	0.51	0.45	0.46	0.43	0.774

Table 10 – intracortical excitability (expressed a ratio of conditioned stimulus to test stimulus) over 6 months

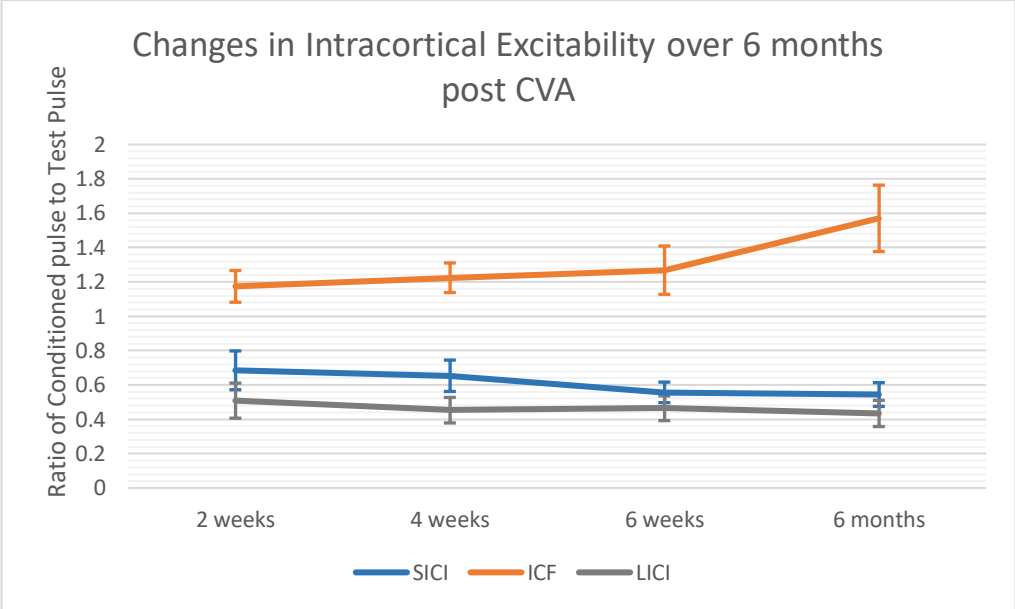


Fig. 4.4 – changes in cortical excitability (expressed as the ratio of conditioned stimulus to test stimulus) over 6 months (2ms and 3ms, and 10ms and 15ms combined to create mean values)

Response to cTBS as a Predictor of Recovery

The relationship between plasticity and clinical improvement was assessed using a bivariate correlation analysis. Scores were not interval data, nor normality distributed on a Kolmogorov-Smirnov test, and so a non-parametric test was utilised (Spearman's rho). Recovery in upper limb motor function (defined as the change in the Fugl-Meyers Score between the first and last session) was not correlated with Individual responses to cTBS (defined as the ratio of averaged MEPs post stimulation to baseline MEP) at either the week 2 session (r_s=0.017, p=0.931) or across the first six weeks of the plasticity window (r_s=0.005, p=0.981).

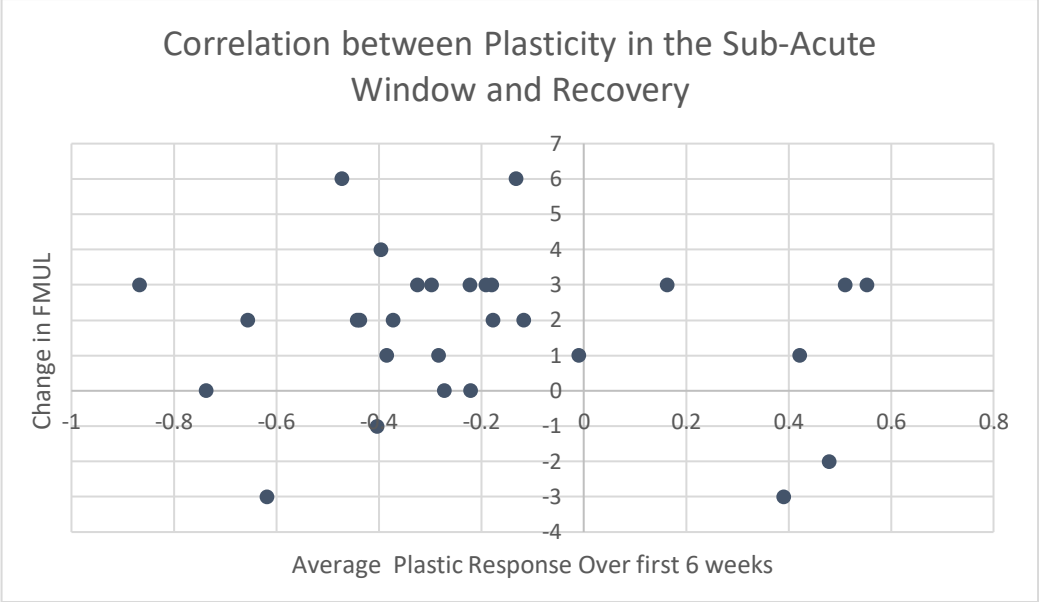


Fig. 4.5 – scatter plot correlating averaged response to cTBS (averaged post-stimulation MEPs over 30 minutes) over the first 3 recording sessions with change in FMUL between first session and 6 months

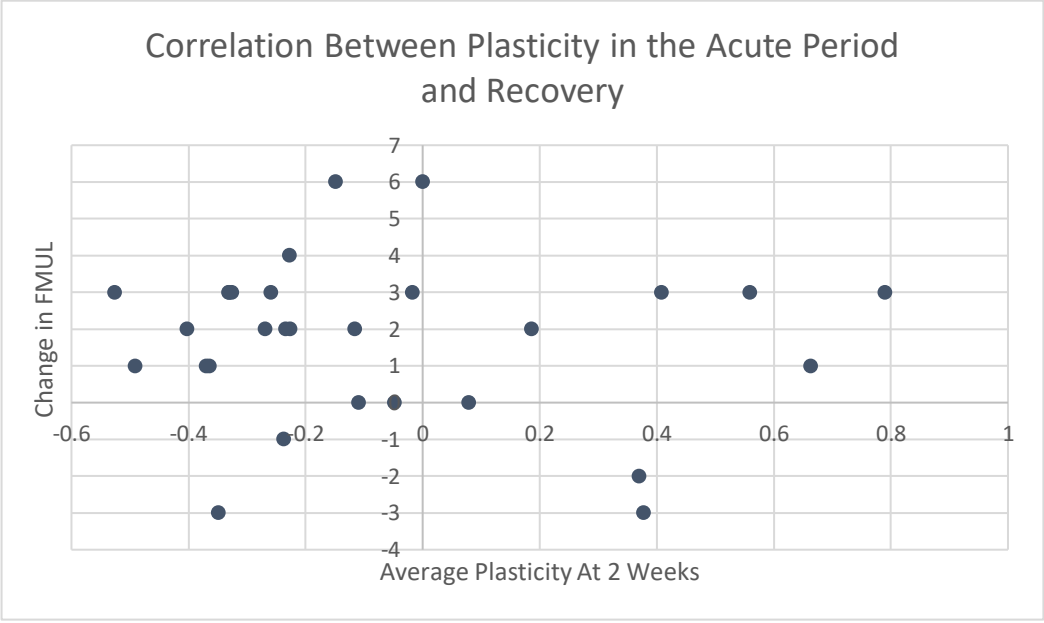


Fig. 4.6 – scatter plot correlating response to cTBS at first session (averaged post-stimulation MEPs over 30 minutes) with change in FMUL between first session and 6 months

There was no correlation between either RMT in the affected hemisphere or Lateralisation Index (difference in RMT between the two hemispheres) at week 2, and either absolute score or improvement in Fugl Meyers Scale at 6 months (all values $p > 0.05$).

Discussion

These data represent the first neurophysiological data in for a window of enhanced plasticity following stroke in humans. The study by Di Lazzaro et al (2010) which found an association between plasticity at day 10 and recovery at 6 months did not include serial measures and so do not give any information as to the duration of any possible critical period. Whilst Di Lazzaro et al (2015) did find a significantly increased response to iTBS in the ipsilesional hemisphere at 1 week compared to 3 months post stroke, these patients had received 5 days of anodal TDCS immediately prior to the week 1 readings, creating a significant confound for time in this group. Indeed, this effect was not observed in the comparator group of patients in the same study who had received sham TDCS. Other previous studies probing post-stroke plasticity have mostly focused on the comparison between stroke patients and healthy controls, often using chronic stroke patients several years after their infarct, introducing multiple potential confounds. Age-matched healthy volunteers are a poor-control group for stroke patients due to mismatch in general health, most particularly the burden of chronic ischaemic small vessel disease as explored in these patients.

Likewise, the impact of motor deficit, learnt non-use and long-standing immobility on response to non-invasive stimulation cannot readily be controlled for at present. By using stroke patients as their own comparator here, these factors can be more effectively controlled for (in addition to the considerable level of inter-individual variability in terms of rTMS response), as well as allowing a more precise

delineation of the time window of enhanced plasticity to be identified through the use of serial readings.

The present data support the hypothesis derived from clinical data that plasticity declines over the first six months following stroke. Significant functional recovery (as opposed to improved behavioural compensation) is considered uncommon after six months and from this we would extrapolate that plasticity in stroke patients is stable thereafter (or may perhaps show a continued slow decline (Kraemer et al, 2004). Clearly these data give no indication as to what this relationship might be after 6 months, which will require further longitudinal studies, with the associated obstacles to patient follow-up over prolonged time periods. Intriguingly, our data suggests (at borderline significance) that plasticity peaks at or before day 14, and then declines significantly over the course of the first six weeks, consistent with the results from experimental animal data, which suggest window as brief as of 5-14 days (Biernaskie, Szymanska, Windle, & Corbett, 2005; Carmichael et al, 2005).

It is worth noting that this is also the first study to directly investigate changes in plasticity in the contralesional hemisphere in human stroke patients. Di Lazzaro et al (2010) had compared response to facilitatory iTBS to the ipsilesional cortex in the first 10 days post-stroke and found facilitation of MEPs in the ipsilesioned hemisphere and inhibition in the contralesional hemisphere (similar effects have also been demonstrated in healthy volunteers -Suppa et al (2008)). The interhemispheric disparity in MEPs in response to iTBS to the ipsilesional hemisphere was inversely correlated with recovery, although this was demonstrated through a binary split of patients into “full” and “partial” recovery based on the modified Rankin Scale, strictly speaking a measure of disability rather than functional deficit which will be heavily influenced by co-existing cognitive impairment and shaped by the individuals social environment – for example many patients will not achieve a score of 0 on the mRS unless and until they are able to return to driving, irrespective of their level of motor recovery.

Unpublished data examining response to cTBS in ipsilesional cortex have been collected by our collaborators at the University of Adelaide, which have shown a significant effect of SESSION, although peak response to cTBS was delayed slightly, between week 2 and week 4 with only a weak response at day 7. Whilst our own data effectively pool data from week 1 and week 2 (first readings were taken between day 7 and day 14), it would not be entirely counter-intuitive to see a neuroplastic response emerge earlier in the intact cortex of the contralesional hemisphere than in the ipsilesional cortex, where response to cTBS may be predicated upon functional restoration of surviving circuits and resolution of surrounding perilesional oedema.

In the present study thresholds of excitability did not change significantly in either hemisphere, contradicting the prevailing hypothesis (Di Lazzaro et al., 2010; Swayne et al., 2008) that the contralesional hemisphere experiences significant disinhibition following an infarct that resolves as the ipsilesional cortex recovers. It is worth noting that other studies with well recovered patients (or with patients who went on to make a good recovery – Delvaux et al, (2003); Freundlieb et al, (2015)) have nonetheless produced similar findings to our own. To muddy the waters yet further, other studies have found persistently decreased RMT in the contralesional hemisphere despite apparent full clinical recovery (Pennisi et al, 2002) – it seems that the relationship between RMT/laterality index and recovery may not be a predictable or reliable one. Supporting our focus on the contralesional hemisphere, Simis et al (2015) found that RMT in the ipsilesional hemisphere predicted recovering, the strength of which relationship was correlated with measures of EEG coherence in the *contralesional* hemisphere. Other studies have proposed that ICI or ICF (Huynh, Vucic, Krishnan, Lin, & Kiernan, 2016), or perhaps even cortical silent period (Lamola et al, 2016) are better discriminators.

Neither Swayne et al (2008) nor Di Lazzaro et al (2010) found any significant correlation between RMT in the acute phase and recovery, similar to our findings. A recent systematic review (Rosso & Lamy, 2018) concluded that RMT was associated with motor function in the upper limb post stroke, but fell short of supporting its predictive value in the acute (< 7 days) period due to small sample sizes. Di Lazzaro et al (2016) speculated that hemispheric asymmetry in terms of response to TMS would be predictive of functional outcomes, but only found a significant correlation in females and not males, postulating stronger interhemispheric connections in females. Whilst it would perhaps be surprising for gender to have such a significant effect on neurophysiology, it may be that our predominantly male sample failed to capture such an effect. It may also be that our largely well-recovered cohort of patients (average FMUL 63.8 out of 66 and mRS 1.1 at 6 months) did not include those who would have exhibited the greatest hemispheric imbalance from the outset. (Such patients, although not explicitly excluded from our sample, frequently have large disabling infarcts and would likely have struggled to attend the laboratories for TMS at day 14 post infarct, potentially explaining the preponderance of well recovered subjects in our sample). Patients with unelicitable MEPs post stroke tend to have the most severe motor deficits and consequently a poor functional prognosis (McDonnell & Stinear, 2017), so it appears that once threshold exceeds the a certain point (corresponding to complete abolition of the corticospinal tract for that region of cortex) then this has serious repercussions for recovery, whereas at sub-maximal thresholds RMT does not reliably discriminate between better and worse recovery.

