# MOTOR SYSTEM PLASTICITY INDUCED BY NON-INVASIVE STIMULI

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Thesis submitted for the degree of

*Doctor of Medicine*

February 2019

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# Abstract

Precisely timed paired stimulation protocols can change cortical and subcortical excitability.

In the first study, induction of plastic changes in the long-latency stretch reflex (LLSR) by pairing non-invasive stimuli was attempted, at timings predicted to cause spike-timing dependent plasticity (STDP) in the brainstem. LLSR in human elbow muscles depends on multiple pathways; one possible contributor is the reticulospinal tract. The stimuli used are known to activate reticulospinal pathways. In healthy human subjects, reflex responses in flexor muscles were recorded following extension perturbations at the elbow. Subjects were then fitted with a portable device which delivered auditory click stimuli, and electrical stimuli to biceps muscle. The LLSR was significantly enhanced or suppressed in the biceps muscle depending on the intervention protocol. No changes were observed in the unstimulated brachioradialis muscle. Although contributions from the spinal or cortical pathways cannot be excluded, the results were consistent with STDP in reticulospinal circuits.

In the second study, baseline TMS responses were recorded from two intrinsic hand muscles, flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC). In the first phase, paired associative stimulation (PAS) was delivered by pairing motor point stimulation of FDS or EDC with TMS. Responses were then remeasured. Increases were greatest in the hand muscles, smaller in FDS, and non-significant in EDC. In the second phase, intermittent theta-burst rapid-rate TMS was applied instead of PAS. In this case, all muscles showed similar increases in TMS responses. This study showed that potential plasticity in motor cortical output has a gradient: hand muscles > flexors > extensors. However, this was only seen in a protocol which requires integration of sensory input (PAS), and not when plasticity was induced purely by cortical stimulation (rapid rate TMS).

In the third study, motor imagery was paired with TMS in healthy human subjects. They were asked to imagine wrist flexion or extension movement, while TMS was delivered to the motor cortex. Six different protocols were tested, but only flexor imagination with TMS and extensor imagination with TMS showed significant facilitation following the test. Flexor imagination with TMS increased motor evoked potential (MEP) in all four

muscles with maximum changes towards flexor, whereas extensor imagination with TMS increased MEP only in extensor.

Above changes in the cortical or subcortical excitability evoked by non-invasive stimulation protocols were consistent with long term potentiation and long-term depression mediated plastic changes.

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# Figures and Table

# Publications

## Papers

**Published in the Journal of Neuroscience:**

FOYSAL, K. M., DE CARVALHO, F. & BAKER, S. N. 2016. Spike Timing-Dependent Plasticity in the Long-Latency Stretch Reflex Following Paired Stimulation from a Wearable Electronic Device. J Neurosci, 36, 10823-10830.

https://doi.org/10.1523/JNEUROSCI.1414-16.2016

**Published in the Journal of Physiology:**

FOYSAL, K. M. and BAKER, S. N. (2019), A hierarchy of corticospinal plasticity in human hand and forearm muscles. J Physiol. doi:10.1113/JP277462

**Work in progress:**

FOYSAL, K. M. & BAKER, S. N. Induction of Plasticity in the Motor Output with Paired Motor Imagery and Transcranial Magnetic Stimulation. Newcastle upon Tyne, 2019

## Abstracts

FOYSAL, K. M. & BAKER, S. N. Spike-Timing Dependent Plasticity in the Long Latency Stretch Reflex Following Paired Stimulation from a Wearable Electronic Device. Society for Neuroscience. San Diego. 2016

FOYSAL, K. M. & BAKER, S. N. A Hierarchy of Plasticity In The Human Hand And Forearm: Smaller Changes In Extensors Than Flexors/Intrinsic Hand Muscles. Sensory Motor Conference, Newcastle upon Tyne. 2016

FOYSAL, K. M. & BAKER, S. N. Rewiring the Nervous System Using A Wearable Electronic Device Rewiring the Nervous System Using A Wearable Electronic Device. North East Postgraduate Conference. 2015

FOYSAL, K. M. & BAKER, S. N. Effects of a paired stimulation paradigm on biceps long latency reflexes investigated using a wearable electronic device. Sensory Motor Conference, Leeds. 2014

# Acknowledgment

The intensive work would not have been possible without the generous help and support from many people.

Firstly, I would like to thank my supervisor, Stuart Baker, for his exceptional support, encouragement, continual patience and tolerance, all of which allowed me to undertake and continue this thesis. Special thanks go to Mark Baker who has also provided me excellent support, opportunities to engage myself into other clinical studies and has been a fascinating guide who helped me in finding the right directions.

My special thanks go to Mark Cunningham, who has been very supportive, helpful and encouraged me a lot. His pieces of advice were priceless.

I want to thank Anna Basu, who was very supportive, and her advice encouraged me a lot.

Special thanks go to Gavin Clowry, who guided me learning immunofluorescence techniques, slide preparation and staining protocols. It was a pleasure working in his laboratory.

I would like to thank Andrew Jackson and Demetris Soteropoulos for their motivational discussions and talks on neuroscience.

Special thanks go to Grainne Gorman for her support, advice and encouragement.

I am thankful to Felipe De Carvalho who designed and made the electronic circuitry for the wearable device experiment. I would like to thank Norman Charlton for building the robotic arm chair that I used to measure stretch reflex.

I would like to take the opportunity to thank current and former members of the motor laboratory: Bonne Habekost, Boubker Zaaimi, Claire Witham, Damar Susilaradeya, Edina Tozser, Ed Hodkin, Ferran Galan, Georgia Collins, Jesús Tapia-López, Isabel Glover, Karen Fisher, Lauren Dean, Stefane A. Aguiar, Stephan Jaiser, Supriyo Choudhury, Thomas Hall, Terri Jackson, Wei Xu, Alison Liddle and others.

Special thanks to Boubker Zaaimi for helping me with Matlab coding, to Karen Fisher for explaining complicated protocols in my early days in the laboratory and to Damar Susilaradeya for helping me in pilot experiments.

My thanks go to Beckie Hedley for her tremendous support and help. I would like to thank Ann Fitchett, Carole Ainsley, Glynis Mitchinson, Karen Bai, Karen Curry and Mel Leitch for their help.

I am grateful to all those people and patients who participated in the studies and invested their valuable time for neuroscience, and this work would not have been possible without their interest in helping people.

Finally, I want to thank my parents for their continued support over the years and special thanks go to Syema for her love, patience, and encouragement.

# Chapter I

# BACKGROUND

## 1.1 General Introduction and Plasticity

The human motor system is an integral component of the extremely complex and dynamic central nervous system, which ultimately regulates movement and motor behaviour. It had been a widely believed misconception until the late 19th century, that once developed and matured, interconnected neuronal circuits in the brain and spinal cord leave very little to no scope for further modification in adults. It took centuries before researchers could finally establish the idea that the brain exhibits more plasticity than people have ever predicted. In neuroscience, plasticity can be generally defined as the adaptation or remodelling of the nervous system in dynamic response to physiological, environmental or pathological changes.

The brain's ability to adapt and reorganize itself following lesions was experimented as far back as the early 19th century by a French physiologist Pierre Flourens, who closely observed brain's capability of regaining function after partial lobectomy in birds. He believed that the brain's redundancy mostly accounted for the restitution of functions after such lesions (Levin and Grafman, 2000). Fluorens's concept of brain redundancy was later supported by other researchers (Lashley, 1929, Franz, 1912). Some scattered case reports and scientific studies in the 19th century and early 20th century described the potential ability of the brain's adaptation and plastic changes in adverse physiological or pathological conditions. Later on, it has been documented by neurosurgeons and other scientists that the brain can effectively recover some of its functionality largely mediated by reorganization and adaptation in the events of a severely damaged hemisphere or even after hemispherectomy (Nielsen, 1946, Hillier, 1954, Crockett and Estridge, 1951, Zollinger, 1935). Kennard (1938), from a series of experiments in primates, found that both infant and adult monkey regained function after brain lesions, but recovery became less and less obvious with increasing age and spasticity developed more severely in adult monkeys than their newborn counterparts (Kennard, 1940). Kennard also suggested that reorganization in the brain could involve a shift of functionality to a neighbouring area in the cortex (Kennard, 1942). It was

eventually becoming clearer to researchers that successive partial damages over a prolonged period caused much less loss of function than a sudden complete lesion since each partial damage allowed the brain to have sufficient time recovering some of its functions through reorganization after each event (Travis and Woolsey, 1956). Similarly, a slowly grown tumour causes less functional impairment than the damage caused by a similarly sized lesion from acute stroke (Anderson et al., 1990). However, the idea of brain plasticity was largely viewed as changes in the early phases of life during development, or in certain cases, as a means of redundancy and adaptability to compensate the loss of function which occurs only after brain damage (Van der Loos and Woolsey, 1973).

Brown and Sherrington (1912) were among the first believers in a mechanism that supports cortical plasticity in the adult brain. They observed interesting cortical effects after running a series of experiments with direct cortical stimulation and peripheral nerve stimulation. They found it was possible to inhibit or facilitate the response of the targeted muscles in primate forearm with specially designed protocols of stimulation and they described the short-lasting changes as a cortical reaction.

Later, more evidence supporting reorganization in the sensory-motor area or motor cortex mediated by exposure to repeated natural stimulation, various types of invasive and non-invasive stimulation to peripheral nerves, motor point or cortex and by other methods of stimulation began to emerge. In an experiment, Pascual-Leone et al. (1993) found a significantly enlarged cortical representation of the index finger in the reading hand while they mapped the cortical areas of proficient braille readers using Transcranial Magnetic Stimulation (TMS). Another related example of plastic changes triggered by afferent input from hand in congenitally blind patients was described by Sadato et al. (1996). To determine whether the visual cortex receives input from the somatosensory system, they used positron emission tomography (PET) to measure response in the primary and secondary visual cortex during a tactile reading task and found that blind subjects showed activation in those areas, while normal subjects showed deactivation.

It is now evident that some natural, altered or artificial physiological conditions can initiate changes in the synaptic junctions (synaptic plasticity) or in the overall neuronal connections (metaplasticity). Examples of such conditioning include lesions (Donoghue

et al., 1990, Rauschecker, 1995, Calford and Tweedale, 1991, Kolarik et al., 1994, Silva et al., 1996, Merzenich et al., 1983b), amputation (Calford and Tweedale, 1988, Borsook et al., 1998, Chen et al., 1998), skill developing or learning (Grafton et al., 1992, Recanzone et al., 1992, Pascual-Leone et al., 1994, Kami et al., 1995, Nudo et al., 1996, Taubert et al., 2010) or deafferentation by ischaemia (Ziemann et al., 1998b, Brasil-Neto et al., 1992, Brasil-Neto et al., 1993, Ridding and Rothwell, 1995, Ridding and Rothwell, 1997, Ziemann et al., 1998a).