The current data show no significant change in ICI and ICF across the studied time period, supporting the findings of Swayne et al (2008) who found no change in intracortical excitability in either hemisphere. In their study SICI and LICI in the lesioned hemisphere were reduced compared to healthy controls, whilst ICF was normal, in keeping with other studies (Cicinelli et al., 2003; Liepert, Bauder, Miltner, Taub, & Weiller, 2000; Manganotti et al., 2002). A meta-analysis by McDonnell & Stinear (2017) concluded that intracortical excitability in the contralesional

hemisphere did not differ from healthy controls in either the acute or chronic stroke period, but did not directly explore longitudinal relationships. Huynh et al., (2016) found no significant change in SICI or SICF in the contralesional hemisphere, but demonstrated increasing intracortical inhibition over time in the contralesional hemisphere in subgroups with either mild-moderate impairment or subcortical infarcts: the sample of patients here, with mild impairment and a significant proportion of subcortical infarcts (Appendix A), nonetheless likewise showed no significant change. Since not all subjects have MRI allowing precise delineation of the infarction, and since this was not one of our a priori hypotheses, a subgroup analysis has not been performed with these data. Overall the published literature suggest that intracortical excitability is increased (i.e. inhibition is reduced) in the contralesional hemisphere, usually when compared with the ipsilesional hemisphere, for which we lack data from the current sample for a comparison.

Small vessel disease did not correspond with plastic response when included as a covariate, despite good theoretical reasons to think chronic ischaemic damage, with disruption of cortical architecture and associated loss of dendritic branches, might reduce the capacity for NIBS-induced plasticity (Silasi, She, Boyd, Xue, & Murphy, 2015). Whereas our sample consisted of predominantly well recovered patients, nonetheless there was considerable variation in the degree of small vessel disease demonstrated on MRI. It is known that signs of small vessel disease on MRI correlate poorly with cognitive impairment clinically (Cees De Groot et al., 2000), and so it may the case changes on MRI likewise correlate poorly with measures of synaptic plasticity. Nonetheless leukoaraiosis, a major radiological component of cerebral small vessel disease, has been correlated with poor 90 day outcomes in patients receiving endovascular thrombectomy for ischaemic stroke (J. Zhang, Puri, Goddeau, Henninger, & Khan, 2014) and also iv thrombolysis (McAlpine et al, 2014), although it has been suggested that this may be due to an increased rate of haemorrhagic transformation of the infarct in such patients (Palumbo, Boulanger, Hill, Inzitari, & Buchan, (2007); Shi et al, (2012)). Thus, SVD could engender worse outcomes through increasing the sensitivity of brain parenchyma to acute ischaemia rather than impaired synaptic plasticity. As far as small vessel disease

impedes recovery from stroke, these data do not support a role for reduced synaptic plasticity as a mechanism.

For the first time there now is longitudinal evidence for a decline in plasticity over 6 months following stroke in humans, with a decline that approaches significance even over the first 6 weeks. This would be consistent with the behavioural data from stroke patients which show that recovery is optimal within the first 6-8 weeks and then declines significantly thereafter (Cortes et al, 2017; Jørgensen et al, 1999; Kwakkel, Kollen, & Twisk, 2006). It has been suggested that recovery proceeds for longer in more severely affected patients (Semrau et al., 2015), and it would be useful to repeat the study in a more severely disabled patient population and with additional time points, despite the inevitable technical challenges this would present. Plastic response was not correlated with recovery in our sample, possibly due to a ceiling effect in terms of recovery, so it cannot be concluded definitively that the neuroplasticity demonstrated here is responsible for recovery rather than an epiphenomenon, although this remains biological plausible.

It is important to note that when we consider 'plasticity' we are in fact seeing the sum of multiple processes rather than a single discreet mechanism. Much of the change seen in animal studies during these early weeks comprises dendritic branching and the formation of new synapses, and we cannot distinguish with our present methodology between anatomical plasticity (development of new synapses) and Hebbian plasticity (changes at existing synapses), although it would seem likely that these two processes interact with each other. 'Virgin' synapses produced during the plasticity window might feasibly be more sensitive to the induction of LTD and LTP during this early period, but could continue to be molded into new circuits well beyond it. There is no particular reason why we should expect solely LTD to be increased following a stroke: LTP is likely also to have a role in the process, given the weight of data supporting a role for LTP in physiological motor learning (Teo, Swayne, Cheeran, Greenwood, & Rothwell, 2011). Thus, we might

expect a similar window of plasticity using an excitatory TMS protocol such as iTBS, although this could conceivably lag behind the window of LTD-like plasticity demonstrated here – it would seem possible that downregulating of redundant circuits could precede the experience-driven process of establishing new pathways. The obstacles to studying both inhibitory and facilitatory protocols in the same subjects simultaneously would be substantial however, given the known interaction between protocols and the uncertain duration of any "washout" period, making any such data a challenge to interpret.

Our cohort represents a well-recovered population, and it is known that good recovery is associated with a reversal of plastic changes seen in the contralesional hemisphere (Ward, 2011). The drop off in plasticity seen in our patients' contralesional hemisphere might simply represent the fact that most were already close to completing their recovery during the first 6 weeks studied here. A fuller survey of subjects across a wider range of disabilities might produce evidence of a longer critical window or even confirm that the window is longer with greater impairment. Such a study would likely need to be imbedded within the running of an inpatient rehabilitation programme in order not to interfere with the intensive treatment regime such patients are likely to require.

The failure to reach the target sample size is a potential criticism of the study. It is worth noting that the estimated effect size used in calculating our target sample was somewhat modest (0.05) and so this represents a rather conservative estimate. Whilst the present study will ideally be replicated in larger studies, this is still a considerably larger sample than most of the existing TMS studies in acute stroke to date. Acute stroke patients are by their very nature not ideal subjects for TMS as they are often poorly mobile, and the prospect of bedside testing with current equipment is problematic. As our very hypothesis suggests, this is also the period when physical therapy is largely focused and so time constraints are also a factor, particularly when the levels of fatigue reported by patients undergoing stroke recovery (De Doncker, Dantzer, Ormstad, & Kuppuswamy, 2018). As the clinical

utility of TMS increases it is hoped that testing models will evolve to be less intrusive in a busy clinical setting.

A separate problem is that many eligible subjects who were keen to take part in the present study but were precluded from doing so by their enrolment in various clinical trials, which often offered greater prospect of enhanced recovery than the purely observational design of this experiment. With the expansion of funding for stroke research and a large number of clinical trials now active this may represent a significant obstacle to the recruitment of stroke patients into observational TMS studies in the future.

Another possible criticism of these data is that the increased response to cTBS in the non-stroke hemisphere may represent primarily the results of increased activity in the non-stroke limb following an infarct rather than any endogenously driven neuroplasticity. It is conceivable that motor learning (LTP) in cortical circuits in the contralesional M1 would render the cortex more susceptible to LTD-inducing NIBS through homeostatic plasticity, as has been shown in healthy subjects (Stöckel et al., (2015), Ziemann et al., (2004)). It is difficult to discount this argument, given that it would be expected to see an increase in use of the non-paretic limb in the weeks following a stroke: indeed, it would be difficult to disentangle the diverse effects of use-dependent and primary post-stroke plasticity in any population and with any present technique. Against this argument would stand the fact that these subjects represent a well recovered and minimally disabled population, with FMUL close to ceiling in the affected limb even at the point of the first testing session. Furthermore, a majority of patients had already been discharged from active physiotherapy by the time they were tested, and were not being exposed to any supervised motor learning comparable to the healthy subjects in the studies mentioned. Nevertheless, it is not possible to completely exclude this as an explanation of our findings.

Conclusions

Spaced cTBS demonstrated a significantly enhanced inhibitory response in weeks 2 – 6 (acute window) compared to 6 months (chronic stage). Within the acute window there remained a borderline-significant decrease in plasticity, with peak inhibitory response at the first session (week 2). Radiological markers of cerebral small vessel ischaemia were not significantly correlated with response to cTBS. Intracortical excitability in the contralesional hemisphere did not change over the time course studied. Neither response to spaced cTBS nor baseline markers of corticospinal excitability at week 2 were predictors of recovery or functional performance at 6 months.

If cTBS can reveal changes in metaplasticity that are associated with stroke recovery, it may prove to be useful tool to test potentially plasticity-modifying medications, such as fluoxetine. Recent studies (Mead et al., 2012) have suggested that serotonin-selective reuptake inhibitors (SSRIs) can boost recovery after stroke, and amongst the putative mechanisms for this is by the enhancement of synaptic plasticity. In the next chapter we examine an experiment probing the neuroplastic effect of fluoxetine 20mg, currently one of the front runners in the search for therapeutic plasticity modulators for stroke patients.

Chapter Five

Experiment 3

Effect of cortical priming with single dose of fluoxetine on cTBS in healthy volunteers

Background

Hyper-acute reperfusion therapy has revolutionised stroke care in recent years, with significant gains in improved survival and reduced disability achieved through the use of intravenous thrombolysis (Lees et al., 2016) and endovascular clot extraction (Saver et al., 2016). However, many patients are left with ongoing deficit despite receiving cerebral reperfusion therapies, and many more are unable to access them at the point of care. There is consequently an urgent search for interventions which might enhance or extend the window of plasticity to improve recovery in the days and weeks following brain injury. A variety of drugs have been tried in small scale clinical trials, including levodopa, reboxetine and citalopram (Liepert, 2008), but the present leader in the field is fluoxetine, a selective serotonin reuptake inhibitor. Fluoxetine has been licensed since the 1980s for the treatment of depression, and is a cheap, safe and well tolerated medication, making it an ideal Investigational Medicinal Product for use in clinical trials. Furthermore, there is a wealth of laboratory data to support its use to enhance stroke recovery (Y. Sun et al., 2017).

Much interest was generated when the FLAME clinical trial (Chollet et al., 2011) reported that out of 110 stroke patients, 26% of those who received fluoxetine had a modified Rankin score (mRS) of 0 to 2 (no dependency on other people) at three months, compared 9% of those allocated to placebo (Chollet et al., 2011), a benefit which appeared to be independent of any effect as an antidepressant. This concept has now been expanded in to a series of international Phase III randomised placebo-controlled clinical trials to test the rehabilitative effect of 6 months of fluoxetine in acute stroke (Mead et al., 2015). The first of these (FOCUS, a study of 3127 patients from the UK) has recently reported a negative outcome (Dennis et al., 2019), with no benefit in terms of modified Rankin Score at 6 months in those receiving fluoxetine 20mg. The study design was pragmatic, with no particular emphasis on attempting to provide evidence for any specific possible mechanism, although a significantly lower incidence of post stroke depression was observed in patients randomised to fluoxetine. It remains to be seen whether the remaining

Phase III trials will produce different results but there still remains good experimental evidence to suggest a number of possible mechanisms whereby fluoxetine could promote stroke recovery.

Firstly, fluoxetine appears to be directly neuroprotective against ischaemic damage following an infarct, with reduced infarct volume when it is infused into rodents immediately following an experimental infarct (Lim et al., 2009; Shin et al., 2009). Whilst this is a promising line of enquiry, and may prove preferable to the current resource-intensive neuroprotective strategy of using medically-induced hypothermia, it is unlikely to underly the benefits seen with chronic administration for 3 months in FLAME, since patients in this study were not randomised to fluoxetine or placebo until day 5 post stroke.

Secondly and more promisingly, fluoxetine has been shown to have a neurotrophic effect, promoting neurite development, dendritic branching and even stimulating neurogenesis in the hippocampus, similar to the response seen in rodents following ischaemic insult (Encinas, Vaahtokari, & Enikolopov, 2006; Sun et al., 2015). However, it is difficult to distinguish between a direct pharmacological effect of the drug here versus any indirect effect induced via behaviour change. There is evidence that the effect is mediated through expression of BDNF (Li et al., 2015; Maya Vetencourt et al., 2008; Junjian Zhang et al., 2014), and in rat visual cortex, SSRIs appeared able to re-open critical period plasticity through reduced GABAergic inhibition (Maya Vetencourt et al., 2008). Fluoxetine treatment produced enhanced LTP (not normally seen in adult rat visual cortex) and reduced LTD. Most intriguingly of all, (Ng et al., 2015) found that the sensitive period of increased responsiveness to motor training post-stroke could be extended in mice using chronic administration of fluoxetine, even if the motor training was itself delayed. Interestingly it appeared that if administration of fluoxetine was delayed until day 7 the effect was lost: the sensitive period could not be ‘re-opened’.

This possibility that fluoxetine might re-open or extend the sensitive period in stroke patients that seems to be so critical to recovery is driving much of the

interest surrounding the current critical trials, and inspired this current experiment. If fluoxetine has been observed to extend the critical period of enhanced plasticity in mice, and we have been able to demonstrate a similar critical period in stroke patients using cTBS, it may prove possible to capture the neuroplastic effect of fluoxetine using the same methodology.

An interaction between TMS and fluoxetine is also suggested by their use in clinical depression. Plasticity-modulating NIBS have been shown to be effective in the treatment of major depression (Brunoni et al., 2016) leading to the suggestion that depression can be modelled as a disorder of aberrant cortical plasticity, which can potentially be restored via pharmacological manipulation of plasticity via serotonergic neurotransmission (Umemori, Winkel, Didio, Llach Pou, & Castrén, 2018). However, both the data from clinical depression and from small scale clinical trials in stroke patients would suggest chronic administration of an SSRI is required to produce a meaningful modulation of clinical outcomes. It would not prove feasible to recruit a significant proportion of stroke patients receiving fluoxetine given the multicentre nature of the current clinical trials, and ethically speaking, it was not felt justifiable to expose volunteers to 6 months administration of a drug with potentially significant side effects. To study the effects of SSRIs in those receiving it in long term treatment for major depression would itself introduce a number of insurmountable confounds.

Fortunately, the existing data suggest it may be possible to demonstrate an effect in volunteers exposed to just a single dose of SSRI. A single dose of a citalopram (an alternative SSRI) has been shown to affect cortical excitability in healthy controls (Robol et al., 2004), and a single dose was likewise able to modulate the response to cortical plasticity induced with TDCS to motor cortex (Nitsche et al., 2009). Nitsche et al (2009) found an enhanced facilitatory response to anodal TDCS, whilst citalopram converted an inhibitory protocol (cathodal TDCS) into facilitation. The same group (Batsikadze, Paulus, Kuo, & Nitsche, 2013) found a similar response

(reduction of inhibitory PAS and a trend towards amplification of excitatory PAS) with a single dose of citalopram using a Paired Afferent Stimulation protocol (PAS: Stefan et al, 2000), and also with chronic (35 days) administration of citalopram and TDCS (Kuo et al., 2016). However, all of these studies involved small sample sizes (12-16 subjects) and frequently were only single-blinded: future studies should aim to engage double-blinding, and use appropriately powered sample numbers based on calculations from existing data.

No study to date has probed the effect of fluoxetine in humans using repetitive stimulation, although a further study by Kuo et al., (2017) again found an enhanced facilitatory effect and reversed inhibitory effect of TDCS using following 21 days of reboxetine, a related anti-depressant drug with somewhat greater affinity for noradrenaline. Animal studies likewise tend to show that serotonin increase LTP and inhibit LTD *in vitro* (Holderbach, Clark, Moreau, Bischofberger, & Normann, 2007) suggesting that an effect similar to that seen by Nitsche et al. (2009) might be replicated with TBS. This hypothesis is yet to be tested in humans, but may provide important insight into the mechanism of SSRIs in stroke recovery, which will be of particularly importance if the remaining phase III clinical trials demonstrate clinical benefit and the drug moves over into the therapeutic domain.

It is therefore proposed in this third experiment to assess the impact of fluoxetine 20mg versus placebo on the response of healthy volunteers to spaced continuous theta burst stimulation (cTBS). Although findings in animal studies have been somewhat contradictory, the existing human data would support the generation of a hypothesis that fluoxetine will attenuate or abolish the inhibitory response to cTBS. Experimental data supports a mechanism of increased intracortical excitability and suppression of GABAergic interneurons (Maya Vetencourt et al., 2008), and so it is further hypothesized that increased ICF and supressed ICI will be observed in the fluoxetine-medicated recordings.

Hypotheses

Primary Hypothesis:

- Spaced cTBS will produce reduced suppression of MEPs when accompanied by fluoxetine compared with placebo.

Secondary Hypothesis:

- Subjects will show reduced ICI (LICI and SICI) and enhanced ICF with fluoxetine
- Subjects will not be able to reliably distinguish between fluoxetine and the placebo IMP on a forced-choice questionnaire

Methodology

Participants

31 healthy volunteers (average age 26.3 +/- 5.6, range 20-47; 30 RH 1LH, 20M 11 F) were recruited from the staff and students of the Institute of Neurology or by word of mouth. Subjects were screened for any chronic neurological conditions and for any contra-indication to receiving either non-invasive brain stimulation or a single dose of fluoxetine. Subjects with a history of significant mood disorder or epilepsy, or currently taking antidepressant medication, were excluded from the study.

Power Calculation

Based on data from Experiment 1, pertaining to the standard deviation (0.2) and intraclass correlation (0.5) of the primary outcome measure (average change in MEP amplitude over 30 minutes) using spaced continuous theta burst stimulation, and anticipating a modest effect size (0.2) then a target sample size of 31 subjects was considered to be sufficient to detect a significant difference with 80% power.

Experimental Protocol

Subjects were given either fluoxetine 20mg in liquid form or placebo (mint syrup) under double-blind conditions and then asked to return to the laboratory for testing 6 hours later (to conincide with peak plasma concentrations following oral administration – (Johnson, Lewis, & Angier, 2007)). Consequently, all testing took place in the afternoon or early evening. Subjects were be asked to refrain from alcohol and caffeinated drinks on the day of testing and to ensure they were well rested overnight (Manganotti et al., 2001). FDI hotspot in dominant hemisphere motor cortex was targeted with neuronavigation as in earlier experiments. After baseline MEPs are recorded, a random-sequence paired pulse protocol probing SICI and ICF was delivered, with identical parameters to those used in Experiment 2. Subjects then received the standardised spaced-cTBS protocol as per previous experiments. Based on a recent study (Cuypers et al., 2014) which found reduced MEP variability using 30 MEP sample in comparison to the 20 MEPs used in Experiments 1 + 2, a sample of 30 MEPs at 1mV intensity was collected at each time point (every 5 minutes up until 30 minutes) post stimulation (readings were more frequent in this experiment as the whole protocol is shorter (less than 60 minutes in total) and therefore subject fatigue was less of a concern).

All subjects returned for a second testing session a minimum of 72 hours later (average interval 23 days, range 3-65), where they received the other alternative of either fluoxetine or placebo (order was randomly allocated for each subject by a non-blinded investigator). The second session was otherwise identical to the first. After the second session subjects were given a forced-choice question asking them whether they thought that they received fluoxetine or placebo in each session.

Data Analysis and Statistics

Statistical Analysis

Raw data were inspected for non-normality and appropriate transformations were applied, as outlined in the results section. An individual's MEP amplitudes at each time point was averaged to produce a single value. Average MEPs were then be normalised to baseline in each individual to correct for baseline variation. A two-way repeat-measures ANOVA was be performed with factors DRUG (fluoxetine/placebo) and TIME (baseline, 0m, 5m, 10m, 15m, 20m, 25m, and 30m). Secondary analyses included a paired sample t-test to compare RMT between groups and to compare the effects of paired pulse data. The proportion of correct answers on the forced-choice questionnaire was analysed using a Chi-Square test.

Results

There were no reported adverse effects from either the IMP or from TMS. All subjects completed the study. On the forced-choice questionnaire subjects performed no better than chance (19 out of 31 correct, $\chi^2 = 0.7877$, $p = 0.375$), suggesting the subject-blinding was successful.

Baseline Parameters

Resting Motor Threshold did not differ between sessions on a paired-samples t-test using natural-logarithm transformed data ($T_{(30)} = 0.706$, $p=0.485$). This remained the case when comparing RMT measured using with the monophasic pulse of the MagStim Rapid^{TM} used to calculated stimulus intensity for theta-burst stimulation ($T_{(30)} = 1.114$, $p =0.274$; again using natural logarithm transformed data).

Pre-stimulation baseline 1mV MEPs did not differ significantly in peak-to-peak amplitude across sessions (fluoxetine 1.30mV s.d. 0.54; placebo 1.25 s.d. 0.68: $T_{(30)}=0.517$, $p=0.609$) in paired sample t-test using untransformed data.

	Fluoxetine	Placebo	T	P
RMT	45.0 (+/- 7.9)	45.71 (+/- 8.5)	0.706	0.485
RMT (biphasic)	63.0 (+/- 12.8)	63.3 (+/- 10.8)	1.114	0.274
Test Pulse	56.3 (+/- 8.5)	57.1 (+/- 8.8)	0.354	0.725
Test pulse MEP (mV)	1.30 (+/- 0.54)	1.25 (+/- 0.68)	0.517	0.609

Table 11 - motor threshold and stimulation parameters in each session (percentage of maximum stimulator output)

Intracortical Excitability

The peak-to-peak amplitude of the unconditioned 1mV test stimulus used for the paired pulse protocol likewise did not differ between sessions in a repeat-measures one-way ANOVA ($F_{(1.8,53.3)}$=1.652, p=0.204). Comparing intracortical inhibition, there was no difference in mean SICI (average of 2ms and 3ms ISI) between sessions (placebo 0.51, fluoxetine 0.52, Z=-0.196, p =-0.845 using a Wilcoxon Signed Ranks Test - data could not be transformed to meet the assumptions of the parametric model). Using parametric tests (paired-sample t-test) on natural logarithm transformed data for individual ISIs still found no effect of fluoxetine at either 2ms ($T_{(30)}$=-0.558, p =0.581)or 3ms conditioning stimulus ($T_{(30)}$=0.02, p=0.984). LICI likewise did not differ between sessions ($T_{(30)}$=1.647, p =0.11).

Intracortical facilitation was also unaffected by fluoxetine (mean conditioned pulse:test pulse with placebo 1.28, mean with fluoxetine 1.38, 1.$T_{(30)}$=0.757, p=0.455, paired sample t-test using natural logarithm transformed data), with no effect evident when values were again broken down to individual 10ms ($T_{(30)}$=0.883, p=0.384) and 15ms ($T_{(30)}$=0.283, p=0.779) interstimulus intervals (Table 12).

ISI	*2ms*	*3ms*	*SICI*	*10ms*	*15ms*	*ICF*	*150ms*
Placebo	0.532	0.490	0.511	1.292	1.27	1.279	0.288
Fluoxetine	0.505	0.537	0.521	1.427	1.338	1.382	0.398
Sig. (p-value)	0.581	0.984	0.845	0.384	0.799	0.455	0.110

Table 12 - intracortical excitability (expressed as ratio of conditioned stimulus to test stimulus) in both sessions

Response to cTBS

Comparing post c-TBS MEPs in a two-way repeat measures ANOVA using natural logarithm transformed data, there was a significant effect of TIME ($F_{(3.6,108.3)}$=5.888, p <0.001), but no effect of DRUG ($F_{(1,30)}$=2.14, p=0.154) or DRUG*TIME interaction ($F_{(4.2, 126.9)}$=0.80, p=0.533), suggesting that cTBS was influencing MEP amplitude, but that fluoxetine was not affecting MEPs or influencing the effect of cTBS. This remained the case when the data were normalised to pre-stimulation baseline, with a significant effect of TIME ($F_{(3.6, 108.5)}$=7.145, p<0.001) but no effect of DRUG ($F_{(1,30)}$=0.14, p=0.711) or DRUG*TIME interaction ($F_{(3.8,112.9)}$=0.882, p=0.472).