The organization and projection of neuronal pools in the cortical and subcortical area have a major role to play when synaptic plasticity and remodelling is concerned. The topography of skeletal muscle and sensory organs in the somatosensory and motor cortex is extremely complex, and they tend to overlap with each other, opposing the famous and influential homunculus idea of having precisely ordered representation for each body part (Schott, 1993). Evidences from some recent studies using advanced imaging, intracortical electrical stimulation via microelectrodes inserted into focal cortical areas and other sophisticated methods suggested that the internal organization of brain areas representing sensory organs and muscles can rather be described as a dynamic network of neuronal pools arranged in their region and sub-regions in such a manner that allows reorganization. This modification in their synaptic excitability or internal connectivity occurs to compensate functional changes following injury or in altered physiological conditions, promoting metaplasticity in general (Sanes and Donoghue, 1997, Sanes and Donoghue, 2000, Stoney Jr et al., 1968, Nudo et al., 1992, Penfield and Boldrey, 1937, Schieber and Poliakov, 1998). This dynamic nature of the central nervous system controlling motor output and expected behaviour provides substantial flexibility for plastic changes in animals, and to a greater extent in primates and humans. The motor cortex is highly capable of retaining its functional outcome through reorganization and plasticity, but for this, it has to receive translated feedback from sensory inputs reflecting behavioural or functional changes. Typically, a lesion, and theoretically an afferent input can produce such a feedforward loop to promote plasticity. In other words, to maintain homeostasis and to obtain plasticity motor cortex relies on sensory inputs to a great extent. Therefore, afferent pathways that convey upstream volleys towards sensory motor areas can be potentially exploited to relay abrupt functional changes, altered inputs or artificial stimulation. In that regard, peripheral afferent stimulation is already known to modulate cortical excitability,

thereby it can affect motor performance after a lesion in the brain and spinal cord (McDonnell et al., 2007a, Liao et al., 2014, Roy et al., 2010, Lala et al., 2016). Peripheral sensory deafferentation is also capable of reorganizing cortical and subcortical connections exhibiting plasticity (Merzenich et al., 1983a, Merzenich et al., 1983b, Kaas et al., 1983, Sanes et al., 1988, Sanes et al., 1990). This leaves a very good reason to study different interventional methods to test scopes of plasticity in the human motor system using non-invasive stimulation capable of changing cortical and subcortical excitability.

## 1.2 Main Aims and Questions

### 1.2.1 Aims

The primary aim of this thesis is to explore the potential effect of specially designed non-invasive stimulation protocols in human motor system and to understand the basic mechanisms of plasticity at the cortical and subcortical level.

I intended to observe the characteristics of plasticity induced by simultaneous sensory input and cortical stimulation in motor cortical output and how it may express changes in different groups of muscles (forearm flexor, extensor and hand muscles in this case). I was also very keen to see how it differs if the plasticity is induced purely by cortical stimulation in motor cortical output measuring from those muscles.

Another objective was to introduce novel methods of delivering plasticity inducing protocols that may lead to the development of future translational therapies, which will help to facilitate functional recovery after certain brain or spinal cord lesion.

It was also a target of this research to deliver realistic and practical methods of providing stimulation, monitoring, measuring and comparing observed changes among a considerable number of healthy human subjects within a short period.

This approach to induce plasticity in the human motor system with non-invasive stimulation may arise a few questions, some of which are addressed below, and the rests will be discussed in the relevant part of this thesis.

## 1.2.2 Questions

**Which kind of techniques were used and why?**

For all the experiments in this research only non-invasive stimulation techniques such as superficial electrical stimulation, auditory stimulation and transcranial magnetic stimulation (TMS) were used. This served following benefits over any invasive procedure:

- Suitable to study plasticity in humans in vivo
- Purely non-surgical methods
- Safe and painless techniques
- No requirement of anaesthesia
- Easily applicable, effective and less cumbersome
- Ideal to use in patients as in therapy or rehabilitation programmes
- Considerably less ethical issues
- Highly cost effective
- Saves preparation time

All procedures described in this thesis were approved by the Local Research Ethics Committee before any experiment. Full written consent from each participant was obtained from each participant after explaining each procedure in detail.

**How to induce plasticity in the human motor system and where does this plasticity occur?**

In recent years, several stimulation protocols have been utilized in studies to map somatosensory and motor areas, to test cortical and subcortical excitability and to explore neural pathways under numerous experimental conditions. Generally, it has been possible to induce plasticity in the human brain and spinal cord using various forms of non-invasive stimulation. Applied stimulus to an afferent nerve fiber or sensory receptors usually generates a compound action potential sending an afferent volley upwards. This can be achieved by using a variety of stimulus alone or in combination

with others such as fine or crude touch, vibration, tactile, pain or electrical stimulation (Passmore et al., 2014). Via the dorsal root ganglion, the afferent volley then ascends to the medulla where those afferent nerve fibers synapse with ipsilateral dorsal column nuclei. After decussation in the medial lemniscus, second order nerve fibers reach to the contralateral ventral posterior lateral nucleus of the thalamus. From the thalamus, third order neurons take the afferent input towards somatosensory areas and then to the motor cortex where signals are processed to execute the desired movement (Leeman, 2007). However, interrelated parallel pathways connecting somatosensory, motor cortex, thalamus, brain stem and spinal cord motor neurons are incredibly complex in primates and human. In the past few decades, a substantial amount of research has been carried out to investigate the role of corticospinal and other descending pathways that control motor behaviour, balance, posture and reflexes. The structural organization of the motor system is highly hierarchical. The somatosensory-motor bridge connections, corticospinal and other pathways sending downward volley to spinal neurons also maintain some hierarchy. Major descending pathways and their anatomical organization were studied closely in Hans Kuyper's seminal experiments investigating nerve tracts using tracing methods (Kuypers, 1981, Kuypers, 1964). In primates, the motor component of descending pathways can be divided into three groups: ventromedial brainstem pathways, dorsolateral brainstem pathways and corticospinal-corticobulbar pathways (Kuypers and Brinkman, 1970). Ventromedial pathways convey the vestibulospinal (both lateral and medial) tracts, the tectospinal tracts arising from the midbrain, and the reticulospinal and bulbospinal tracts arising from the pontine and medullary reticular formation (Matsuyama and Drew, 1997, Shinoda et al., 1992, Lemon, 2008). Dorsolateral pathways consist of the rubrospinal tract, arising from the red nucleus, and pontospinal tract, arriving from pontine tegmentum (Kennedy, 1990, Kuchler et al., 2002, Lemon, 2008). These descending motor pathways, arising from different regions of the brain, send downward volleys to finally reach topographically arranged alpha and gamma motor neurons and interneurons at multiple levels of the spinal cord. The schematic drawing in Figure 1.1 outlines the pathways of major descending tracts in human.

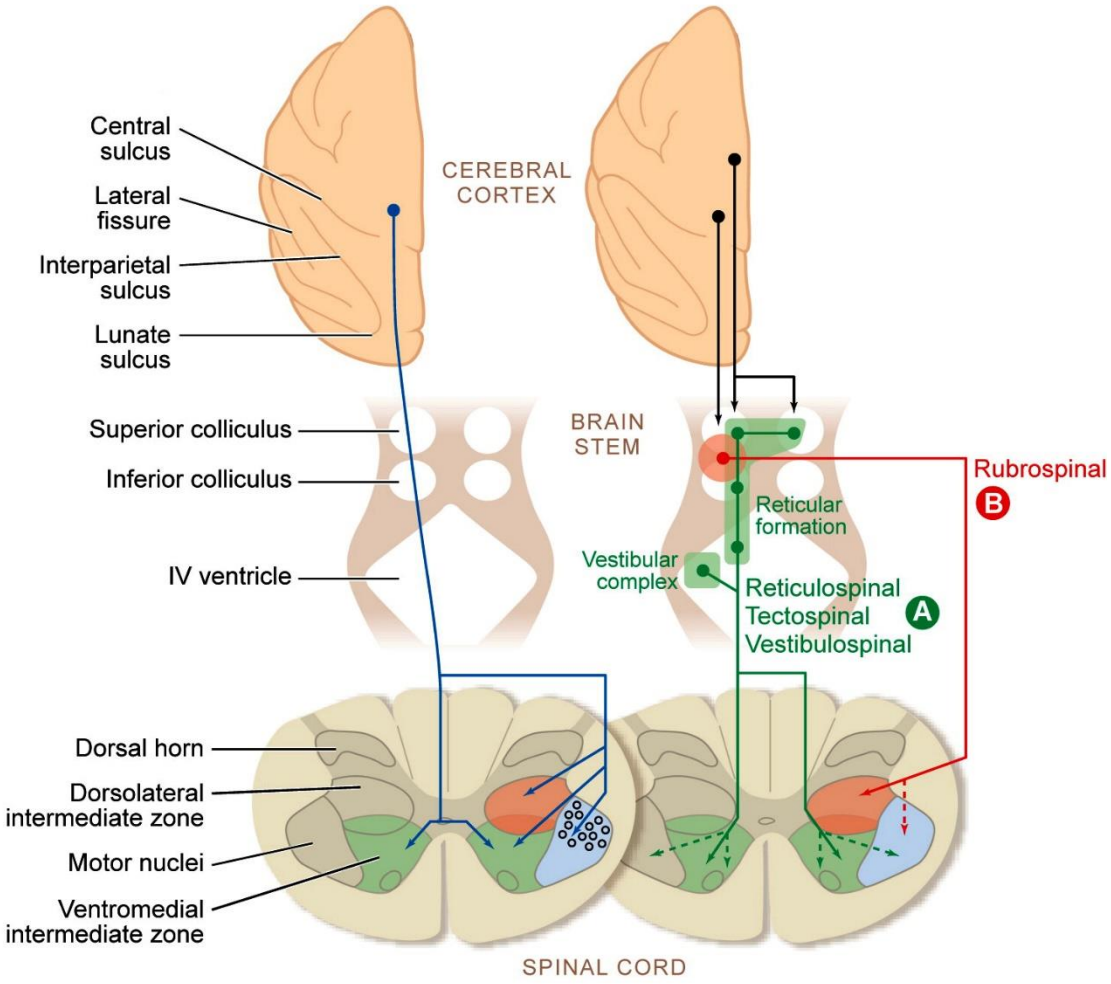


***Figure 1.1. Schematic representation of the descending tracts, their origin and termination.***
Corticospinal (on the left) and other major descending motor pathways (on the right); Figure modified from (Lemon, 2008).

While the largest proportion of the corticospinal tract arises from the primary motor cortex, it also gets contributions from supplementary motor area and somatosensory area (S1) (Murray and Coulter, 1981, Galea and Darian-Smith, 1994). Most of the corticospinal tract arising from the motor cortex (M1) and Supplementary Motor Area (SMA) send projections to the intermediate and ventral zone of the spinal cord. The corticospinal tract originating from S1 sends projections to the dorsal horn of the spinal cord and is known to control posture, gait, balance, spinal reflexes and hand functions. In an experiment, Lawrence and Kuypers (1968) observed that after surgical lesions of CST, rubrospinal tract took part in regaining locomotion and grasping function in macaques. However, a combined lesion of the corticospinal and rubrospinal tract in

these animals caused complete loss of grasp function, but some gripping function remained to allow them to climb. A possible explanation of this finding could be the surviving reticulospinal tract contributed substantially in performing the gripping function in the absence of corticospinal and rubrospinal tract. Current evidence suggests that the rubrospinal tract is absent in human (Nathan and Smith, 1955), which left the reticulospinal tract to play a major role in regaining hand function after recovery from the brain or corticospinal tract lesion in patients (Baker, 2011).

To find and compare the relative association and function of the reticulospinal tract in controlling distal and hand muscles, Riddle et al. (2009) carried out an experiment where they measured reticulospinal inputs to antidromically identified cervical ventral horn motoneurons projecting towards distal and intrinsic hand muscles in anaesthetized macaque monkeys. They performed intracellular recordings and found that motoneuronal projections towards distal hand muscles generated both mono and disynaptic excitatory postsynaptic potentials following stimulation to medial longitudinal fasciculus (reticular cells) in the brainstem region (Fig. 1.2). These findings suggest that the reticulospinal tract in primates not only contributes to controlling axial and proximal muscles but also govern some of the functions in distal hand muscles. In other words, it is possible that a parallel pathway of reticulospinal connections runs along the corticospinal tract in the spinal cord. Since upper limb motoneuron with projections towards forearm flexor muscles received short latency monosynaptic input from the reticulospinal connections, Riddle et al. (2009) suggested a potential role of the reticulospinal tract in recovery after lesions affecting the corticospinal tract.