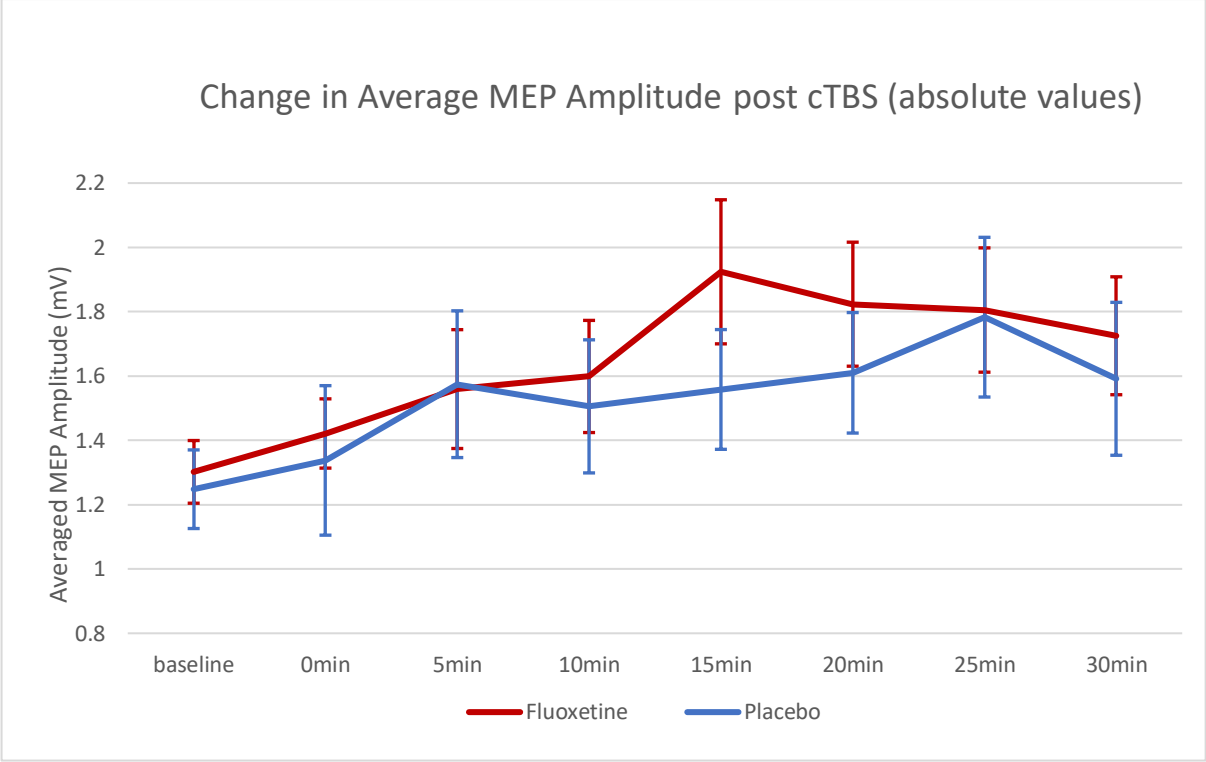


Fig. 5.1 Change in MEPs amplitude following spaced cTBS – fluoxetine vs placebo

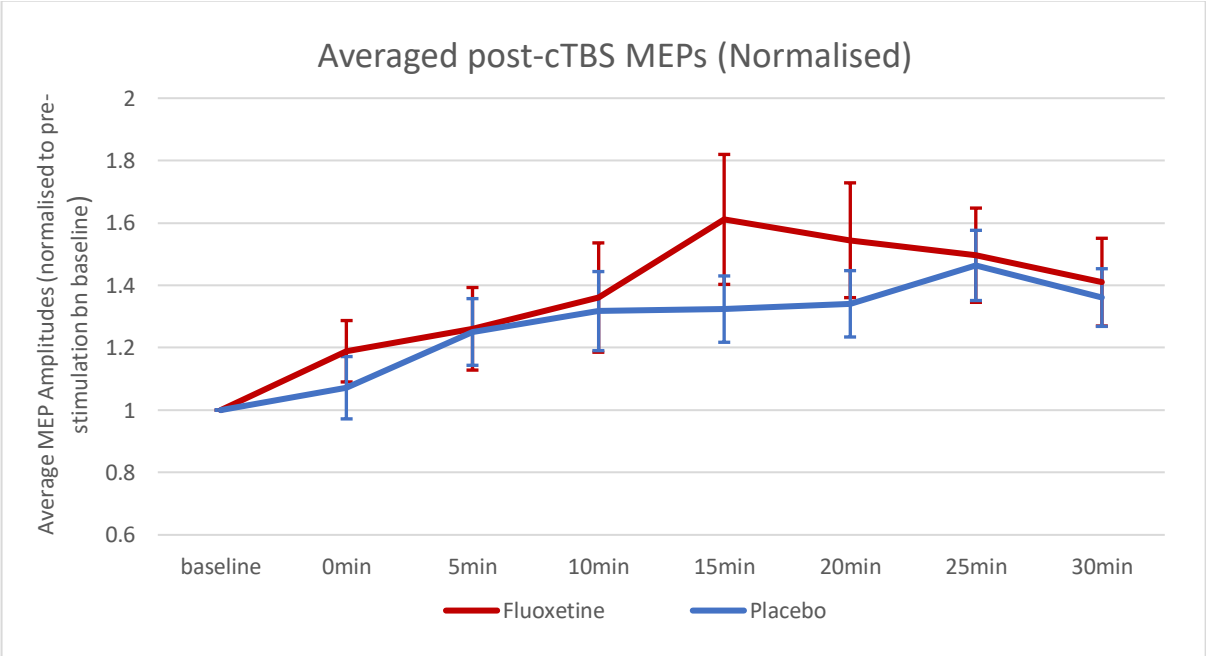


Fig. 5.2 Change in MEPs relative to baseline following spaced cTBS – fluoxetine vs placebo

Variability

Individual response to cTBS was predominantly facilitatory in both sessions, with only 11 out of 31 subjects showing inhibition from cTBS with placebo and 10 showing inhibition with fluoxetine. However, response was highly concordant, with 26 out of 31 (83.9%) showing the same response (facilitation/inhibition) in both sessions. Despite differing pharmacological conditions, intraclass correlation between the two recordings was good (ICC = 0.592 CI[0.154, 0.803] p=0.008), in keeping with our previous data in this age group. ICC improved yet further when data from the first 3 time points (first 10 minutes) were used, improving to 0.651 (CI[0.277-0.832], p = 0.003).

Given that the high proportion of non-canonical responders in this sample, we repeated the ANOVA using RESPONDER as a covariate to see if this could better capture any effect of fluoxetine. Using their response across 30 minutes in the placebo session as the determinant of whether they were classified as RESPONDER or NON-RESPONDER, adding this factor as a covariate did not affect the analysis significantly, with no SESSION*TIME*RESPONDER interaction ($F_{(3.9, 112.5)}$=1.389, p = 0.243). Thus, the effect of fluoxetine on response to cTBS did not differ between supposed responders and non-responders.

As a final exploratory analysis, the repeat measures ANOVA was repeated using just the data for the 11 canonical responders. This found no effect of SESSION ($F_{(1,10)}$=0.516, p=0.489) and no TIME*SESSION interaction ($F_{(6,60)}$=0.643, p=0.695). Hence there was no effect of fluoxetine, even amongst canonical responders to cTBS.

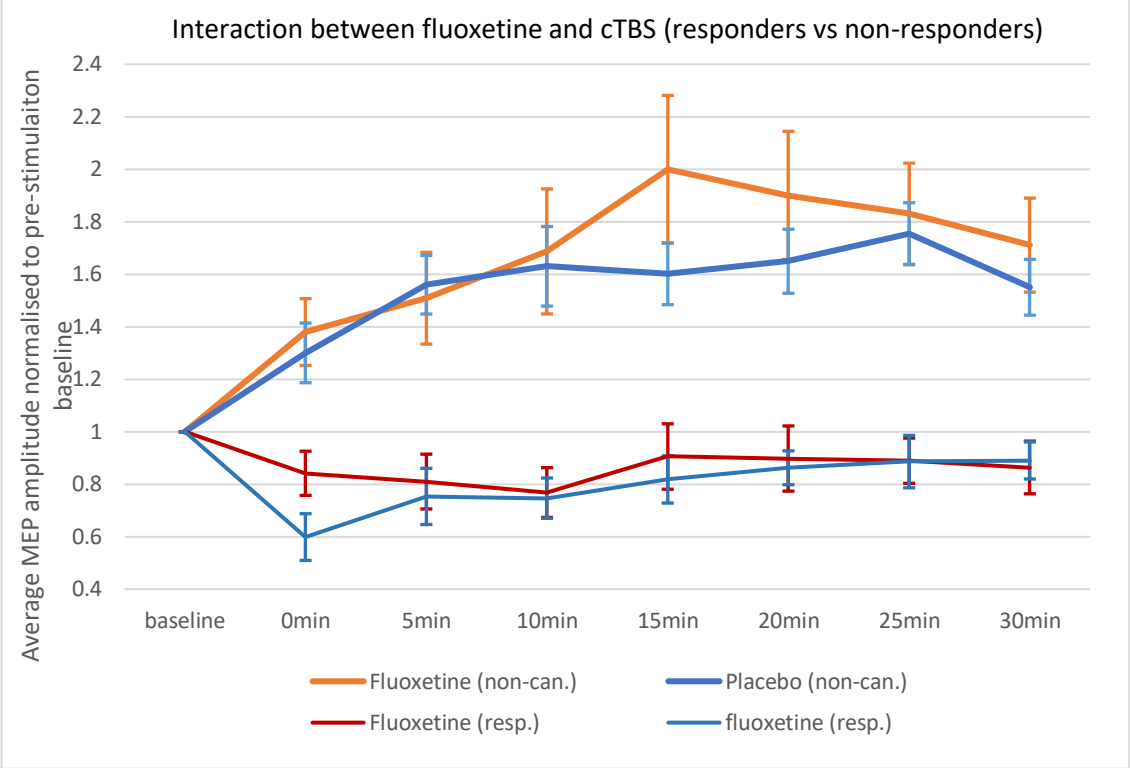


Fig 5.3. effect on cTBS of fluoxetine vs placebo – canonical responders vs non-canonical responders

Discussion

These data suggest that a single dose of fluoxetine 20mg does not influence the effect of cTBS on human motor cortex. This result was somewhat surprising, given the significant effect of both chronic and single administration of other SSRIs demonstrated by the group in Göttingen using a variety of other non-invasive brain stimulation methodologies. There was also no effect on either corticospinal or intracortical excitability, despite good evidence for this in animal models. Resting motor threshold did not differ between sessions, which is in keeping with the existing human data, which do not support an effect on RMT of either single dose (McDonnell, Zipser, Darmani, Ziemann, & Müller-Dahlhaus, 2018; Pleger, Schwenkreis, Grünberg, Malin, & Tegenthoff, 2004), or chronic administration (Lagas et al., 2016) of fluoxetine, although Pleger et al., (2004) did report an expansion of cortical representations in motor cortex suggestive of a generalised heightened cortical excitability. Results with citalopram have produced quite

different findings, with Robol et al (2004) finding an increase in motor threshold, as well as an increased SICI. A similar increase in RMT was found in the unaffected hemisphere of stroke patients receiving chronic administration of citalopram (Acler, Robol, Fiaschi, & Manganotti, 2009). These findings with citalopram would appear to be at themselves at odds with the hypothesis that serotonin increases cortical excitability. On the other hand, Eichhammer et al. (2003) found no effect of citalopram on motor thresholds but did find an increase in SICI in those subjects with the l1 polymorphism in the serotonin transporter gene 5-HTTLPR (a polymorphism that has been associated with improved response to SSRIs (Pollock et al., 2000)). Batsikadze et al. (2013) did not measure resting threshold, but found no difference in the 1mV simulation intensity between placebo and citalopram medicated PAS sessions, implying no alteration in baseline corticospinal excitability with citalopram.

Ilic, Korchounov, & Ziemann (2002) had earlier also found no effect on RMT with sertraline (an alternative SSRI), but an increased gradient in the TMS recruitment curve, implying an effect on the distribution of excitability in the pool of corticospinal neurones. This study found an increase in ICF but no effect on SICI. Münchau et al. (2005) also found no increase in RMT or AMT and no impact on SICI or ICF with mirtazapine, an alternative SSRI. Paroxetine, yet another SSRI, also had no effect on RMT (Gerdelat-Mas et al., 2005), with a significant boost to ICF but no significant effect on SICI or LICI. Paroxetine also produced a flattening of the MEP recruitment curve, the opposite of the effect seen with sertraline in Ilic et al (2002). The paroxetine-mediated increase in ICF in this study was correlated with improved performance on a finger tapping test, which could be evidence of an effect mediating motor cortical plasticity (Loubinoux et al., 2005) although since neither effect was significant with regards to fluoxetine in this study we have not explored the relationship between ICF and plasticity here.

There is thus a significant discrepancy in the existing human data as to the effects of SSRIs on corticospinal excitability, without even a clear trend towards increased or decreased cortical excitability. Changes in baseline corticospinal excitability with different SSRIs are variable, with perhaps the most consistent data for citalopram suggesting a decrease in cortical excitability and an increase in intracortical inhibition. Fluoxetine has however consistently failed to alter human resting motor threshold in three existing studies plus the present one, even when administered for 19 consecutive days (Lagas et al., 2016). Fluoxetine, paroxetine, and sertraline all exert an effect through upregulation of a1 and downregulation of a2 adenosine receptors, and on norepinephrine, acetylcholine, and dopamine reuptake mechanisms. Citalopram on the other hand is a much more selective 5-HT reuptake inhibitor, which might be partially responsible for the discordant results.

The present data suggest no effect of fluoxetine on LTD-like plasticity in human motor cortex, and the only other neurophysiological studies to date to investigate fluoxetine metaplasticity in healthy humans have also found negative results (McDonnell et al., 2018). McDonnell et al (2018) assessed the impact of cTBS on performance on a ballistic thumb movement task, and found no effect of fluoxetine on motor learning in a thumb-abduction task, a process which has been shown to be dependent on LTP plasticity in motor cortex, and which produces an LTP-like increase in MEPs which can be reversed with cTBS (Cantarero, Lloyd, & Celnik, 2013). Likewise the study by Pleger et al., (2004) found no effect of a single dose of fluoxetine on a motor learning tasking involving thumb abduction. Thus, there is some additional indirect evidence that fluoxetine does not influence LTP in human motor cortex either (and that if it did we might expect the effect to be detectable using cTBS). Loubinoux, Pariente, Rascol, Celsis, & Chollet (2002) however did find an effect of a single dose of citalopram on a motor learning on a manual dexterity task, suggesting once again there may be a possible difference in efficacy between these two drugs with regards to motor plasticity.

The one exception to this pattern seems to be when fluoxetine was used on patients during the post-stroke sensitive period (Pariente et al., 2001). This study demonstrated a benefit of 20mg fluoxetine on motor learning in the finger-tapping test. Thus the effect of fluoxetine on plasticity may be restricted to the post-stroke critical period, as suggested by the work in rodents by (Ng et al., 2015).

The failure of the current study to replicate the results of Nitsche et al. (2009) could be potentially be attributed to either the difference in pharmacological agent or the different mode of non-invasive brain stimulation, as this is the first study to examine the effect of SSRIs using repetitive TMS. As mentioned above, fluoxetine and citalopram differ in their pharmacodynamics, which could potentially explain their different interaction with NIBS. Equally the sample size in Nitsche et al (2009) was only 12 subjects, suggesting this study may have been significantly underpowered given the ICC of TDCS is broadly comparable to cTBS (discussed in Chapter Three). Whilst both inhibitory protocols, Cathodal TDCS and cTBS have different mechanisms of action, as TDCS does not cause neuronal depolarization. Rather, it modulates the neuronal membrane potential by weak constant direct current, thereby influencing the levels of excitability and modulating the spontaneous firing rate of neurons. Furthermore, TDCS has a broader (and thus less defined) spatial resolution. Nevertheless, both produce a demonstrable impact on motor cortex plasticity, and there is no *a priori* reason why TDCS should capture an effect of SSRIs not seen with TBS.

In one regard however the present data could be said to have reproduced the results of Nitsche et al (2009), in that the combination of SSRI with an inhibitory NIBS protocol produced a paradoxical facilitatory effect: perhaps the most remarkable result in this present study is the facilitatory effect seen with cTBS in the placebo session! Why such a result has been seen with the subjects in this present study but not our demographically similar healthy controls in Experiment 1 is unclear. Such a seemingly paradoxical response to cTBS has also been previously

demonstrated when cTBS was primed with a preceding run of cTBS (Gamboa, Antal, Moliadze, & Paulus, 2010), although in this study the two cTBS protocols ran back-to-back as a continuous train without any inter-train interval, unlike the 10 minute interval in our protocol, which has previously been found to produce prolonged inhibition (Goldsworthy et al., 2012). The paradoxical effect in the current study seems to have been driven by an unexpected large number of subjects showing a consistent non-canonical response under both experimental conditions. The consistence of response across the two sessions (Intraclass Correlation 0-59 - 0.65) implies that this is a genuine physiological response being captured and not merely 'noise' arising out of the baseline variability in the MEP. Furthermore, it would suggest that this effect is due to an inter-subject variable (and would further support a suggestion for no modulation of response with fluoxetine).

It is not clear why this present sample should contain such a high number of 'non-responders', although only 35% of subjects showed a 'canonical' response to single (non-spaced) cTBS in a recent study (Hordacre et al., 2017), and so such response rates are certainly not unheard of. This was attributed by these authors to variation in baseline MEP amplitude, with those with low baseline variability tending to manifest a facilitatory response to cTBS. Equally, in the study by Huang, Rothwell, Edwards, & Chen (2008), modest contraction (10% of maximal) of the FDI muscle during cTBS (such as is often used to set parameters for repetitive stimulation via Active Motor Threshold) was able to negate the neuromodulator effect of cTBS in healthy volunteers, and 1 minute of voluntary muscle contraction immediate after cTBS was sufficient to convert its inhibitory effect to facilitation, confirming that the response is malleable even within individuals. This concept, that neuroplasticity is itself malleable and plastic, has been termed "metaplasticity" (Hassanzahraee, Zoghi, & Jaberzadeh, 2018), and is the rationale underpinning not only the use of a spaced cTBS protocol, but also for the study of pharmacological effects on cTBS.

Variability in response to NIBS has bedevilled the field since its origins, and has driven much of the refinements in technique in recent years. López-Alonso et al. (2014) found that response to facilitatory NIBS was 40-45% regardless of which tool was used, with 55-60% showing either inhibition or no effect. Hamada, Murase, Hasan, Balaratnam, & Rothwell (2013) found 47% of subjects showed a canonical response to cTBS and 52% to iTBS, but only 25% showed the orthodox response to both protocols. However, it has frequently been noted (Vallence et al., 2015) that response is fairly consistent within subjects across time (as seen here), implying that when experimental parameters are kept constant, non-canonical response is determined by intrinsic biological factors rather than statistical 'noise'. Response to PAS (Hamada et al., 2014) and TDCS (Wiethoff, Hamada, & Rothwell, 2014) were not superior to TBS in this regard, with a recent critical review citing broadly similar responder rates across the three modalities (Guerra, López-Alonso, Cheeran, & Suppa, 2018), suggesting that the findings of Nitsche et al (2009) cannot easily be attributed to the technical superiority of their choice of NIBS.

Genetic polymorphism may be contributing to the existing disparity in response to SSRIs, as suggested by the results of Eichhammer et al. (2003), who found the effect of citalopram on SICI was modulated by polymorphisms in the serotonin transporter protein 5HTTLPR. As well as 5HTTLPR, polymorphisms in the 5-HT1A receptor have also been shown to modulate response to SSRI in clinical depression (Hong, Chen, Yu, & Tsai, 2006). Polymorphisms in both these regions have been found to also modulate the efficacy of rTMS in treatment of depression (Zanardi et al., 2007) suggesting that such genetic polymorphisms could explain the lack of a drug-stimulation interaction in our experimental subjects. Response to cTBS has repeatedly been linked to polymorphisms in BDNF (Antal et al., 2010; Cheeran et al., 2008; Puri et al., 2015) that are linked with reduced activity-dependent production of BDNF in mice (Chen et al., 2006). The same BDNF polymorphism (Val66Met) that blocked the effect of cTBS (Cheeran et al., 2008) has also been linked to blunted response to fluoxetine in rodent models of depression (Chen et al., 2006) and in human patients (Zou et al., 2010). Thus, BDNF polymorphisms in

our population could potentially have the “double-whammy” effect of impaired response to fluoxetine and impaired (or perhaps paradoxical?) response to cTBS. As more genetic influences on plasticity are being identified and as testing subjects becomes quicker and cheaper, routine genotyping of subjects prior to NIBS studies is likely to become commonplace and may hopefully shed light on this perplexing issue.

The administration of fluoxetine in this experiment was only of a single dose on the day of testing, whereas it is well established in clinical practice that the benefits in depressed patients stem from chronic administration, and the same thinking has underpinned clinical trials of fluoxetine in stroke. Likewise, the dramatic benefits seen in mouse stroke models were as a result of prolonged administration. When the SSRIs were first employed for treatment for clinical depression it was observed that there was even sometimes paradoxical worsening in depressive symptoms in the first days of administration (Maes et al., 2009) suggesting there may be some kind of biphasic effect of chronic administration on neural function. Chronic administration of SSRIs is known to enhance the cAMP-dependent phosphorylation of proteins such as BDNF (Duman, Heninger, & Nestler, 1997) and a single dose of fluoxetine may have been insufficient to modulate the BDNF-driven effect of cTBS. Nevertheless it is worth repeating that other NIBS investigations have demonstrated an effect with single doses of citalopram (Batsikadze et al., 2013; Nitsche et al., 2009).

Comparing the present study with the critical study by Ng et al (2015), as well as many other of the studies in rodent models, rodent models typically use a dose of fluoxetine (10mg/Kg) an order of magnitude greater than the concentration likely to be achieved with 20mg in an adult human being. Such doses would be considered a large overdose in humans, with a high likelihood of significant adverse, or even lethal, side effects (Borys et al., 1992) although none of the above-mentioned studies report any adverse effects of such high doses in their rodent

populations. This may prove yet another obstacle to translating the encouraging results seen in animals into the clinical setting.

Conclusions

Fluoxetine had no effect on cortical excitability or LTD-dependent plasticity. Whilst other SSRIs, particularly citalopram, have been found to demonstrate metaplastic effects, the existing data for fluoxetine do not support a role in modulating plasticity in human motor cortex. Any benefit in stroke patients may be due to a specific interaction with factors unique to the post-stroke sensitive period ("critical window") or may act via an indirect mechanism such as through reducing the incidence of post-stroke depression. At present there is insufficient evidence to recommend the use of fluoxetine to enhance post-stroke recovery of motor function.

cTBS produced an unexpected non-cannonical response in a majority of subjects. This may be due to underlying genetic polymorphism in the current cohort.

Chapter Six

Discussion

It is possible to summarise our three presented data sets thus:

- spaced cTBS is a reliable measure with which to probe serial measures of cortical plasticity in a stroke-aged human population
- spaced cTBS demonstrates a window of declining LTD-like plasticity in stroke patients in the weeks following a cerebral infarct
- Fluoxetine does not influence this measure of cortical plasticity in healthy human controls

Where then does this leave us? We will outline the implications of these three data sets and their broader impact on the state of neuroscience research in 2019. How do our data suggesting a decline in plasticity over the first six weeks post stroke fit with the other data from humans? And how far does this begin to explain the relative contribution to post stroke recovery? Finally, what if anything can we conclude from this regarding the planning and delivery of stroke rehabilitation services?

cTBS versus other Protocols and Methodologies - Variability

Firstly, we have demonstrated reasonable interclass correlation for spaced cTBS as a longitudinal measure of plastic response in motor cortex over six months. When the spaced protocol was first explored (Goldsworthy, Pitcher, & Ridding, 2013) it showed promise for greater response rates and more prolonged inhibition than conventional TBS. However, the present data suggest that intrasubject variability is comparable to that seen with other rTMS protocols and also that seen with TDCS. Thus, whilst no direct comparison with single burst cTBS has been performed, the present data do not support the use of a spaced protocol over a single one, particularly given the extra time and additional theoretical risk introduced by repeating cTBS trains. ICCs for both cTBS and comparable protocols are in the fair to

good range (mostly the former), making them a valid, if not ideal, tool for tracking longitudinal change in plasticity. However, the modest longitudinal correlation (ICC of the order of 0.5 to 0.6) has significant impact on the design of related experiments, with anticipated projected sample sizes in the range of 30-40 (Brown et al., 2017), well above the average size in the published literature. This has serious implications for the design and funding of rTMS research in the future.

Are future refinements on the horizon that might significantly advance this issue? Quadripulse TMS (QPS (Nakamura et al., 2016)), a relatively newer form of rTMS consisting of 4 pulses at a constant interstimulus interval repeated with an inter-burst interval (IBI) of 5s (i.e., 0.2 Hz) for 30 min (i.e., 1440 pulses in total) claims reduced variability compared to older protocols (up to 96% with an inhibitory protocol), with response rate in the region of 80% - although this success failed to be reproduced in a Caucasian population (Simeoni et al., 2016). The test-retest reliability of this measure is still yet to be recorded, and it remains to be seemed whether this will allow an improvement in reproducibility sufficient to facilitate clinical TMS studies with realistic sample sizes.

Precision Stimulation

Various measures are underway to attempt to improve the variability problem in rTMS. The use of neuro-navigation as utilised here has been proposed. The use of MRI-guided neuronavigation particularly (Sparing, Buelte, Meister, Paus, & Fink, 2008) may lead to even greater spatial resolution and improved intra-subject variability, but will come at a substantially increased cost in terms of resources. Technological advances should allow increasingly sophisticated computer modelling of the NIBS-induced electrical field, to more accurately incorporate anatomical features such as bone thickness, CSF volume, and gyral folding of the individual brain to factor in elements contributing to between-subjects variability (Opitz, Windhoff, Heidemann, Turner, & Thielscher, 2011).

Another important factor, both within and between subjects, that is more challenging to control for than neuroanatomical variation, is background cortical activity. TMS-EEG (Izumi et al., 1997) is a useful, and potentially more sensitive, technique that goes some way to addressing this. Simultaneous EEG recording allows for the incorporating of differences in oscillatory brain activity (Karabanov, Thielscher, & Siebner, 2016), whilst also allowing the detection of TMS-induced activity in regions other than motor cortex and at lower stimulation intensities than those required to evoke an MEP. TMS pulse delivery can be co-ordinated with real-time EEG data to facilitate pulse delivery at pre-defined states (Zrenner, Belardinelli, Müller-Dahlhaus, & Ziemann, 2016) – termed State Dependent Brain Stimulation (SDBS) which could potentially significantly reduce the pulse to pulse variation in MEP size. This technique also allows for investigation of plasticity outside of primary motor cortex, since TMS-Evoked Potentials (TEPs) on EEG - representing the transmission of evoked potentials to connected regions of cortex rather than to corticospinal end-effectors - can be demonstrated in and elicited from many regions other than motor cortex. Hence TMS-EEG could prove invaluable for piecing together the impact of anatomically distributed post-stroke plasticity on recovery, and may help overcome the potential obstacles to studying patients with impairment too severe to generate MEPs from ipsilesional motor cortex. TEPs have been shown to be sensitive to changes in cortical excitability induced by NIBS (Casula et al., 2014), and seem to be a robust and reliable indicator of cortical excitability (Kerwin, Keller, Wu, Narayan, & Etkin, 2018), but whether this would improve the test-retest reliability of NIBS such as cTBS is yet to be ascertained. Currently the TEP is not yet an established alternative to the MEP as a measure for detecting changes in cortical plasticity, but may gain favour once technical refinements in optimal stimulation parameters result from advances in the spatial resolution of stimulation (Kerwin et al., 2018).

TMS Probe

The choice of the TMS probe has likewise been suggested as a way of improving the test-retest reliability of rTMS, as demonstrated by the use of an expanded 30 MEP

sample in Experiment 3, which nonetheless had similar intraclass correlation to our earlier experiments (and a highly unexpected outcome!). Likewise, the use of a test pulse intensity set relative to RMT has been suggested (Vallence & Ridding, 2014), although in our own data set the use of an arbitrary 1mV test pulse was nevertheless shown to be relatively consistent in proportion to RMT. Previous studies have used active motor threshold as an arbitrary point to either measure corticospinal excitability or by which to set a standardised test pulse. However, in addition to the problems outlined by Goldsworthy, Müller-Dahlhaus, Ridding, & Ziemann, (2014) whereby prior muscle activation obliterated the effect of cTBS, use of this measure introduces more potential sources for variation. Published studies vary in their choice of background muscle activation used in defining AMT (20%, 10% or even 5% of maximal MEP amplitude) and there is little evidence as to how consistently this is enforced within studies. The issue of muscle fatigue is a significant one, particularly when studying patient groups and in prolonged protocols.

The use of an arbitrary 1mV MEP has strengths and weaknesses, and the most attractive alternative of performing a range of MEPs along a recruitment curve has serious limitations in terms of time demands. However, if the peak effect of protocols such as theta-burst can be consistently isolated to a specific time window (e.g. 10 minutes post stimulation) this may avoid the need to repeat serial measures post stimulation, reduced the burden of MEP collection and allowing a more sophisticated probe (or probes) to be used. Pellegrini, Zoghi, & Jaberzadeh (2018) have looked at the choice of test stimulus and found that variability in the probe decreased between 120% RMT and 135% RMT. However, they also found that MEP amplitude did not significantly increased after 135% RMT, implying that this constitutes the top of recruitment curve. The danger is therefore that any plasticity-inducing effect of NIBS might be drowned out by supra-maximal stimulation beyond this threshold (i.e. a ceiling effect). However, Vallence et al. (2015) found that the inhibitory effect of cTBS was most marked upon MEPs elicited at 150% RMT, with superior consistency across subjects and sessions. Different

NIBS have differential effects on late and early I-waves, with TDCS preferentially modulating early I-waves (Lang et al., 2011), and iTBS modulating late-I waves (Di Lazzaro et al., 2008) suggesting that different intensity probes may be preferable for different protocols. cTBS, the protocol utilised in these studies, has been shown to have a preferentially effect on I1 waves (Di Lazzaro et al., 2005), a finding seemingly at odds with the finding by Vallence et al of superior reliability at 150% toward the top of the recruitment curve, which they speculate may be due to less variability in the recruitment of late I waves at this intensity increasing the sensitivity to change in early I(1) wave recruitment. It has been proposed to more reliably assess the effects of NIBS on different parts of the MEP recruitment by adjusting coil orientation for the test pulse to produce an MEP consisting of more or less early, late, or direct waves (Hordacre et al., 2017). However, such a technique is technically challenging and requires subjects to maintain a constant level of muscle contraction, and may prove difficult to reproduce in patient populations.