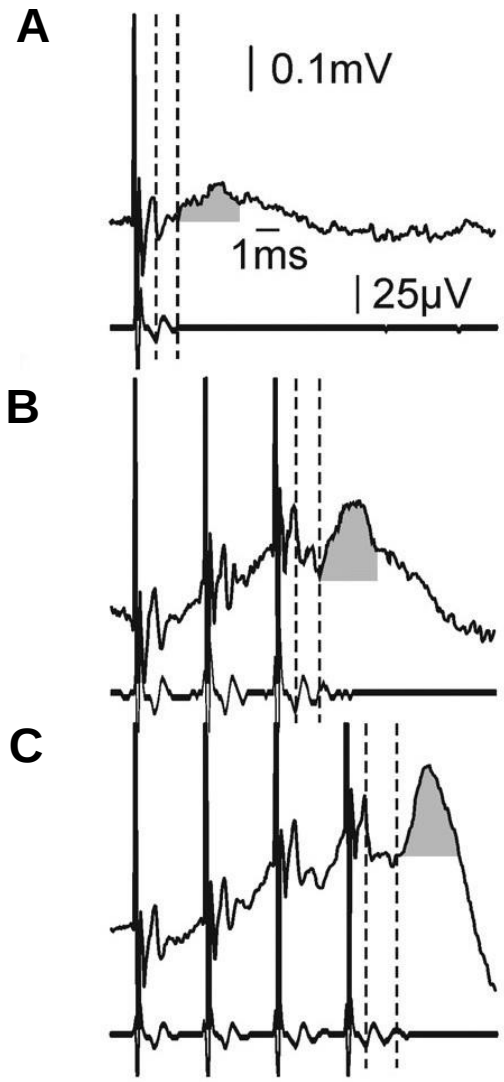


***Figure 1.2. Reticular activation: forearm flexor receives reticulospinal input.***
Intracellular recordings from cervical motoneurons projecting to forearm flexor following stimulation to the reticular cells. A, Reticulospinal excitatory post synaptic potential (EPSP) following single medial longitudinal fasciculus (MLF) stimulus (300 μA); B, Similar recordings following train of three stimuli; C, Similar recordings following train of four stimuli. Each panel shows averaged intracellular records (top) with simultaneously recorded epidural volleys below. Vertical dashed lines highlight the segmental latency of the response. Figure modified from (Riddle et al., 2009).

There is also evidence that connections from the reticulospinal and the corticospinal axons can converge into the same interneuron in the spinal cord providing overlapping effects in the motor control and function of the distal hand muscles (Riddle and Baker, 2010).

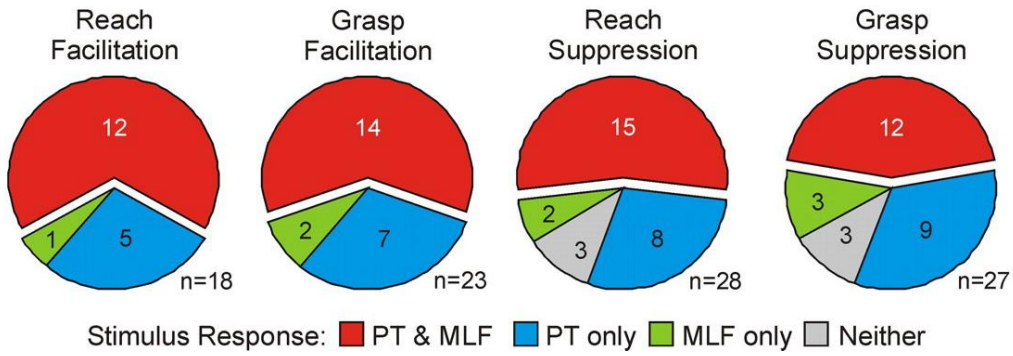


***Figure 1.3. Convergent and overlapping.***
Relative incidence of convergent corticospinal (PT, pyramidal tract) and brainstem inputs (MLF, medial longitudinal fasciculus) to spinal interneurons during reach and grasp task; based on cell numbers, the firing rate of which facilitated or suppressed during the reach or grasp phase. Figure from Riddle and Baker (2010)

This convergent and overlapping of corticospinal and reticulospinal inputs in the spinal motoneurons also denotes a key role of reticulospinal projections during recovery after some brain lesions, for instance, stroke in primary motor cortex or damage to corticospinal tract after spinal cord injury. Therefore, it is possible that subcortical extra-pyramidal connections, especially reticulospinal tract mediated projections affect reorganization and plasticity towards motor outputs at least to some extent if not extensively (Baker, 2011).

**What is the importance of nerve conduction speed in the plasticity inducing protocols?**

Specificity and success of a paired stimulation protocol modulating corticomotoneuronal excitability depend on the interstimulus interval.

Based on Hebb's initial postulate (Hebb, 1949), several studies demonstrated that long term potentiation (LTP) and long term depression (LTD) can be mediated by a mechanism called Spike-Timing Dependent Plasticity which relies on the temporal order and time-spacing of pre-synaptic and post-synaptic inputs (Song et al., 2000, Bi and Poo, 1998, Drew and Abbott, 2006). The connection can be potentiated if presynaptic spiking is preceded by postsynaptic spiking within a constrained window of

time (LTP), or it can be suppressed if the order of input is reversed (LTD) (Caporale and Dan, 2008, Levy and Steward, 1983, Markram et al., 1997, Ridding and Rothwell, 2007, Wolters et al., 2003). Inducing plasticity in the motoneuronal output exploiting this STDP mechanism by a paired stimulation protocol was extensively tested by in a number or previous studies (Stefan et al., 2000, Baranyi and Szente, 1987, Hess et al., 1996, Iriki et al., 1989)

Latencies of cortical response following an afferent input also depend on modalities of somatosensory receptors, characteristics of their nerve fiber and diameter, for example, thick fibers are faster than thin ones. In mammals, conduction velocity varies to a good extent in different fibers carrying afferent volleys; where motor fibers conduct slower than cutaneous fiber and muscle afferent is the fastest, but in humans those differences in conduction velocity in afferent fibers are much smaller than in animals. In human, Shefner and Logigian (1994) have found a conduction velocity of 57.6m/s in muscle afferent, 55.1m/s in cutaneous afferent and 56.3m/s in mixed nerve. Table 1 illustrates a brief summary of the conduction speed for various nerve fibers.

Table 1. Modality, type, diameter and conduction speed of different nerve fibers and their somatosensory receptors that can be activated using peripheral stimuli. These stimuli include electrical stimulation at nerve/motor point/skin, pressure, vibration or stroke (Kandel et al., 2000).

| Receptor | Fiber group | Fiber name | Modality | Diameter (μm) | Nerve conduction velocity (m/s) |
|---|---|---|---|---|---|
| Muscle and skeletal Mechanoreceptors: | | | | | |
| Muscle spindle primary | Aα | Ia | Muscle length and speed | 1-20 | 72–120 |
| Muscle spindle secondary | Aβ | II | Muscle stretch | 6-12 | 36–72 |
| Golgi tendon organ | Aα | Ib | Muscle contraction | 12-20 | 72–120 |
| Joint capsule receptors | Aβ | II | Joint angle | 6-12 | 36-72 |
| Stretch-sensitive free endings | Aδ | III | Excess stretch or force | 1-6 | 4–36 |
| Cutaneous and subcutaneous mechanoreceptors: | | | | | |
| Meissner corpuscle | Aα,β | RA1 | Stroking | 6-12 | 30-70 |
| Merkel disk receptor | Aα,β | SA1 | Pressure, texture | 6-12 | 30-70 |
| Pacinian corpuscle | Aα,β | RA2 | Vibration | 6-12 | 30-70 |
| Ruffini ending | Aα,β | SA2 | Skin stretch | 6-12 | 30-70 |

Interestingly, the difference of latencies in somatosensory potentials evoked by stimulating the whole peripheral nerve, muscle or cutaneous nerve close to wrist area is negligible, and latencies in the mentioned three categories of afferent inputs stay in the 19.0ms-20.3ms range (Gandevia et al., 1984). This observation supports the idea that afferent volley from muscle afferents reaches to the sensorimotor area via a similar route utilized by mixed afferent or whole nerve stimulation.

**Are there any limitations in the currently available plasticity protocols?**

There are many plasticity protocols that use non-invasive stimulation, but there are also limitations in the currently available approaches.

Most of the methods suffer from variability in test results, and even in healthy subjects, the evoked changes in brain's response can fluctuate to the extent that can limit its therapeutic specificity and sensitivity. A wide range of factors can affect changes in corticospinal or subcortical excitability; for example, age (Muller-Dahlhaus et al., 2008, Todd et al., 2010), sex (Tecchio et al., 2008), genetics (Cheeran et al., 2008), priming with another stimulation (Iyer et al., 2003, Nitsche et al., 2007, Ragert et al., 2009), prior voluntary motor activity (Stefan et al., 2006, Ziemann et al., 2004, Iezzi et al., 2008), parallel motor activity (Huang et al., 2008), drug (Wolters et al., 2003, Kuo et al., 2008, McDonnell et al., 2007b, Ziemann et al., 2002), aerobic exercise (Cirillo et al., 2009), diurnal variation (Sale et al., 2008) or attention (Stefan et al., 2004, Conte et al., 2007).

Almost all of the currently known stimulation protocols are applicable in the specialized laboratories, and in most of those procedures are not suitable to test a large number of healthy human subjects daily to design effective translational rehabilitative protocols. There is no standardized guideline of procedures or protocols to utilize plasticity inducing stimulation protocols in clinical settings to facilitate motor recovery. Number of studies with translational approach is also scarce.

Neuronal plasticity and adaptation processes are vastly complex to the extent that available studies and data set are unable to determine the exact nature, scopes and boundaries of sensory stimulation when utilized to reorganize the nervous system. More detailed knowledge about the human motor control, cortical and subcortical connections, and the spinal pathways are mandatory to develop sophisticated plasticity protocols. Despite recent advancement into the neuroscience, current knowledge in human motor control and connections lacks major key components required to design effective translational methods and calls for further research in this field.

**Why is it necessary to compare the effects of different plasticity protocols in different muscles?**

In a previous experiment, while studying the properties of descending brainstem pathways and their possible contribution in reorganizing motor outputs, Zaaimi et al. (2012) found that reticulospinal tract strengthens selectively to flexors and intrinsic hand muscles and not to extensor muscles in the upper limb. This was observed during recovery following corticospinal tract lesion in macaque monkeys. After six months of

the surgical lesion in the corticospinal tract, they stimulated ipsilateral and contralateral medial longitudinal fasciculus (MLF) and recorded elicited potentials from intracellular electrodes placed in 167 spinal motoneurons that innervated forearm and hand muscles. Then they compared it with the data recorded from 207 motor neurons in control monkeys. Interestingly, in the lesioned monkeys they found significant facilitation in monosynaptic excitatory post synaptic potential (EPSP) recorded from motoneurons projecting towards the forearm flexor and intrinsic hand muscles following MLF input. For flexors, contralateral response showed bigger changes than the response from the ipsilateral or lesioned side. In contrast, there was no significant change in ipsilateral or contralateral monosynaptic EPSP evoked from motoneurons controlling forearm extensors.