Other Methodologies

NIBS need not be seen as the exclusive methodology for studying post-stroke plasticity: other studies have studied the phenomenon of post-stroke plasticity using neuroimaging, predominantly Magnetic Resonance Imaging. Plasticity has been defined both as anatomical change in (such as the famous study identifying enlargement of the hippocampus in London taxi drivers studying The Knowledge (Maguire et al., 2000)), or more commonly in changes in patterns of cortical activation on fMRI. Both methodologies have been shown to demonstrate changes in response to motor learning (Groussard et al., (2014), Kami et al., (1995)) and have been shown to map changes in cortical organisation post-stroke (Abela et al., 2015, Yu et al., 2017). Magnetic Resonance Spectroscopy (Floyer-Lea, Wylezinska, Kincses, & Matthews, 2006) is a related methodology that allows pattern of neurochemical change to be mapped helping to elucidate the time line of individual biochemical processes and their relative contribution to recovery, with potential interaction with NIBS to study real-time changes in brain biochemistry (Stagg & Johansen-Berg, 2013). As demonstrated, none of these methodologies need be

mutually exclusive, although once again the incorporation of imaging technologies in to TMS comes at significant financial cost.

Interpretation of cTBS data in a stroke population – Hebbian vs Homeostatic Plasticity

As mentioned previously, our data only explore changes in LTD-like plasticity in the contralesional hemisphere, and so should be interpreted with caution in terms of their relationship to stroke recovery. LTD and LTP are both forms of Hebbian synaptic plasticity, and whilst LTP-like plasticity is more popularly associated with motor learning, there must surely be a role for LTD-like plasticity as well, both in motor learning and in neurorehabilitation.

Similarly, the reason for the terms 'LTD-like' and 'LTP-like' plasticity stems from the fact that whilst the changes in cortical excitability induced by NIBS in many ways mimic these phenomena (Cirillo et al., 2017), at present there is only circumstantial data to infer what is occurring at the synaptic level. Furthermore, the time course of NIBS-induced plasticity does not quite correlate with observed synaptic phenomena. Emerging evidence breaks down synaptic plasticity to a number of stages, with NIBS induced plasticity seemingly reproducing the early stages, but not the later stages where changes in synaptic excitability are consolidated. This could explain the short duration of NIBS induced plasticity and possibility the modest effect observed in clinical trials. The time-scale of facilitatory protocols fits best with what is termed Early LTP (distinct from the proteinsynthesis-requiring later stages of LTP) which occurs within 30 minutes of synaptic activation, and unlike late-LTP seems to be ephemeral (Lisman, 2017). Interestingly, late stage LTP appears to be dependent on BDNF (Pang, Nagappan, Guo, & Lu, 2016), and also possibly dopamine (Angenstein, Matthies, Staeck, Reymann, & Staak, 1992). Whilst it would appear that the transient and more-or-less immediate effect of facilitatory protocols matches well with the phenomenon of early LTP, equivalent biphasic

phenomena have not been observed with LTD, and its temporal relationship with inhibitory NIBS is less clear.

Homeostatic Plasticity

It was rapidly observed following publication of Donald Hebb's rule of synaptic plasticity that it could not be the only concept at work, as it does not allow for any limit on increasing synaptic plasticity, producing an unstable model. The concept of homeostatic plasticity, whereby physiological processes work to return the firing rate of a neuron back to a predetermined point is seemingly at odds with the concept of increased plasticity following cerebral insult. Indeed, the precise relationship between Hebbian and homeostatic plasticity in normal circumstances is still poorly understood. It appears that different relationships exist between critical periods of development and later stages, with the post-stroke window potentially mimicking a critical development period, as has been suggested previously. Indeed one theory is that critical periods represent a period of Hebbian plasticity that is then closed by the restoration of homeostatic plasticity that is slower to re-establish itself (Turrigiano, 2017). The role and contribution of Hebbian and homeostatic plasticity need not be entirely equal and opposite, given that an innate saturability of LTP and limit to the induction of new dendritic expansion (Matsuzaki, Honkura, Ellis-Davies, & Kasai, 2004) provide an existing check to excitotoxicity from LTP. Homeostatic mechanisms therefore may exist solely to 'reset' the synapse or circuit to allow further learning to be encoded, once existing learning has been successful consolidation. Furthermore, an overlap between LTD and a homeostatic response to LTP clearly exists, as some of the molecular mechanisms are shared (Fox & Stryker, 2017), but is yet to be clearly delineated.

History of Neuronal Activity and Metaplasticity – turning LTD into LTP?

As mentioned above, it is proposed (Hassanzahraee et al., 2018) that neurons in a circuit have an in-built maximal firing rate and that the threshold for inducing LTP

increases as this rate is approached. The existence of such homeostatic plasticity mechanisms has been postulated as underlying the frequently paradoxical responses to rTMS such as was demonstrated in our fluoxetine study. It has already been noted that previous level of physical activity prior to rTMS – both immediately prior to stimulation with regards to muscle activation, or in the hours and days before a session – can influence the response, although such a phenomenon is easier to recognise than to control for in human subjects, particular since it may be motor practice rather than motor activity *per se* that is the major confound (Ziemann et al., 2004). Suffice to say, subjects in NIBS experiments are likely to each have cortical neurons that are on different portion of the Hebbian plasticity recruitment curve and are nearer or further away from saturation point – and consequently more or less resistant to NIBS-induced plasticity. As a source of variability this can potentially be controlled for by good randomisation of subjects in a study, but will still vary from session to session, and as mentioned earlier it is not clear to what extent activity diaries from subjects allow meaningful control of this variable.

Changing Trains - Intertrain Intervals

Metaplastic interactions between NIBS protocols also underlined our choice of a spaced TBS protocol. One might expect homeostatic mechanisms to blunt the effect of a second LTD-inducing train of cTBS, as is indeed the case when the two trains are delivered back to back (Gamboa et al., 2010). The 10-minute intertrain interval was derived from TDCS data (Monte-Silva et al., 2010), and is unlikely to be the optimal interval for cTBS (Gamboa et al., 2011). Spaced cTBS is clearly capable of producing paradoxical results such as in our third experiment, and it is as yet unclear whether this 10-minute interval (or any subsequent refinement of it) is an innate quality of motor cortex or whether this may vary across subjects (e.g. due to genetic factors) or across time (e.g. due to history of cortical activity – although our data showed fair concordance across large time windows, even in those subjects showing a non-canonical response). The field is busy with research at both

molecular and behavioural level studying the time scale of varying plasticity mechanisms (Turrigiano, 2017) and a clearer picture will hopefully soon emerge.

Interhemispheric Competition Model

Whilst there has been much interest in changes in the contralesional hemisphere following stroke, our present findings would appear at face value to be somewhat at odds with the prevailing theory of Interhemispheric Competition, whereby loss of transcallosal inhibition from the lesioned hemisphere produces disinhibition with the contralesional hemisphere, whereas we have found no evidence of a disinhibited contralesional M1. There are few possible explanations here: firstly, our well recovered sample possibly did not experience significant disruption of interhemispheric inhibition as their (often subcortical) infarcts may have left them with a significant degree of functioning motor cortex in the ipsilesional hemisphere.

However, it is within the realms of possibility that the present enhanced LTD-like plasticity demonstrated in the contralesional hemisphere is related to a reduction in interhemispheric inhibition. LTP has been shown to be enhanced in disinhibited motor cortex (Cash, Murakami, Chen, Thickbroom, & Ziemann, 2016), although the effect on LTD is less clear. In rodent auditory cortex *in vitro* LTD was abolished by the presence of a GABA antagonist (Watanabe, Kamatani, Hishida, Kudoh, & Shibuki, 2007), whilst low-dose ethanol administration enhanced PAS-induced LTD in human subjects (Fuhl, Müller-Dahlhaus, Lücke, Toennes, & Ziemann, 2015). Thus, it might be expected to see reduced LTD in the disinhibited cortex, unlike our own data. It would certainly be informative to see to what extend LTP-like plasticity would track the changes in inhibitory plasticity in contralesional cortex seen here, although the obvious interaction between the two protocols would make it very challenging to study both phenomena in the same subjects simultaneously.

Alternatively, the closing of the development critical period in visual cortex has been shown to be triggered by GABA (Iwai, Fagiolini, Obata, & Hensch, 2003), and so disruption of interhemispheric inhibition could be responsible for prolonging the critical period post-stroke (and provides a rationale as to why the critical period post stroke might follow a different time course in the two hemispheres).

Motor Practice in the Non-Paretic Limb

As mentioned, there is a link between motor practice and cortical metaplasticity, and of course it can be argued that perhaps motor practice with the non-paretic limb during the course of stroke recovery underpins the changes observed in LTD. This would explain why LTD-like response is greatest at week 2, corresponding to the greatest deficit in the paretic upper limb. To counter this, as argued in Chapter 4, many subjects had stroke affecting their non-dominant upper limb (15/29), and it is hard to see a stroke affecting the non-dominant hand would result in significantly increased levels of motor practice with the dominant upper limb. It is worth noting that human beings are unique in this respect, possibly reducing the validity of extrapolating findings from animal studies into clinical practice. This confound could be partially controlled for by excluding patients with dominant hemisphere infarcts, but once again this would reduce the availability of subjects in what is already a crowded field.

Intracortical Excitability

The failure to find changes in intra-cortical circuits reproduces much previous data and seems to be a fairly consistent finding. Paired-pulse protocols have however been shown to have reasonable test-retest reliability in a subacute stroke population (Schambra et al., 2015) with ICCs in the range of 0.55-0.75, comparable to that seen in healthy volunteers with our primary measure - spaced cTBS. Criticism has been made of the use of a standard test pulse in the measurement of intracortical inhibitory and excitatory microcircuits, with the emergence of

threshold tracking possibly more reliable (Samusyte, Bostock, Rothwell, & Koltzenburg, 2018) and has shown diagnostic utility in patients with amyotrophic lateral sclerosis (Vucic, Cheah, Yiannikas, & Kiernan, 2011). As well as being quicker to perform than conventional SICI, this has shown greater reproducibility and may be a useful tool with which track longitudinal changes in stroke patients. However, this technique may struggle with the high motor thresholds often exhibited in stroke patients, with coil overheating a significant issue. Hopefully technological advancements may obviate this in the future.

Cortical Target for NIBS

It should also be considered whether contralesional M1 is the optimal target for NIBS to measure the critical period post stroke. Experimental animal data and imaging studies in human stroke patients show changes in activity in a variety of regions including secondary motor cortices, sensory cortex and the cerebellum – areas which are often spared in middle cerebral artery occlusion (the most common form of disabling stroke) and consequently are potentially suitable for study in ipsilesional cortex – although the obstacle of eliciting an MEP from damaged M1 would remain, although potentially surmountable through use of TMS-EEG. Interestingly, in healthy volunteers reduced motor activity in the upper limb impaired inhibitory NIBS in a homeostatic interaction (Rosenkranz, Seibel, Kacar, & Rothwell, 2014) – thus we might expect to see impaired LTD in the ipsilesional cortex controlling a paretic limb separate from any endogenous plasticity effect.

As mentioned above, changes in ipsilesional cortex need not follow the same time course as in contralesional hemisphere, so it may be more appropriate to talk of 'critical periods' than of a single time window. At the clinical level this would still manifest as a period of enhanced recovery, but importantly one that closes slowly and sequentially rather than abruptly.

FDI as Target

Likewise, the choice of a single hand muscle such as FDI may vary too much between individuals to be a reliable standard measure, and perhaps measurement from multiple electrodes on multiple muscles may allow a more stable measure (albeit with an increased family wise error rate). Equally, small muscles of the hand are continuously being used in fine motor skills for daily activities, which will frequently encompass some degree of motor learning that will interfere with the effect of NIBS on their cortical regions (Rioult-Pedotti, Friedman, & Donoghue, 2000, Ziemann et al., 2004, Rosenkranz, Kacar, & Rothwell, 2007). In healthy volunteers this may similarly be controlled for to an extent by investigating the non-dominant hand (which nonetheless is still capable of a significant element of skill-learning for both unimanual and bimanual tasks).

Potential Refinements to Our Study Design

Bearing much of this in mind, it is possible to suggest in retrospect refinements to our study design. The present study would ideally be expanded using a larger and more varied sample of stroke patients, particularly those with more severe impairment. However, as was noted in Chapter Four, these patients are precisely those least likely to be able to engage with research of this nature, both due to their lack of mobility and (hopefully) the intensity of their rehabilitation regime. Such patients are also more likely to have significant cognitive impairment from their stroke and medical comorbidities that will limit their capacity to take part. By using portable equipment and embedding researchers within an inpatient rehabilitation unit, it might be possible to allow greater recruitment from such a sample. Portable equipment suitable for testing in a ward environment is currently being developed, such as the Deymed™ DuoMAG XT which we were able to 'test drive' at the Sobell Department during my Fellowship. However, the potential for ongoing motor learning could represent a more significant confound in such a population undergoing daily rehabilitative therapy.

Greater London is not well suited for such an approach, however, as stroke patients are admitted to centralised Hyper Acute Stroke Units for the initial part of their hospital stay and then repatriated to local General Hospitals for rehabilitation, so that any continuity for a longitudinal study such as this is lost. Smaller urban centres, where all services exist on a single site, are better suited for continuity in this regard, but of course require a trade off in terms of numbers, such that a study might need to be run concurrently at more than one location to achieve an adequate sample.

Window of Recovery

It is worth repeating that this study is the first to demonstrate using TMS that a critical period of plasticity exists in humans after stroke, which had previously been hypothesised from animal data and from behavioural observation in humans. It appears that the first six weeks following stroke represent a unique environment for stroke rehabilitation, and whilst causation and correlation cannot be untangled at present, it would seem eminently plausible that such heightened synaptic plasticity is the substrate that drives the optimal recovery during this period. It follows that the conclusions of Biernaskie, Chernenko, & Corbett (2004) that failing to optimise recovery during this period leads to permanent deficits that cannot be overcome apply similarly to human stroke patients. In light of this it would seem perverse that the bulk of studies of rehabilitative therapies in stroke patients are performed in chronic stroke survivors, often years after their initial infarct: indeed, a recent meta-analysis found just 6% of stroke rehabilitation trials took place within this acute time window, and that many of these engaged small sample sizes (Stinear, Ackerley, & Byblow, 2013). Similarly, clinical resources are often not focused on what ought to be a period of intensive therapy. A study in 2004 (Bernhardt, Dewey, Thrift, & Donnan, 2004) found that in the first 14 days following a stroke, patients were only physically active for little over an hour a day, and recent studies using modern activity monitors (accelerometers) confirm the problem has not gone away (Strømmen, Christensen, & Jensen, 2014). Given that the evidence in humans suggests a possible dose response to physical therapy for

stroke recovery (Lang, Lohse, & Birkenmeier, 2015, Veerbeek et al., 2014), it would seem deeply regrettable that further physical therapy is not targeted at this window. Furthermore, it is not clear that the therapy received during these critical early weeks is of a type necessary to harness such additional plasticity. Lang et al., (2009) found that during acute stroke rehabilitation, the average number of repetitions of an upper limb movement was 32, a stark contrast to the average number of repetitions necessary to be performed in animal studies in order to induce plastic change and meaningful recovery (Friel et al., 2007; Plautz, Milliken, Nudo, et al., 2000). However, the challenges to intensive therapy in the immediate post-stroke period are not purely attributable to resources, but significantly affected by the patient's medical condition, cognitive function and general level of impairment – a patient who lacks the balance to sit up without assistance and who is being artificially fed through a nasogastric tube may well struggle to complete a rigorous schedule of activity every day (although arguably in their interest to manage as much as possible). These are the precisely the population with potentially the most to gain (and lose), and so a greater understanding of how recovery mechanisms might differ between mildly and severely impaired patients is urgently needed.

Boosting cortical plasticity post stroke – a renewed focus?

We found no evidence for an effect of fluoxetine on LTD-like cortical plasticity, in keeping with indirect evidence for an absence of effect on LTP-like plasticity as well (McDonnell et al., 2018; Pleger et al., 2004). If the results of FOCUS (Dennis et al., 2019), that fluoxetine has no benefit on functional stroke recovery, are confirmed with the publication of the AFFINITY and EFFECTS trials, then this would provide additional evidence against an ability for fluoxetine to mediate cortical plasticity in the post-stroke period in humans. The results of this clinical trial have come as major disappointment in the field of translational medicine given what looked like accumulating evidence for a beneficial effect of fluoxetine on plasticity and on recovery. Thus, if fluoxetine did enhance the post stroke critical period in some way then this ought to have been borne out in the clinical data. Ng et al, (2015) found

that fluoxetine was not effective on stroke recovery if delayed until day 7: FOCUS allowed enrolment from day 2 up to day 15, so it is arguable that some of these subjects may have been enrolled too late to receive the benefit of fluoxetine, although the groups were balanced to contain equal amounts of early and late enrolment. The investigators in FOCUS plan subgroup analyses to assess if there was any greater effect of fluoxetine with the early randomisation. However, our own data from this experiment suggest that the critical period in humans extends well beyond day 7. The only possible caveat, as discussed in Chapter 5, is the choice of 20mg as the dose of fluoxetine, much lower than the dosage used in most animal models. However, would seem unlikely that a clinically meaningful benefit would be seen at 40mg (the maximum licensed dose) given that such a large study found no effect at 20mg. Should meta-analysis of the three studies remain negative then this would be extremely hard to justify. Given the safety concerns over high doses of fluoxetine in humans, it is possible that non-human primate studies could help bridge the gap, although this would go against the grain of the prevailing trend in experimental stroke research.

Might an alternative SSRI prove more advantageous? Fluoxetine has been the head of the field for several years now, but as outlined in Chapter Five there are alternative SSRIs with evidence for modulating synaptic plasticity. In their Cochrane Review, Mead, Hsieh, & Hackett (2013) broke down the evidence by compound. Fluoxetine had by far the best evidence, with additional evidence to support reduced disability with sertraline (OR 1.38 [0.99, 1.76]) and paroxetine (OR 1.31 [0.67, 1.95]) but not citalopram (OR 1.18 [-0.22, 2.58]). The same meta-analysis yielded evidence for reduced neurological deficit with citalopram (-1.43 [-2.25, -0.60]) and paroxetine (-1.21 [-1.68, -0.74]) but not sertraline (-0.26 [-0.96, 0.45]). Overall no single SSRI performed better than its peers. Furthermore, there is no clear evidence from clinical data to suggest that a different SSRI would have been more effective choice for FOCUS. Nor was there any evidence in the meta-analysis to suggest that an alternative SSRI would have been better tolerated by the study population, with no difference in drop-out rates. Mead et al went on to point out

that since both citalopram and escitalopram can cause an increased risk of arrythmias, these remained a second choice behind fluoxetine in a population likely to have an excess burden of heart disease by nature of their condition, since stroke and heart disease share a number of risk factors.

Alternatives to SSRIs

Amphetamines were originally the source of much excitement in the search for pharmacological interventions for stroke recovery, after being shown to accelerate recovery in rats when combined with rehabilitative activity (Feeney, Gonzalez, & Law, 1982). Successive clinical trials in humans have failed to demonstrate its efficacy (Goldstein, 2009). Likewise, small positive trials of the use of dopamine have failed to be borne out in larger clinical trials (Cramer, Dobkin, Noser, Rodriguez, & Enney, 2009).

Other emerging pharmacological targets include using GABA antagonists or AMPA receptor allosteric modulators to reverse the excess inhibition seen in peri-infarct cortex (Krakauer and Carmichael, 2017). These drugs appear to have a critical window of their own when they are effective, as reversing cortical inhibition too soon after stroke may have the effect of compounding excitotoxicity and increasing stroke volume.

A criticism of all pharmacological interventions in humans, particularly when observing their failure to replicate the successes seen with animal experiments, is that the dose of rehabilitative therapy, even in clinical trials, does not come anywhere near the intensity achieved training post stroke animals. Emerging data supports the view that physical recovery following stroke shows dose dependency with rehabilitation (Altman, Swick, & Malec, 2013), but that the majority of stroke patients in real world scenarios receive minimal physical therapy if any. There is not currently even an agreed standard of physical therapy received by the patients receiving drugs such as fluoxetine in clinical trials. It is equally clear that present healthcare resources are woefully insufficient to meet the demands of increased

physical therapy, at least in terms of man-hours. It does not appear feasible that a rehabilitation regime equivalent to that experience by animal stroke models will be deliverable within present healthcare frameworks, and so it seems that alternative, patient-driven programmes will need to be developed. Technical advances with improved software and robotic technology may allow patients to follow an autonomous therapy programme, but this approach will likewise not be without considerable cost. Furthermore, those patients with significant cognitive impairment, potentially most in need of intensive rehabilitation, are likely to continue to be reliant on one-to-one personal intervention from a human therapist.

Conclusions

Despite experimental evidence to support a role for serotonin in enhancing synaptic plasticity (Normann & Clark, 2005; Ögren et al., 2008), we have failed to find an effect from fluoxetine in modulating NIBS in the largest and best controlled human study to date. The discrepancy between these data and the previous literature is puzzling, but the most that can be said at present is that the effect does not appear to carry across the whole class of SSRIs, nor with varied forms of NIBS. Anomalous results such as this are perhaps unsurprising given the degree of variability in responses to NIBS, and the role of serotonin in the physiology of neuroplasticity will perhaps best be answered by basic scientists for the time being.

Measures are ongoing to refine the technique of TMS and NIBS in order to reduce this variability, but are likely to result in the trade off with its economy and convenience as a research tool. A major investment in TMS technologies may eventually circumnavigate this, but would appear unlikely unless and until a clear, consistent and clinically meaningful benefit for patients such as those who took part in our study can be demonstrated.

Identifying a short period of enhanced plasticity post stroke heightens the urgency for researchers and service providers to focus their efforts on this window of

opportunity. The results of the recent FOCUS trial came as a blow to clinicians who thought they had seen an accumulating body of data to support the use of fluoxetine to support stroke recovery, and whilst there are still other clinical trials waiting to report, FOCUS was the largest and most pragmatic of the trials and it would seem unlikely that meta-analysis in the near future will overturn the result. Indeed, existing data on proportional recovery (Prabhakaran et al., 2008) has been interpreted by some to suggest that stroke recovery is a somewhat predetermined process, and that even ubiquitous measures such as physical therapy do not necessarily alter the final outcome. Whether sufficient therapy intervention to replicate that seen in animals and potentially replicate their successes can be delivered in the context of a 21st Century healthcare system remains to be seen.

Nevertheless, it seems that interventions to enhance recovery in the post-acute stroke phase are likely to yield more modest benefits than the recent advances in hyperacute care, despite (or because of) being several orders of magnitude more complex in their delivery. Still, the evidence for a critical period of plasticity post-stroke during which recovery is optimal is supported by our data, and it is our belief that scientific investigation should focus more on this period in humans. This will require a more sophisticated cooperation between clinicians and scientists than has hitherto been the rule, in order for acute stroke patients and academics to have ready access to one another. The number of cases of acute stroke is expected to continue to rise for a number of years to come, and many countries and regions are still in the process of redesigning their services to accommodate the advances of recent years, and this is hopefully being taken into account.

The therapeutic nihilism that underscored stroke care for many decades has however now been discarded and a paradigm shift in our approach to stroke care has ensued. The advances of the next decade will likely be slower and more laborious than of the years that have just passed, but progress is continuing and will continue for as long as there are scientists prepared to forgo received wisdom and ask awkward questions. The future will belong to those who have done the most for the sake of suffering humanity.

Appendix A

Patient Characteristics

	age	sex	CVA - territory	Stroke imaging	thrombolysis	NIHSS (admission)	NIHSS (wk 2)	FMUL	MHI-5	mRS	medication
S001	53	F	R MCA	right precentral gyrus	Y	2	2	61	24	1	fluoxetine 20mg
S002	46	M	L MCA	left corpus striatum, left insular cortex, left superior and middle frontal cortex and left pre and post central gyrus regions.	N	8	1	65	28	2	
S003	78	F	L borderzone	left parieto-occipital region, left precentral gyrus and centrum semiovale	N	2	2	62	28	2	
S004	60	F	L MCA	Left Thalamus	Y	2	2	58	16	2	
S005			*withdrew*								
S006	63	M	R MCA	right precentral, subcentral and middle frontal and gyri and corona radiata	Y	4	4	60	12	1	
S007			*withdrew*								
S008	82	M	L MCA	no MRI	N	4	3	65	29	2	
S009	64	M	R MCA	posterolateral aspect of the corona radiata	N	1	1	65	28	1	
S010	68	F	R MCA	no MRI	N	5	5	56	27	2	
S011	60	M	R MCA	right middle and inferior frontal gyri	N	5	3	59	24	3	

	age	sex	CVA - territory	Stroke imaging	thrombolysis	NIHSS (admission)	NIHSS (week 2)	FMUL	MHI-5	mRS	medication
S012	64	F	L PCA	lateral left thalamus and posterior hippocampus	N	2	2	60	18	2	citalopram 20 mg
S013	48	M	L MCA	left corona radiata and posterior lentiform nucleus, insular and left temporal parietal lobe	Y	7	3	62	30	2	
S014	78	F	L MCA	left thalamic infarct	Y	3	3	65	28	2	
S015	69	F	L PCA	no MRI	N	4	4	63	23	2	
S016			*withdrew*								
S017	53	F	R MCA	Right internal capsule + caudate nucleus	N	3	4	61	29	3	
S018	73	M	L MCA	Left striato-capsular infarct	N	4	4	59	23	2	
S019	71	M	L MCA	L MCA territory	N	5	4	65	20	2	
S020	63	M	L PCA	left dorsal pons	N	1	3	61	25	2	
S021	82	M	L MCA	Left corona radiata	Y	1	1	65	28	2	
S022	78	M	R MCA	Right insula and frontl cortex	Y	3	1	64	28	2	
S023	73	M	L MCA	Left pre-central gyrus	N	2	1	64	26	2	
S024	77	M	R MCA	Right MCA lacunar infarct	N	1	0	66	26	2	
S025	63	M	L MCA	left thalamocapsular infarct	N	4	0	62	24	2	
S026	76	M	L MCA	left peri-rolandic region	N	9	3	65	21	1	
S027	75	M	L MCA	left capsular	N	3	1	66	28	1	

	age	sex	CVA - territory	Stroke imaging	thrombolysis	NIHSS (admission)	NIHSS (week 2)	FMUL	MHI-5	mRS	medication
S028	71	M	R MCA	right parietal and insular infarct	N	7	4	65	29	2	
S029	73	M	R MCA	right capsular	Y	6	1	64	25	2	
S030	70	M	R MCA	right striato-capsular	N	3	5	57	20	3	
S031	67	M	L MCA	left lenticulo-striate	N	12	0	63	26	3	
S032	81	M	L MCA	left pontine	N	1	2	57	27	3	

Appendix B

Patient Testing Proformas

NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

Time: ___ ___:___ ___ [ ]am [ ]pm

Person Administering Scale _____

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).