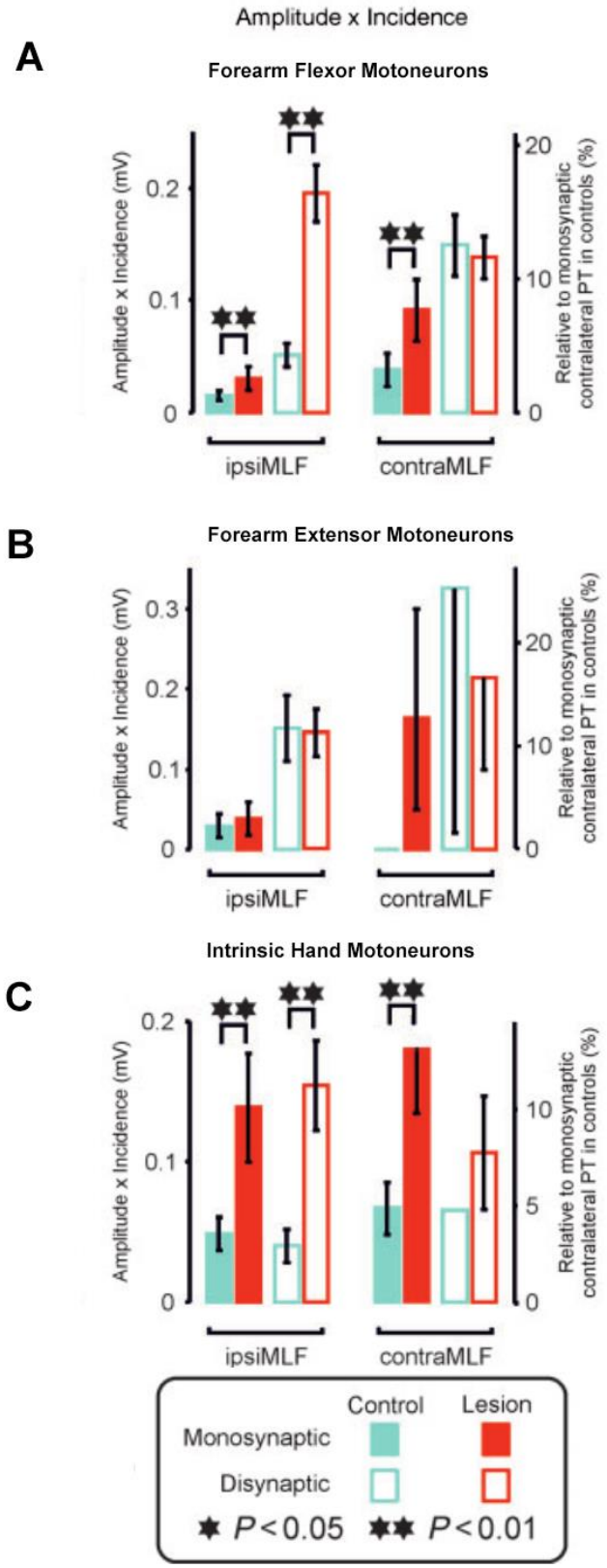


***Figure 1.4. Flexor-extensor bias.***
Facilitated connection towards flexor and intrinsic hand muscles as evidenced from quantitative measurement of input from MLF and recorded from respective

motoneurons. Measurements indicate the product of response amplitude and incidence. A, EPSP recorded from motoneurons innervated forearm flexor; B, EPSP recorded from motoneurons innervated forearm extensor and C, EPSP recorded from motoneurons innervated intrinsic hand muscles. Significant differences between control and lesioned animals (*P < 0.05; **P < 0.01) are marked with asterisks. Figure modified from Zaaimi et al. (2012).

They concluded that reticulospinal tract plays a significant role during recovery after corticospinal tract lesions and strengthened connections mostly towards forearm flexor and intrinsic hand muscles, but not in extensor (Zaaimi et al., 2012). These findings mirror the condition seen in post stroke patients, where they develop flexor spasticity and extensor weakness. The observed bias in strengthening or weakening different muscle groups differently during recovery in lesioned monkeys necessitates further research into the modalities of motor system plasticity in human.

## 1.3 Ways to influence plasticity in humans

### 1.3.1 Sensory Stimulation

Afferent or sensory stimuli are capable of changing excitation in the motor cortical output, which plays a critical role in the brain and spinal cord plasticity. The widely accepted Long Term Potentiation (LTP) and Long Term Depression mechanisms are regarded as the crucial element of the synaptic modification induced by sensory stimulation. Both LTP and LTD affect N-methyl-D-aspartate glutamate receptors to reinforce existing synapses, the formation of the new synapse or unmasking of excitatory amino acid receptors on motor neuron (RF?), triggered by repeated sensory afferent stimulation. Some afferent stimuli, e.g., electrical stimulation, sound, vibration and touch can thereby provide substrates for dynamic interactions between numerous cellular processing and synaptic modification resulting in altered excitability in the cortical sensorimotor area. Consequently, sensory stimulation enhances or suppresses corticomotoneuronal excitability to the downstream connections or body parts. Plenty of studies already suggested that sensory input is a crucial factor enhancing motor performance, learning new skills, recovery after brain or spinal cord lesion (Pearson,

2000, Pavlides et al., 1993, Hamdy and Rothwell, 1998). Some of the recent studies used peripheral nerve stimulation and motor point stimulation to explore the influence of sensory afferent input on the plasticity of motor cortical organization in healthy human (Ridding et al., 2000, McKay et al., 2002b). Sensory input is known to be a factor capable of inducing changes in the motor output by modulating subcortical and spinal interneurons, reflecting the role of sensory input in the modulation of motor reflex and behaviour (Perez et al., 2003).

Previously, a fair number of studies recorded evoked cerebral potentials by stimulating selective motor points (Macefield et al., 1989, Burke and Gandevia, 1988, Abbruzzese et al., 1985). Afferent stimulation, e.g., stimulating a muscle on motor point can send volley towards thalamus (Morioka et al., 1989, Celesia, 1979, Fukushima et al., 1976, Katayama and Tsubokawa, 1987, Suzuki and Mayanagi, 1984). Fast conducting group Ia afferent fibers play a role in relaying afferent volley from motor point stimulation towards thalamus (Fukuda et al., 2000). It is already evident from previous studies that thalamus works as a central relay station with multiple and parallel projections towards sensorimotor and motor cortex. Primary somatosensory cortex sends direct inputs primarily to upper layers of pyramidal neurons of M1 (Kaneko et al., 2000, Hoffer et al., 2003), and also via sensory thalamus (medial subdivision of posterior nucleus, ventrolateral nucleus) (Deschenes et al., 1998, Ohno et al., 2012) and motor thalamus (anteromedial, ventral anterior and ventrolateral nucleus) (Asanuma et al., 1979, Asanuma et al., 1980, Hooks et al., 2013). The ventral intermediate nucleus of thalamus sits between the sensory posterior thalamus and the motor ventrolateral nucleus of the thalamus, possibly receiving and sending fibers to both somatosensory and motor cortex. Projections from sensory thalamus reach mostly to superficial layers in M1 (Mao et al., 2011), whereas inputs from motor thalamus target both superficial layers and L5 in M1 (Herkenham, 1980, Hooks et al., 2013). Overall, thalamus has a considerable contribution in relaying sensory afferent inputs to primary motor areas which also receives inputs from frontal cortex, parietal cortex and primary somatosensory cortex (S1) (Hoffer et al., 2003, Hooks et al., 2013). Additionally, motor point stimulation can send afferent volley to sensory motor area in a similar way that a direct stimulation to a peripheral nerve can achieve.

Sensory input from auditory stimulation is known to increase motor cortical excitability when paired with TMS (Sowman et al., 2014). This describes the existing functional

association between auditory pathways and plasticity in sensorimotor representations. Interestingly, sound can also change excitability in the reticular area. This accidental finding was discovered in a previous study when Fisher et al. (2012) was recording discharge from single units in the pontomedullary reticular formation (PMRF) while stimulating motor cortex (M1) with TMS in anaesthetized macaque. PMRF is known to give rise to axons that later form the reticulospinal tract in the spinal cord (Sakai et al., 2009). In their experiment, it was observed that TMS discharge over M1 elicited short latency response in PMRF cells. This reflected simultaneous activation of cortico-reticular connections since PMRF receives diverse projections from corticomotoneuronal pools including non-primary motor area, supplementary motor area and region F5 (Fisher et al., 2012, Keizer and Kuypers, 1989, Borra et al., 2010). They reported that a long latency response, recorded from the same units, was activated by brief low-intensity sound stimuli; and argued that cochlear and vestibular receptors might contribute to generating action potentials in the reticular cells mediated via relatively unexplored connections. This may include oligospinal and other projections towards the reticular area from higher or surrounding neuronal circuits related to subconscious awareness, or even startle reflex (Davis et al., 1982). Figure 1.5 shows the recorded response from PMRF reticular cells activated by sound of various intensity. This observation of reticular activation by auditory stimulation was utilized to design spike-timing dependent stimulation protocols for intervention in one of my studies, where the auditory stimulation was paired with the electrical stimulation delivered over the motor point of biceps brachii muscle.

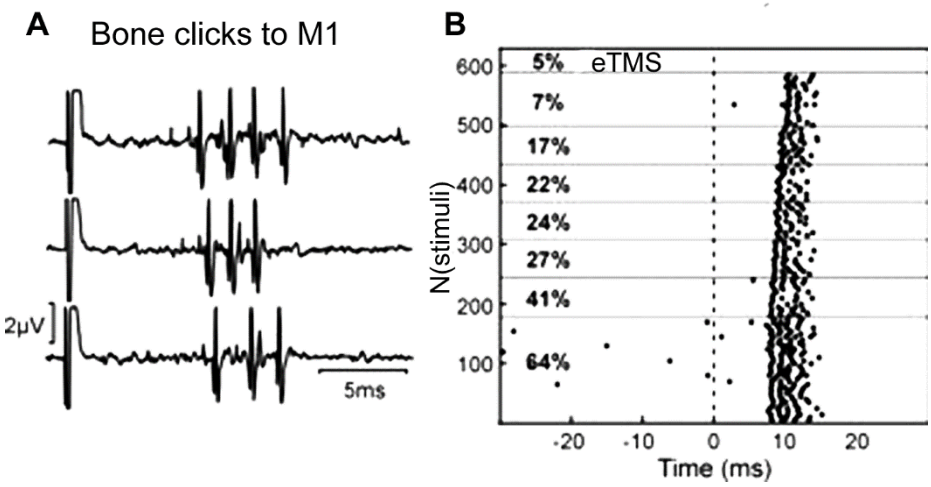


***Figure 1.5. Response evoked from PMRF reticular cell with click stimulation.***

A, sweep responses from single cell. B, responses at different click intensity. eTMS% is the intensity of TMS that produced comparable sound used in this test. Figure from (Fisher et al., 2012)

### 1.3.2 Paired Associative Stimulation (PAS)

PAS is known to induce plasticity in the human motor cortex projections and this process primarily relies on the basic principles of Hebbian's model of neural plasticity. In the original study of PAS, Stefan et al. (2000) utilized a single pulse electrical stimulus to stimulate a peripheral nerve followed by a magnetic stimulation delivered to the contralateral primary motor cortex. In this case, the forearm median nerve near the wrist joint was stimulated to send a presynaptic input arising from the afferent stimulation to the primary motor cortex prior activation of sensory motor cortex with direct magnetic stimulation. Repeated application of the paired stimuli with an interstimulus interval of 25ms and generated from two sources, i.e., electrical stimulation to nerve and magnetic stimulation to motor cortex, over an extended period increased the excitability in the corticomotoneuronal projections. The interstimulus interval between the paired stimuli was adjusted such that the afferent volley arising from the peripheral nerve reaches the motor cortex to be merged with the magnetic stimulation.

In the original description of PAS by Stefan et al. (2000) a paired stimulation of the median nerve at the wrist with Transcranial Magnetic Stimulation (TMS) was delivered to the contralateral motor cortex. In my study, I instead stimulated the motor point of forearm flexor or extensor muscle with superficial electrodes. When working with the intrinsic hand muscles, direct nerve stimulation has several advantages, which were described in Stefan et al. (2000). The nerve at the wrist is in a convenient location for stimulation; for the median nerve, only muscles of the thenar eminence are supplied, so that activation will be focussed to an anatomically-defined muscle group. By contrast, for the forearm flexors or extensors, the relevant nerves (median or radial at the arm) can be relatively deep, such that small movements of the stimulating electrode can generate large changes in efficacy over the extended duration of a plasticity protocol.

Additionally, each nerve innervates multiple muscles (for the median nerve, this includes muscles in both forearm and hand), together with important cutaneous fields. On the other hand, applying stimulation to the motor point of a muscle can be more

consistent and less cumbersome than stimulating a nerve. It is known that motor point stimulation activates large diameter afferent fibers (Bergquist et al., 2011a), and hence should produce a well synchronized afferent volley. Previous studies have used paired motor point stimulation of two muscles to induce a different form of associative plasticity (Ridding and Uy, 2003). In one of my studies, I tested the effect of pairing motor point stimulation with TMS with a view to induce plasticity based on the PAS principals, but more conveniently and effectively.

TMS is a neuro-stimulation and neuro-modulation technique that has a long history of being used as a method of non-invasive stimulation in neuroscience research (Horvath et al., 2011). TMS is also widely used in clinical areas. The basic principles of magnetic stimulation were first discovered by the English physicist Michael Faraday back in 1881. Generally, the TMS device consists of a central unit which stores and discharges energy in terms of electric pulses and a stimulating coil connected to the central unit. The stimulating coil is held over the subject's head and a pulse of current flowing through the coiled wires creates a strong and focal magnetic field (Rossini et al., 1994). As the skull provides very little resistance, this electromagnetic field can reach the brain tissue without major attenuation. By creating a sudden change in the magnitude of the magnetic field, this coil placed overhead is capable of generating electric current in a nearby conductive field, in this case, in brain tissues to effectively depolarise neurons. TMS can be applied as a single pulse (lasting about 100 μs) or as a series of pulses depending on therapeutic or research protocols (Nahas, 2003). Single pulse TMS is regarded as very safe when applied to healthy individuals, whereas series pulse or repetitive TMS with a frequency up to 50 Hz is regarded as safe for healthy humans, although higher frequency at very high intensity poises potential risks of inducing seizures (Pascual-Leone et al., 2000).

Depending on the orientation of the coil and focal electromagnetic field, TMS is believed to be capable of activating the corticospinal tract that receives input from the motor cortex, somatosensory and premotor areas in the brain (Barker et al., 1985, Edgley et al., 1990).

### 1.3.3 Repetitive Transcranial Magnetic Stimulation (rTMS)

It is known that high-frequency rTMS can affect cortical excitability and induces plasticity in the motor output, which can be measured and quantified by recording motor evoked potential (MEP) from the activated muscle (Ridding and Ziemann, 2010). Previously, several studies showed a modulatory effect of rTMS application on the cortical output, but in most cases, the effects were short lasting or inconsistent (Maeda et al., 2000, Hadland et al., 2001, Evers et al., 2001). In a more refined approach, Huang et al. (2005) tested an intermittent theta burst protocol (iTBS) which was delivered over a period of only 190s. This iTBS produced a consistent and lasting effect on cortical plasticity and resulted in significantly larger MEPs after the intervention and the effect lasted for at least 60 minutes after iTBS (Fig. 1.6). They also measured short interval intracortical inhibition and intracortical facilitation and both were affected after the application of rTMS (iTBS). Conversely, spinal H-reflexes were unaffected. From this observation, they concluded that the modulation took place in the motor cortex.

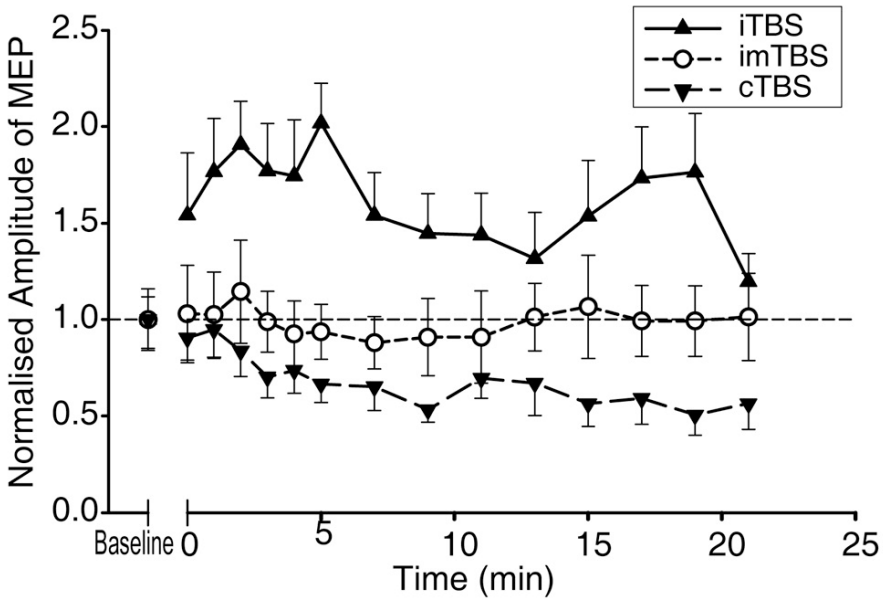


***Figure 1.6. rTMS increases MEP.***

Observed changes in MEP amplitude over time after conditioning with continuous TBS (cTBS), intermittent TBS (iTBS), and intermediate TBS (imTBS). MEP size increased significantly following iTBS which lasted for about 15 minutes, while cTBS caused a significant reduction in MEP amplitude (Huang et al., 2005); TBS, theta burst stimulation. iTBS is a modified protocol of rTMS.

In one of my studies, I used a slightly modified version of the similar protocol to measure and compare changes in cortical excitability for forearm flexor vs. extensor muscles and distal hand muscles.

### 1.3.4 Motor Imagery (MI)

Motor Imagery (MI), or motor imagination, can affect cortico-motoneuronal excitability considerably. MI activates the motor cortex, the premotor cortex and the supplementary motor area as evidenced by fMRI studies (Lotze et al., 1999) (Figure 1.7).

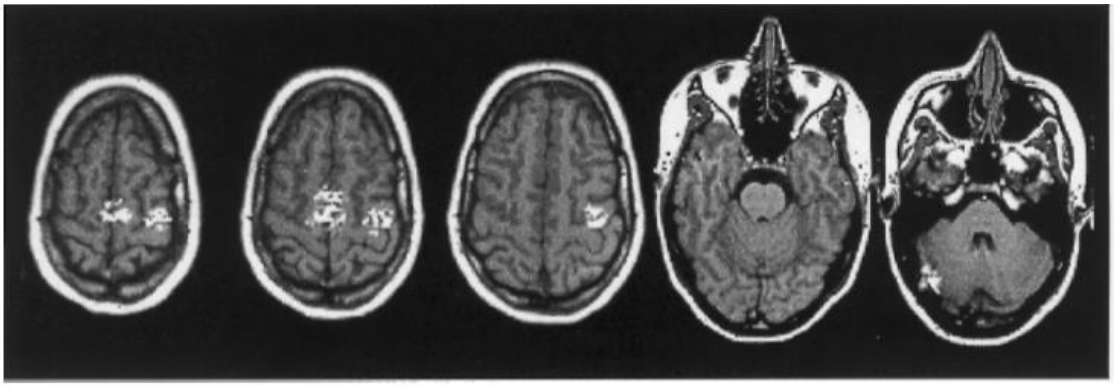

***Figure 1.7. Motor imagery activates primary motor cortex.***
fMRI showing activation of anatomically selected brain regions that include the motor cortex, the premotor cortex and the supplementary motor areas during motor imagery; from Lotze et al. (1999).

Subthreshold activation of motor and sensory-motorneuron pools by MI was also evidenced by many other previous studies (Beyer et al., 1990, Reynolds et al., 2015, Lotze and Halsband, 2006, Jeannerod, 2010). The origin of MI does not evoke enough downward volley towards spinal motor neurons to perform an actual movement, but it instead initiates a central processing mechanism that prepares some neuronal pools in the somatosensory, premotor and motor cortex for the planned action. Therefore, it is theoretically and practically possible to change motor cortical excitability using MI paired or synchronized with other sensory inputs (Villiger et al., 2013, Helm et al., 2015), specialized training protocols (Kaiser et al., 2014), TMS or transcranial Direct Current Stimulation (tDCS) (Lebon et al., 2012, Abbruzzese et al., 1996).

An important observation from Fadiga et al. (1998) should be mentioned here. They found considerably higher MEP amplitude than control in the electromyography (EMG) responses recorded from the flexor muscle during flexion imagination, but MEP amplitudes in the same muscle during extension imagination did not change much (Fig. 1.8) (Fadiga et al., 1998).

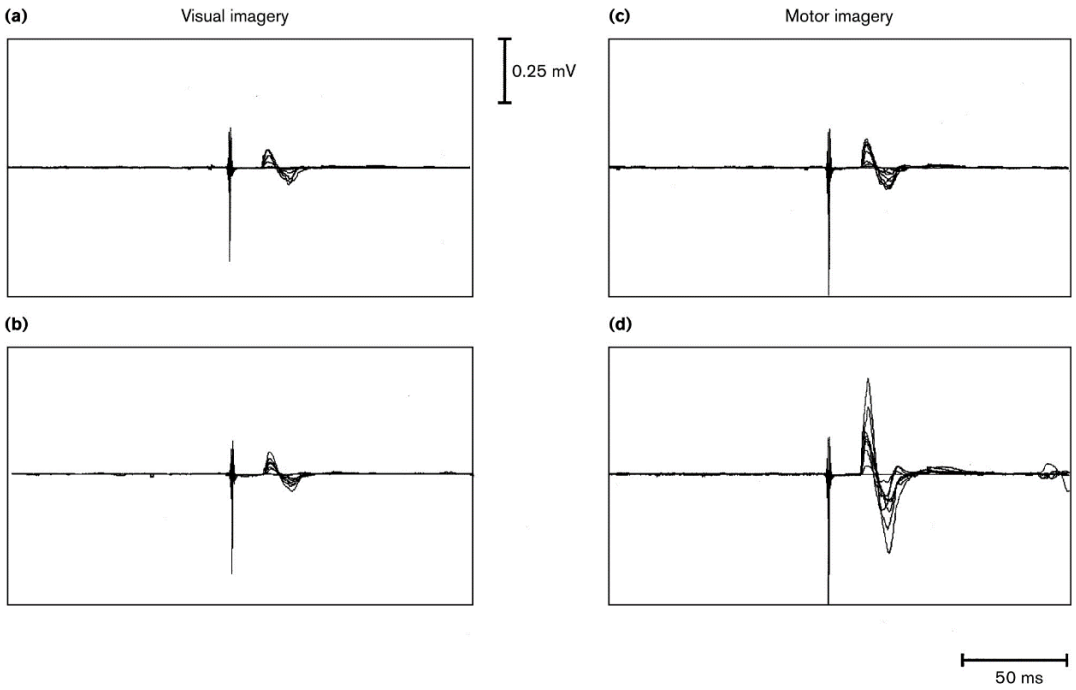


***Figure 1.8. Motor imagery increases MEP.***
TMS evoked MEP recorded from the flexor muscle (biceps brachialis) while subjects were instructed to imagine flexion-extension movement with their right arm and TMS was applied to the left motor cortex. (a,b) Control experiment, where TMS was applied during visual imagery of expanding and shrinking bar respectively. (c) Flexor MEPs recorded following TMS applied during the extension phase of the motor imagery. (d) MEPs recorded following TMS during the flexion phase. Figure from (Fadiga et al., 1998)

There is also evidence showing that precisely timed afferent input generated by peripheral nerve stimulation and concomitant MI can induce plasticity in motor cortex (Mrachacz-Kersting et al., 2012). TMS during MI can increase cortical or subcortical excitability. Evidence of increased MEP amplitude during MI (Jeannerod and Frak, 1999) also suggests that input from an artificial stimulation, e.g., TMS, can be merged with the natural substrates generated by MI to modify corticomotor excitability. My

hypothesis to induce plasticity in the motor system using simultaneous MI and TMS relies on this ground to some extent.