Instructions	Scale Definition	Score
1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = **Alert;** keenly responsive. 1 = **Not alert**; but arousable by minor stimulation to obey, answer, or respond. 2 = **Not alert**; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.	_____
1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = **Answers** both questions correctly. 1 = **Answers** one question correctly. 2 = **Answers** neither question correctly.	_____
1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.	0 = **Performs** both tasks correctly. 1 = **Performs** one task correctly. 2 = **Performs** neither task correctly.	_____
2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	0 = **Normal.** 1 = **Partial gaze palsy;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = **Forced deviation,** or total gaze paresis not overcome by the oculocephalic maneuver.	_____

NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.	0 = **No visual loss.** 1 = **Partial hemianopia.** 2 = **Complete hemianopia.** 3 = **Bilateral hemianopia** (blind including cortical blindness).	_____
4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	0 = **Normal** symmetrical movements. 1 = **Minor paralysis** (flattened nasolabial fold, asymmetry on smiling). 2 = **Partial paralysis** (total or near-total paralysis of lower face). 3 = **Complete paralysis** of one or both sides (absence of facial movement in the upper and lower face).	_____
5. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** limb holds 90 (or 45) degrees for full 10 seconds. 1 = **Drift;** limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support. 2 = **Some effort against gravity;** limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = **No effort against gravity;** limb falls. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **5a. Left Arm** **5b. Right Arm**	_____ _____
6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** leg holds 30-degree position for full 5 seconds. 1 = **Drift;** leg falls by the end of the 5-second period but does not hit bed. 2 = **Some effort against gravity;** leg falls to bed by 5 seconds, but has some effort against gravity. 3 = **No effort against gravity;** leg falls to bed immediately. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **6a. Left Leg** **6b. Right Leg**	_____

NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

		_____
7. Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	0 = **Absent.** 1 = **Present in one limb.** 2 = **Present in two limbs.** UN = **Amputation** or joint fusion, explain: _____	_____
8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.	0 = **Normal;** no sensory loss. 1 = **Mild-to-moderate sensory loss;** patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. 2 = **Severe to total sensory loss;** patient is not aware of being touched in the face, arm, and leg.	_____
9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	0 = **No aphasia;** normal. 1 = **Mild-to-moderate aphasia;** some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. 2 = **Severe aphasia;** all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response. 3 = **Mute, global aphasia;** no usable speech or auditory comprehension.	_____
10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	0 = **Normal.** 1 = **Mild-to-moderate dysarthria;** patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = **Severe dysarthria;** patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. UN = **Intubated** or other physical barrier, explain:_____	_____

NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

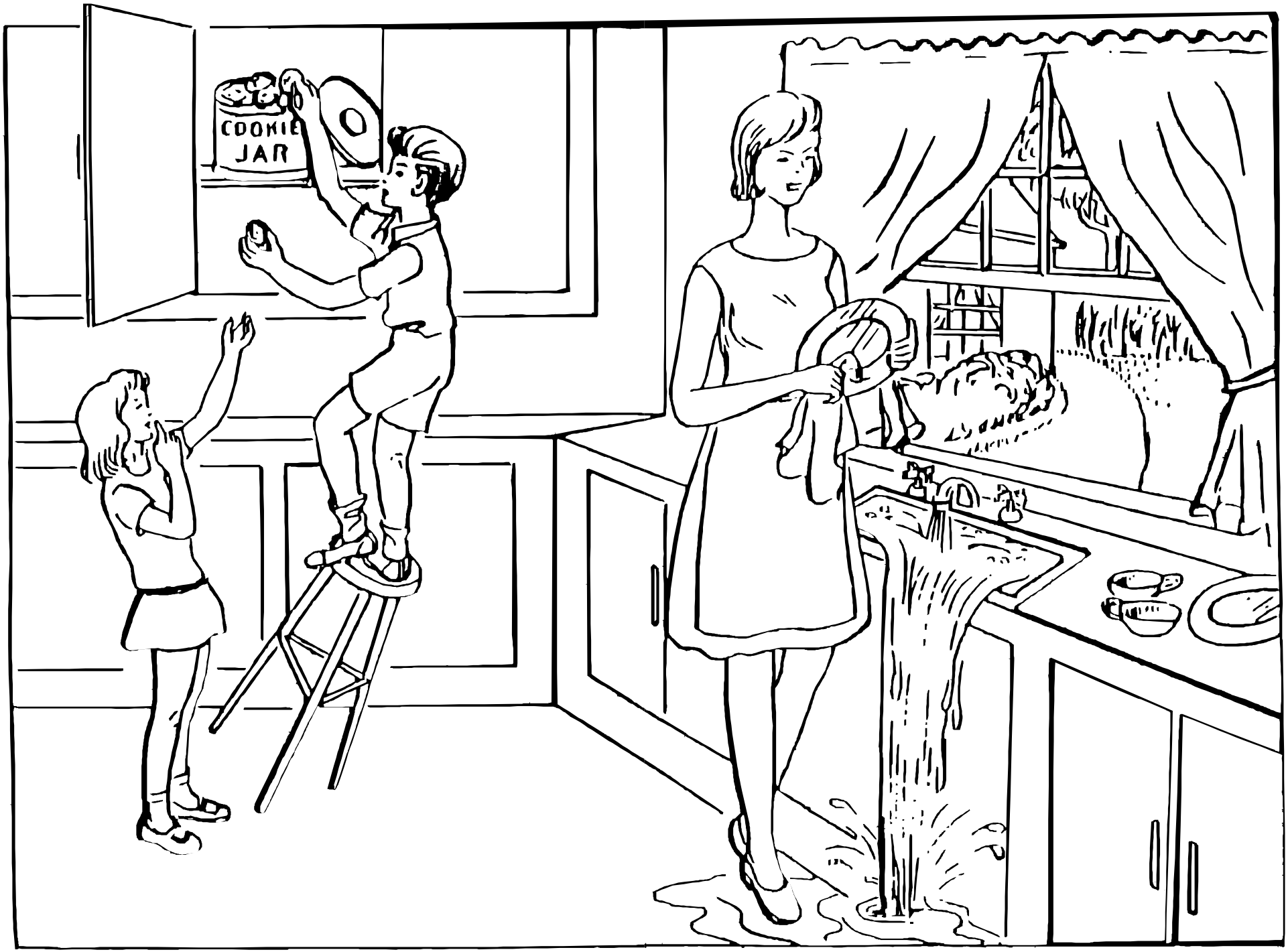
Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	0 = **No abnormality.** 1 = **Visual, tactile, auditory, spatial, or personal inattention** or extinction to bilateral simultaneous stimulation in one of the sensory modalities. 2 = **Profound hemi-inattention or extinction to more than one modality;** does not recognize own hand or orients to only one side of space.	_____

_____

_____


COOKIE
JAR

You know how.

Down to earth.

I got home from work.

Near the table in the dining room.

They heard him speak on the radio last night.

MAMA

TIP – TOP

FIFTY – FIFTY

THANKS

HUCKLEBERRY

BASEBALL PLAYER

FUGL-MEYER ASSESSMENT
UPPER EXTREMITY (FMA-UE)
Assessment of sensorimotor function

ID:
Date:
Examiner:

Fugl-Meyer AR, Jaasko L, Leyman I, Olsson S, Steglind S: The post-stroke hemiplegic patient. A method for evaluation of physical performance. Scand J Rehabil Med 1975, 7:13-31.

A. UPPER EXTREMITY, sitting position

I. Reflex activity		none	can be elicited
Flexors: biceps and finger flexors		0	2
Extensors: triceps		0	2
	Subtotal I (max 4)		

II. Volitional movement within synergies, without gravitational help			none	partial	full
Flexor synergy: Hand from contralateral knee to ipsilateral ear. From extensor synergy (shoulder adduction/ internal rotation, elbow extension, forearm pronation) to flexor synergy (shoulder abduction/ external rotation, elbow flexion, forearm supination). **Extensor synergy:** Hand from ipsilateral ear to the contralateral knee	Shoulder	retraction	0	1	2
		elevation	0	1	2
		abduction (90°)	0	1	2
		external rotation	0	1	2
	Elbow	flexion	0	1	2
	Forearm	supination	0	1	2
	Shoulder	adduction/internal rotation	0	1	2
	Elbow	extension	0	1	2
	Forearm	pronation	0	1	2
		Subtotal II (max 18)			

III. Volitional movement mixing synergies, without compensation		none	partial	full
Hand to lumbar spine	cannot be performed, hand in front of SIAS	0		
	hand behind of SIAS (without compensation)		1	
	hand to lumbar spine (without compensation)			2
Shoulder flexion 0°-90° elbow at 0° pronation-supination 0°	immediate abduction or elbow flexion	0		
	abduction or elbow flexion during movement		1	
	complete flexion 90°, maintains 0° in elbow			2
Pronation-supination elbow at 90° shoulder at 0°	no pronation/supination, starting position impossible	0		
	limited pronation/supination, maintains position		1	
	complete pronation/supination, maintains position			2
	Subtotal III (max 6)			

IV. Volitional movement with little or no synergy		none	partial	full
Shoulder abduction 0 - 90° elbow at 0° forearm pronated	immediate supination or elbow flexion	0		
	supination or elbow flexion during movement		1	
	abduction 90°, maintains extension and pronation			2
Shoulder flexion 90°- 180° elbow at 0° pronation-supination 0°	immediate abduction or elbow flexion	0		
	abduction or elbow flexion during movement		1	
	complete flexion, maintains 0° in elbow			2
Pronation/supination elbow at 0° shoulder at 30°-90° flexion	no pronation/supination, starting position impossible	0		
	limited pronation/supination, maintains extension		1	
	full pronation/supination, maintains elbow extension			2
	Subtotal IV (max 6)			

V. Normal reflex activity evaluated only if full score of 6 points achieved on part IV				
biceps, triceps, finger flexors	0 points on part IV or 2 of 3 reflexes markedly hyperactive	0		
	1 reflex markedly hyperactive or at least 2 reflexes lively		1	
	maximum of 1 reflex lively, none hyperactive			2
	Subtotal V (max 2)			
	Total A (max 36)			

B. WRIST support may be provided at the elbow to take or hold the position, no support at wrist, check the passive range of motion prior testing		**none**	**partial**	**full**
Stability at 15° dorsiflexion elbow at 90°, forearm pronated shoulder at 0°	less than 15° active dorsiflexion dorsiflexion 15°, no resistance is taken maintains position against resistance	0	1	2
Repeated dorsifexion / volar flexion elbow at 90°, forearm pronated shoulder at 0°, slight finger flexion	cannot perform volitionally limited active range of motion full active range of motion, smoothly	0	1	2
Stability at 15° dorsiflexion elbow at 0°, forearm pronated slight shoulder flexion/abduction	less than 15° active dorsiflexion dorsiflexion 15°, no resistance is taken maintains position against resistance	0	1	2
Repeated dorsifexion / volar flexion elbow at 0°, forearm pronated slight shoulder flexion/abduction	cannot perform volitionally limited active range of motion full active range of motion, smoothly	0	1	2
Circumduction	cannot perform volitionally jerky movement or incomplete complete and smooth circumduction	0	1	2
Total B (max 10)				

C. HAND support may be provided at the elbow to keep 90° flexion, no support at the wrist, compare with unaffected hand, the objects are interposed, active grasp		**none**	**partial**	**full**
Mass flexion from full active or passive extension		0	1	2
Mass extension from full active or passive flexion		0	1	2
GRASP				
A – flexion in PIP and DIP (digits II-V) **extension in MCP II-V**	cannot be performed can hold position but weak maintains position against resistance	0	1	2
B – thumb adduction 1-st CMC, MCP, IP at 0°, scrap of paper between thumb and 2-nd MCP joint	cannot be performed can hold paper but not against tug can hold paper against a tug	0	1	2
C - opposition pulpa of the thumb against the pulpa of 2-nd finger, pencil, tug upward	cannot be performed can hold pencil but not against tug can hold pencil against a tug	0	1	2
D – cylinder grip cylinder shaped object (small can) tug upward, opposition in digits I and II	cannot be performed can hold cylinder but not against tug can hold cylinder against a tug	0	1	2
E – spherical grip fingers in abduction/flexion, thumb opposed, tennis ball	cannot be performed can hold ball but not against tug can hold ball against a tug	0	1	2
Total C (max 14)				

D. COORDINATION/SPEED after one trial with both arms, blind-folded, tip of the index finger from knee to nose, 5 times as fast as possible		**marked**	**slight**	**none**
Tremor		0	1	2
Dysmetria	pronounced or unsystematic slight and systematic no dysmetria	0	1	2
		> 5s	**2 - 5s**	**< 1s**
Time	more than 5 seconds slower than unaffected side 2-5 seconds slower than unaffected side maximum difference of1 second between sides	0	1	2
Total D (max 6)				

TOTAL A-D (max 66)	

The instrument contains the following questions:

'How much of the time during the last month have you:

(i) been very nervous?
(ii) felt downhearted and blue?
(iii) felt calm and peaceful?
(iv) felt so down in the dumps that nothing could cheer you up?
(v) been a happy person?'

For each question the subjects were asked to choose one of the following responses:

all of the time (1 point),
most of the time (2 points),
a good bit of the time (3 points)
some of the time (4 points)
a little bit of the time (5 points)
none of the time (6 points).

Because items (iii) and (v) ask about positive feelings, **their scoring is reversed**. The score for the MHI-5 is calculated by summing the scores of each question item and then transforming the raw scores to a 0–100-point scale.

MODIFIED RANKIN SCALE (MRS)

Patient Name: _____

Rater Name: _____

Date: _____

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

TOTAL (0–6): _____

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