# 1.4 Ways to Record Influence of Change in Plasticity

## 1.4.1 Long Latency Stretch Reflex

Stretch reflex, also called the myotatic reflex, is a response of the spinal motoneuron when a sudden stretch is applied to related muscle spindle. Based on the latency of onset, a typical stretch reflex of the upper arm can be further divided into short latency stretch reflex (SLSR) and long latency stretch reflex (LLSR) (Figure 1.9). The short latency reflex starts within 20ms of an applied stretch; it is monosynaptic in nature and connected with spinal motoneuron through Ia fiber (Matthews, 1991a, Pruszynski et al., 2011b). On the other hand, LLSR appears approximately 50ms after triggering the stretch.

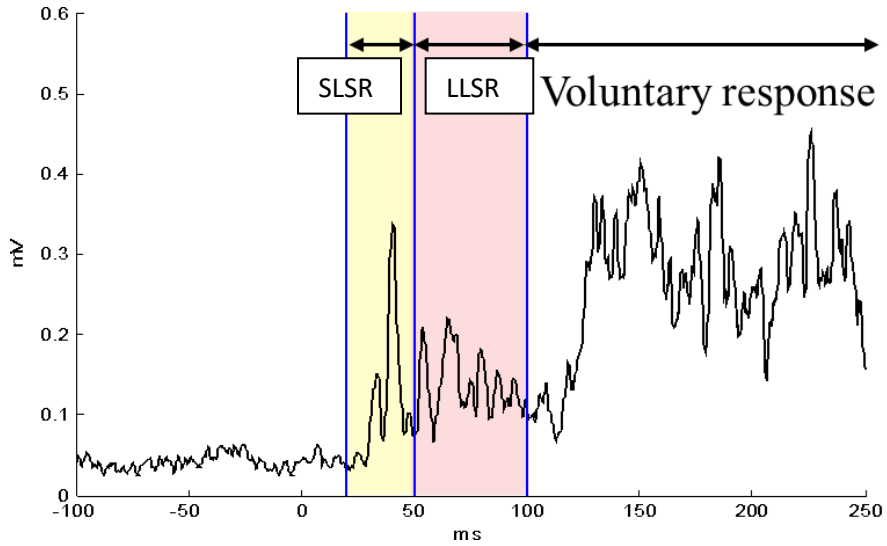


***Figure 1.9. The components of a stretch reflex.***
Raw tracing from rectified EMG recorded from the biceps muscle following a mechanical perturbation. SLSR, corresponds the short latency response with a latency of ~20ms. LLSR, corresponds the long latency response with a latency of ~50ms. The spikes after 100ms show voluntary muscle activity. The onset of stretch is at 0ms.

LLSR is believed to involve transcortical pathways for distal muscles, but its contributions are still controversial for proximal muscles (Matthews, 1991). However, a few studies have established that LLSR is affected by multiple connections including transcortical and subcortical pathways (Petersen et al., 1998, Pruszynski et al., 2011b).

EMG activity with a latency greater than 100ms is usually considered as voluntary response.

In a previous study, Soteropoulos et al. (2012) showed that stretch reflex, which activates hand muscles after a triggering stimulus, can also activate reticular formation cells in the macaques (Fig. 1.10). In their study, after application of stretch to index finger comparable responses with latencies consistent with SLSR and LLSR could be recorded from the pontomedullary reticular formation which gives rise to reticulospinal tract (Soteropoulos et al., 2012) (figure 26). Reticulospinal Tract (RT) originating from pontomedullary reticular formation controls the axial and proximal muscles to maintain balance and gait. The reticulospinal tract has monosynaptic and disynaptic projections to distal muscles of the upper limb, and it plays an important role during recovery from the motor cortex and corticospinal tract lesion (Davidson and Buford, 2006, Riddle et al., 2009, Lemon, 1993). These observations from previous studies suggest that stretch reflex, particularly the LLSR component, can be utilized to measure outputs corticomotoneuronal and reticulospinal tract output. Any changes in the LLSR following the delivery of appropriate plasticity-inducing interventions should reflect the influence of plasticity on the motor cortex, subcortical or reticulospinal pathways.

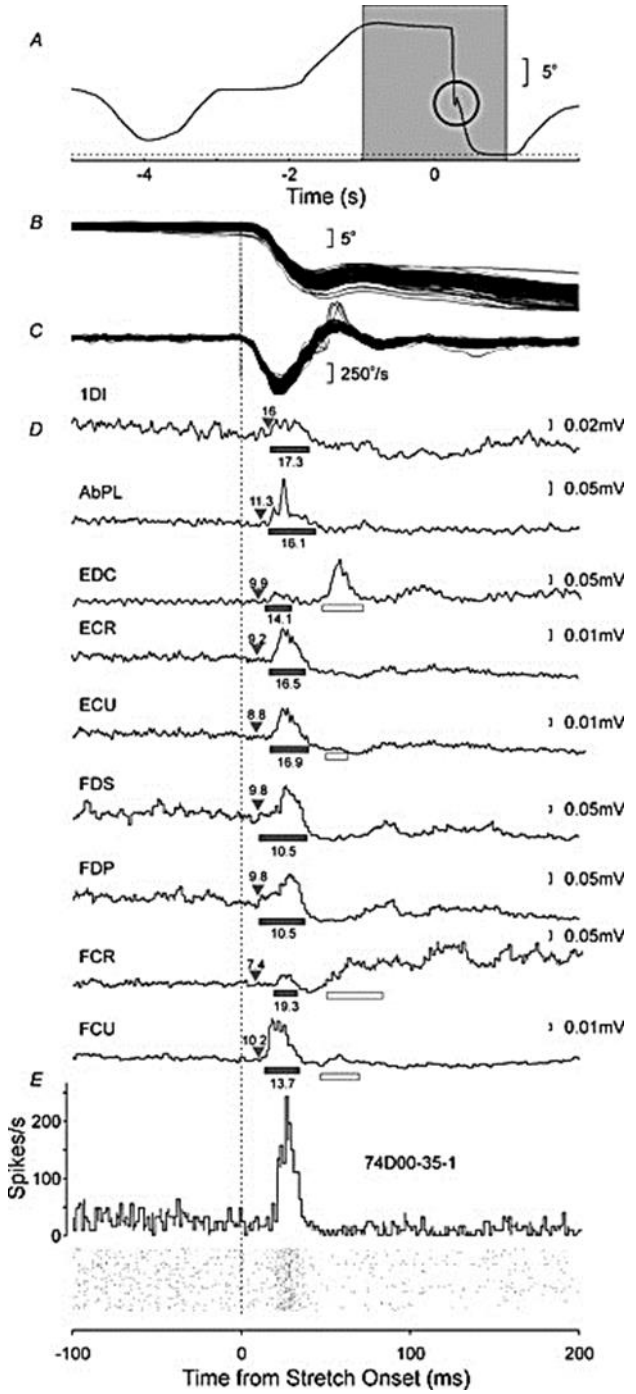


***Figure 1.10. Activation of reticular cells in macaque.***
A, averaged displacement of lever. B, overlain traces of lever displacement of single trials. C, computed velocity. D, mean rectified EMG responses from several forearm and hand muscles with activity aligned to the onset of stretch (dotted vertical line). The responses in the muscles correspond to SLSR and LLSR responses. Grey triangles indicate minimal onset latency, white and grey bars indicate early and late responses respectively. E, peri-stimulus time histogram and raster of pontomedullary reticular formation cell, showing a reticular response to stretch. Plots (B-E) are on the same time scale. Figure modified from Soteropoulos et al. (2012).

### 1.4.2 Motor Evoked Potentials (MEPs)

In most of the non-invasive brain stimulation techniques that use Transcranial Magnetic Stimulation (TMS), action potentials (generated from activated skeletal muscle after stimulation) are measured by placing surface electrodes directly over the skin. These responses are known as motor evoked potential (MEP), which arrive through fast conducting cortico-motoneuronal connections via alpha motoneurons in the contralateral spinal cord (Abbruzzese and Trompetto, 2017). TMS mostly activates axons that stay parallel to this electric field. Activation of motor cortex occurs when axonal potential changes at some point across its length, that runs parallel with the induced electric field. Changes in the electric field in the extracellular substances due to variation in their conductivity can also produce activation of an axon or can induce differences at nerve membranes near synapses (Rothwell, 1997). These responses of motor cortex evoked by a magnetic field can be readily and non-invasively recorded from corresponding muscles by placing surface electrodes directly over the skin. The evoked EMG responses can be recorded, rectified and quantified by measuring peak to peak amplitudes, which can be useful to compare effects of various plasticity protocols.

Interestingly, the application of single pulse TMS or repetitive TMS (rTMS) in the primary motor cortex (M1) elicits several volleys in the cortico-motoneuron pools. Looking at the characteristic features of latency in a typical MEP, an early D-wave and later I-waves can easily be differentiated. Early D-waves are mostly generated from axonal activation of corticospinal neurons, whereas late I-waves originates from activation of mono and polysynaptic inputs (Ridding and Ziemann, 2010). Changes in the MEP amplitude are regarded as a measurable marker or index of changes in cortico-motoneuronal excitability. Interestingly, although the short latency of EMG responses and short central motor conduction time from M1 to spinal motorneurons generally indicates a mono-synaptic downward volley through fast conducting corticospinal tract, disynaptic or oligosynaptic contribution mediated by cortical or subcortical projections to propriospinal connections or interneurons in the spinal cord cannot be excluded (Rothwell, 1997, Mazevet et al., 1996, Burke et al., 1992, Gracies et al., 1994).

The orientation of the TMS coil can influence the pattern and latency of responses by preferentially stimulating cortico-motoneuronal pools. Therefore, MEP outputs from differently oriented coil can be different. It has been found that latero-medially oriented coil evokes short latency D-waves, posterior-anteriorly placed coil recruits long latency I-waves and anterior-posterior orientation is likely to elicit later I-waves with the longest latency (Volz et al., 2015). In all my experiments, the coil was positioned tangentially over the skull while the handle was pointing backward and laterally producing a 45$^{o}$ angle to the sagittal plane, consistent with original PAS studies (Rossini et al., 1994, Stefan et al., 2000). This standard orientation evoked largest potentials and recruited D- and I-waves (Rothwell, 1997).

Di Lazzaro et al. (2001) studied the effect of TMS on human motor cortex by recordings descending volleys with bipolar electrodes placed directly into the cervical epidural space. Simultaneously, they recorded surface EMG from the first dorsal interosseous muscle (FDI) while stimulating primary motor cortex (M1) with TMS. They compared the MEPs induced by TMS during resting and active voluntary contraction phases of the muscle and found that TMS increased the output of motor neurons in the spinal cord during voluntary contraction. They concluded with the notion that prior activation of motoneurons by voluntary effort before cortical stimulation could change subcortical excitability, resulting in increased MEP amplitude (Di Lazzaro et al., 2001, Di Lazzaro et al., 1998). Their study is indicative of a possible scenario where TMS not only activates the M1 but also sends downward volleys via other subcortical projections and pathways that run in parallel with the corticospinal tract and thereby excites spinal motoneurons.

## 1.5 How these approaches may ultimately lead to patient benefit

### 1.5.1 Stroke, Spinal cord injury and disability

It has been estimated that the total number of stroke events in the European Union will rise from 613,148 in 2015 to 819,771 in 2035 (Stevens et al., 2017). According to a report by the World Health Organization, every year between 250,000 and 500,000

people suffer a spinal cord injury worldwide (Organization and Society, 2013). The increasing number of stroke and spinal cord injury patients has already left the world with a massive burden including expense. The current total cost of post stroke care and rehabilitation for patients in the United Kingdom only is calculated as £18 billion over the first five years following stroke (Xu et al., 2017). The condition is even worse and complicated in the underdeveloped and developing country because of lack of clinical therapy, social care, and proper rehabilitation programmes. However, regardless of the region, with currently available measures and therapies, it is nearly impossible to recover full functionality after certain types of brain or spinal cord lesions. This has motivated me to study plasticity in the human motor system, which has been an emerging field in the neuroscience. My goal was to test and implement advanced and novel approaches to influence plasticity in the motor system and to utilize the acquired knowledge for the development of translational therapies. If successful, many patients with stroke and spinal cord injury can be benefited with the recovery of the hand function.

### 1.5.2 The possibility of utilizing plasticity to treat disability in the post-stroke and spinal cord injury patients

Under normal physiological circumstances, a prolonged recovery process typically kicks in after a brain lesion or spinal cord injury. This process is believed to rely on the reorganization of the spared pathways and surrounding neuronal networks. Synaptic plasticity is an integrated and vital component of this reorganization as it strengthens or weakens components of existing pathways, changes excitability of the neuronal circuits and can re-route action potentials by collateral sprouting of the nerve fibers. In the course of recovery, axonal changes and plastic remodelling can occur above and below the lesion, i.e., in cortical and subcortical components within the brain, within the brainstem or within the spinal cord pathways and interneurons. Certain brain and spinal cord lesions leave a large motor and sensory deficits that usually persists throughout life. This disruption of nerve fiber and pathways can result in paraplegia, hemiplegia or quadriplegia and often causes pronounced sensorimotor dysfunction ultimately leading to the development of spasticity (Raineteau and Schwab, 2001). Muscle spasticity, usually accompanied by the weakness of its antagonist muscle, can severely restrict the

range of motion, can cause uncontrolled movement and development of contractures in joints, leading to major disability.

Over the decades, the debilitating factors of brain and spinal cord lesion have prompted many researchers attempting to facilitate functional recovery by enhancing plasticity mediated reorganization. Mainly, plasticity protocols relying on external non-invasive stimulation can be extremely beneficial and suitable since they not only allow extensive control over experimental conditions but also make it possible to test numerous paradigms on a large number of healthy individuals. Human models of brain and spinal cord plasticity from my studies may contribute further information to understand the basic principles of cortical and subcortical remodelling and reorganization following artificial stimulation. This knowledge can be useful to develop new treatment strategies and rehabilitation therapies using non-invasive stimulation protocols capable of inducing plasticity to reorganize neuronal circuits in cortical or subcortical level after a lesion. In my research, I emphasized on testing a variety of plasticity inducing protocols that can be easily delivered via non-invasive, currently available and cost effective yet safe techniques. For instances, one of my studies looked into delivering a specially designed stimulation protocol via a wearable electronic device non-invasively, which a participant can easily wear for a prolonged duration continuing daily activities. Acquired results from this study can provide further knowledge and information to develop more effective ways of delivering such stimulation protocols capable of modifying functional outcome during recovery. In another study, I approached to evaluate the basic mechanism of plasticity in different groups of forearm and hand muscles, where I prompted to test modified methods of providing paired stimuli protocols, which can be easily applicable to subjects on a daily basis. Analysis and results from this basic study can provide important aspects of central nervous system plasticity to assess the feasibility of using such techniques as translational therapy in clinical settings. I also introduced a novel method of utilizing motor imagery as an input and its effect in modulating cortical or subcortical plasticity was observed. The outcome from this research can lead to develop practical strategies of delivering effective plasticity inducing protocols through patients' ability to think a movement where actual motion is compromised after brain or spinal cord lesion.

# Chapter II

# SPIKE-TIMING DEPENDENT PLASTICITY IN THE LONG LATENCY STRETCH REFLEX FOLLOWING PAIRED STIMULATION FROM A WEARABLE ELECTRONIC DEVICE

## 2.1 Introduction

The reticulospinal tract is a major descending motor pathway, which is typically considered to convey commands for gross motor function such as maintaining posture, locomotion and reaching movements (Matsuyama and Drew, 2000, Prentice and Drew, 2001, Dyson et al., 2014, Buford and Davidson, 2004, Davidson and Buford, 2006). Recent work in primates has shown that the reticulospinal tract may also contribute to hand function, in parallel to the more prominent corticospinal tract (Riddle et al., 2009, Soteropoulos et al., 2012, Baker, 2011, Riddle and Baker, 2010). Following corticospinal damage, connections from the reticulospinal tract to motoneurons controlling the upper limb strengthen selectively to flexors (Zaaimi et al., 2012). This probably underlies the selective recovery of function seen after stroke or spinal cord injury, when extensors remain weak, but flexors regain strength, sometimes even to the extent of unhelpful spasticity.

These observations motivate the search for principled interventions to modify reticulospinal connections, which could enhance functional recovery after lesion. Previous work has induced plastic changes in the cortex by consistently pairing stimuli which act on a common circuit. By the principles of spike-timing dependent plasticity, if a post-synaptic neuron is activated consistently after a pre-synaptic input, that input is strengthened; reversing this timing weakens the input (Markram et al., 1997). Plasticity protocols can use pairs of stimuli delivered using microelectrodes (Bi and Poo, 2001), or time one stimulus at a fixed delay after spontaneous neural spikes (Jackson et al., 2006). It is also possible to induce plasticity using non-invasive stimuli. Example paradigms targeting the cortex pair transcranial magnetic brain stimulation with peripheral nerve stimulation (Stefan et al., 2000), or stimulation of the motor points of

two hand muscles (Ridding and Uy, 2003). To date, no reports have attempted to induce plasticity in reticulospinal pathways.

An essential pre-requisite to induce spike-timing dependent plasticity is to find two stimuli which converge on a common target circuit. It is well known that the brainstem nuclei which give rise to the reticulospinal tract receive extensive afferent input (Leiras et al., 2010); electrical stimulation in the periphery will thus generate robust synaptic input. It has been recently demonstrated that primate reticular neurons fire bursts of action potentials after loud auditory clicks (Fisher et al., 2012). I therefore hypothesised that precisely-timed pairing of peripheral shocks with clicks may lead to plasticity in reticulospinal circuits.

Experimental study of plasticity finally requires a way to measure any changes. For the corticospinal system, motor evoked potentials following transcranial magnetic brain stimulation are typically used to assay connectivity. Similarly, unambiguous non-invasive measures of reticulospinal function are not available. One option may to be assess the long-latency stretch reflex (LLSR). For distal muscles acting on the digits or wrist, the LLSR appears to have a substantial component passing via the primary motor cortex and the corticospinal tract (Matthews et al., 1990, Cheney and Fetz, 1984), although even the LLSR in finger muscles has a reticulospinal contribution (Soteropoulos et al., 2012). For muscles acting on the elbow and shoulder, although there is undoubtedly a corticospinal contribution (Pruszynski et al., 2011a, Evarts and Tanji, 1976), there is evidence that this is reduced compared to more distal muscles (Fellows et al., 1996) and that there may also be a sub-cortical component (Kimura et al., 2006). I therefore hypothesised that LLSR in a more proximal muscle might partially measure reticulospinal output (Kurtzer, 2014), and that paired stimuli targeted to induce plasticity in reticulospinal pathways might modify the LLSR.

To date, most experiments on synaptic plasticity have paired stimuli for only short periods, working within the confines of a laboratory setting. Whilst changes may be induced, they typically fade after around an hour. I wished instead to develop protocols which could be applied for many hours while the subject went about their normal daily activities. To this end, I utilized a wearable electronic device, capable of delivering the required stimuli in a portable system. In this study, I describe successful induction of

plasticity using this device measured as a change in LLSR, which may partially reflect spike timing dependent processes within the brainstem.

## 2.2 Materials and Methods

Results were obtained from 74 healthy volunteers (22 male) aged 19-84 years over 89 experiments. All procedures were approved by the local ethical committee of Newcastle University Medical School, and full written consent was obtained from each participant.

### 2.2.1 Measurement of Stretch Reflex

Subjects were seated in a rigid chair, fitted with a five-point harness to prevent trunk and shoulder movements. Electromyogram (EMG) was recorded from the biceps and brachioradialis muscles of the right arm, using adhesive surface electrodes (Kendall H59P for brachioradialis and Kendall H91SSG for biceps) placed over the muscle belly with an interelectrode spacing of 2 cm for brachioradialis and 3-5 cm for biceps. Electrodes were connected to a Digitimer D360 amplifier (gain 1000, bandpass 30 Hz – 2 kHz). The arm was fitted into a robotic device which measured elbow flexion angle, and could generate torques around the elbow using a powerful motor (Maxon part number 353301, with 25:1 planetary gearhead and a further 1.6:1 reduction ratio generated by the gear wheels and belt drive linking motor to drive shaft). The forearm was partially pronated, and the shoulder was flexed at 45º and abducted at 90º (Fig. 2.1A). Subjects were instructed to maintain a 90º flexion against a background torque, by moving a cursor related to elbow angle into a target displayed on a computer screen. With a delay of 1.5-2 s after the target was acquired (chosen at random from a uniform distribution) the motor torque increased to a high level (66 Nm) for 150-200 ms, generating an elbow extension movement with a near-constant velocity of 150-300º/s. This was a little lower than the expected velocity obtained by taking the motor's specified free-running speed, and correcting for the gearing (404 º/s). It is likely therefore that the motor rapidly accelerated the arm to a terminal velocity, in which friction in the gears and bearings was equal and opposite to the motor torque. These perturbations evoked consistent short and long latency reflexes (Trumbower et al., 2013, Thilmann et al., 1991b) in the recorded muscles. Subjects were told to return the arm to

the central target after each perturbation, but were not required to do this within any time constraints. A total of sixty trials were recorded for each session, comprising 20 trials at each of three levels of background torque. Levels of background torque were determined individually for the subject to allow comfortable task performance, and ranged from 0 to 4.5 Nm; the same levels were used for that subject in both recording sessions.

EMG, elbow displacement and motor torque signals together with markers indicating task events were captured to a personal computer (5 kHz sampling rate) using a 1401 interface (Cambridge Electronic Design). Subsequent analysis involved constructing averages of rectified EMG, using custom scripts written in the MATLAB environment.

### 2.2.2 Experimental Protocol

All experiments followed the same general pattern (Fig. 2.1B). Subjects came to the laboratory before 9:30am, and a set of stretch reflex recordings were made. They were then fitted with a wearable electronic device, designed to deliver electrical and auditory stimuli. The wearable device generated constant-current electrical stimuli to the biceps muscle (surface electrodes and placement as above; 220V compliance; 150 μs pulse width; more proximal electrode negative). The intensity was adjusted to be just below motor threshold (defined as a visible muscle twitch); subjects reported a weak paraesthesia produced by the stimulus. Auditory stimuli were generated by delivering a 0.1ms wide, 12V square excitation pulse into a miniature earpiece; this produced a brief click with intensity 110 dB SPL.

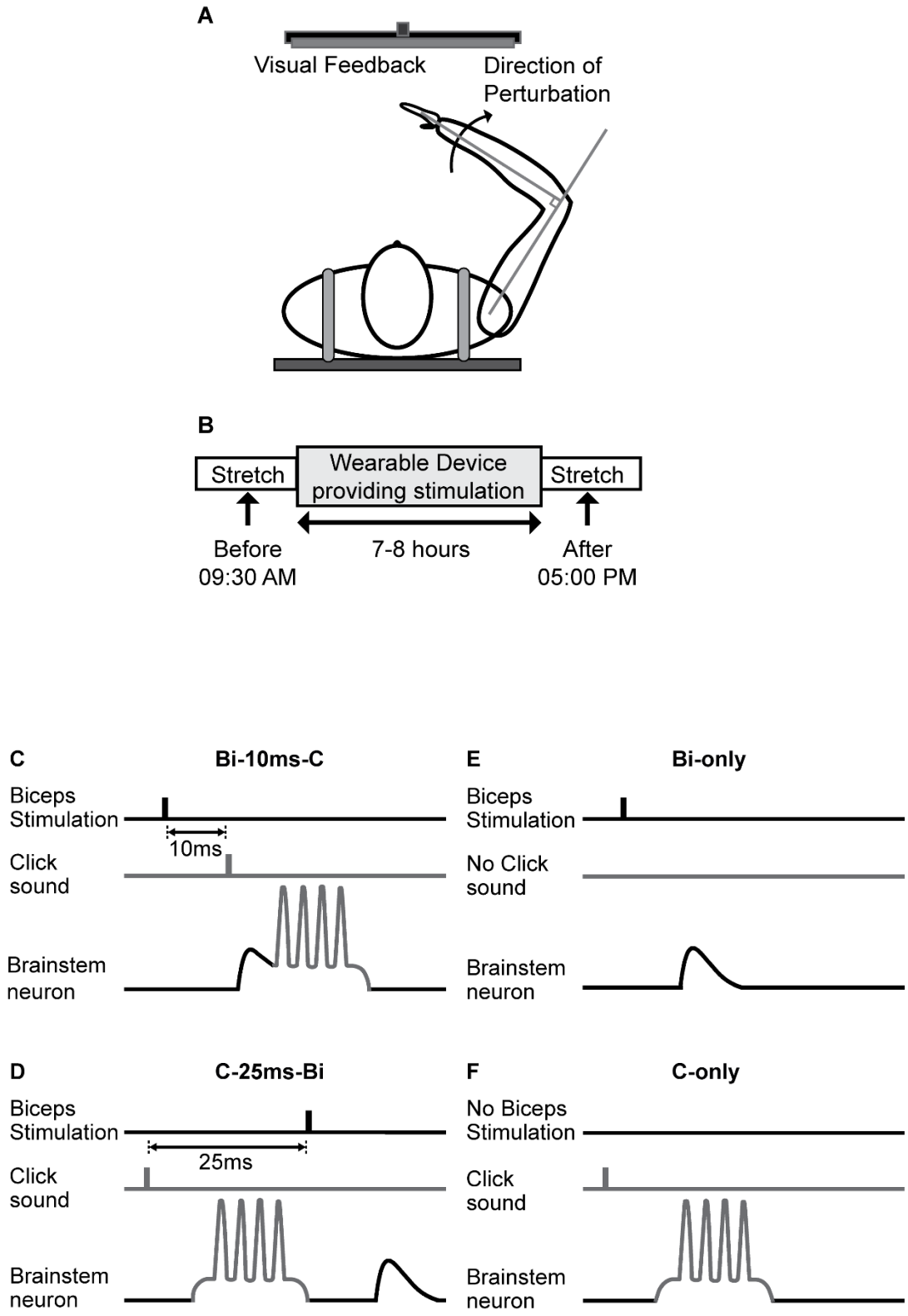


***Figure 2.1. Schematic diagram showing experimental setup and wearable device stimulus conditions.***

A, subjects were strapped into a chair, with their right arm attached to a robotic device capable of delivering extension perturbations at the elbow joint. A computer screen provided visual feedback of elbow angle. B, the general experiment protocol. C-F, the four different stimulus conditions implemented by the wearable device, and their hypothesised effects on a reticulospinal neuron. C, biceps stimulation 10 ms before click; the EPSP elicited by the afferent input arrives just before the click-induced discharge, which should potentiate synapses conveying the EPSP. D, click 25 ms before biceps stimulation; the afferent EPSP arrives after the click-induced discharge, which should lead to depression of the EPSP. E, biceps stimulation alone, F, click stimulation alone. With no stimulus pairing, I expect no change in EPSP amplitude.

Based on calculations provided by Rosengren et al. (2010), this intensity delivered at 0.66 Hz for 8 hours corresponds to an A-weighted intensity of 68 dB $L_{Aeq}$, which is well below the recommended safe limit for hearing of 85 dB $L_{Aeq}$ given by the UK's Control of Noise at Work Regulations (The Stationery Office, 2005). The earpiece was placed in either the left or right ear. I found no consistent differences dependent on which ear was stimulated; recordings from monkey reticular formation also showed that cells could be activated by clicks delivered to a wide area of the scalp by a bone vibrator (K.M. Fisher, B. Zaaimi & S.N. Baker, personal communication). Stimuli were delivered with an inter-stimulus interval of 1.25-1.75 s (chosen at random from a uniform distribution). Subjects then left the laboratory and continued their usual daily activities. As most subjects were staff or students in the university, this typically involved office or laboratory tasks such as typing and soldering. After 5pm, they returned, the wearable device was removed, and a further set of stretch reflex recordings was made. Because the electrodes over biceps were used for both recording reflex responses and for stimulation, they were kept in place for the whole day, ensuring consistency between the morning and evening recordings. For the brachioradialis, either electrodes were also left in place all day, or their location was marked after the morning session with a UV fluorescent marker pen; this ensured that electrodes could be replaced in exactly the same location for the evening reflex recording.

Four different stimulus combinations were tested in different experiments; these are illustrated in Fig. 2.1C-F, together with a schematic which indicates the effects in the brainstem which I hypothesised each would generate given previous work. The first paradigm placed the electrical stimulus 10 ms before the click. I expect the biceps stimulus to generate an EPSP within the reticular formation in a human subject with a

latency of around 10 ms (see Discussion). The click should generate an action potential burst after around 7 ms (Fisher et al., 2012). I predicted therefore that this timing would lead to the EPSP consistently arriving just before the spike burst, and should lead to long term potentiation. By contrast, when the click preceded the electrical stimulus by 25 ms (Fig. 2.2D), this should place the EPSP after the spike burst, and generate long term depression. Two control conditions (Fig. 2.2EF) tested the effect of giving electrical or auditory stimuli alone. Some subjects participated in more than one of these paradigms; at least one week separated different experiments in the same subject.

### 2.2.3 Analysis

Figure 2.2A illustrates ten sweeps of raw EMG from a single subject following the perturbation. An average of the elbow displacement trace revealed a nearly linear ramp perturbation (Fig. 2.2B). One problem which I encountered was considerable variation in the level of background EMG prior to the perturbation onset. Since it is known that the stretch reflex scales with background activation (Matthews, 1986), an inconsistent background would render comparisons of the morning and evening reflex recordings invalid.

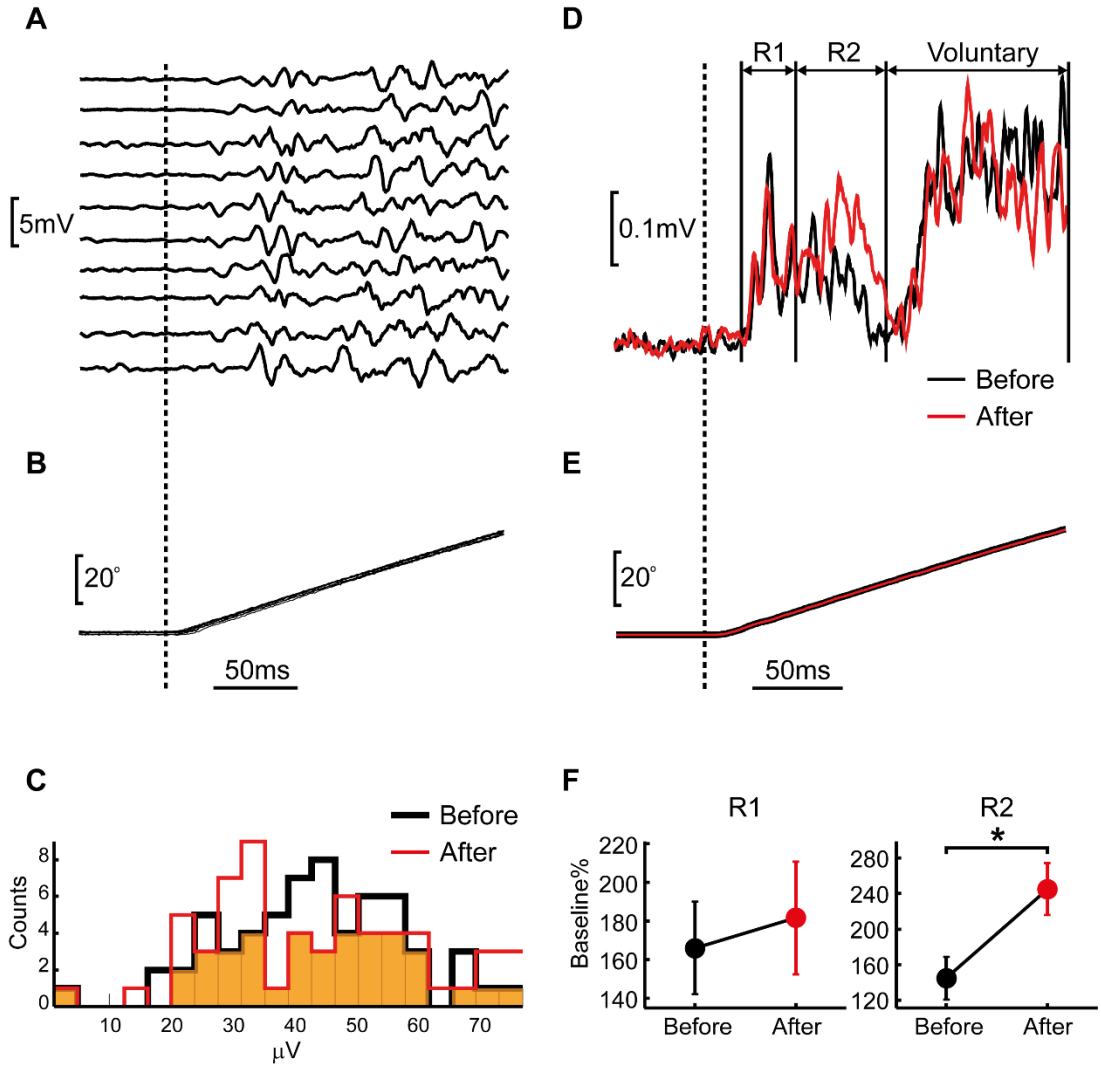


***Figure 2.2. Example result in a single subject.***
A, raw biceps EMG recording after ten perturbations. Dotted line indicates the perturbation onset. B, overlain single sweeps (thin lines) and average (thick line) of elbow angle during the perturbation for the same experiment as (A). C, distribution histograms of mean background EMG in the biceps muscle in one subject, for recordings before and after the wearable device stimulation. Orange shading marks the minimum value of each bin. D, average rectified EMG in the biceps muscle following perturbation onset (dotted line), for recordings before and after wearable device stimulation (protocol Bi-10ms-C, Fig. 2.1C). Average has been compiled using the sweep selection procedure described in the text and illustrated in (C), such that baseline EMG is matched. Short latency (R1), long latency (R2) and voluntary response epochs are marked. E, average elbow angle for the same experiment as (C); traces for measurements before and after wearable device stimulation are overlain, but are barely distinguishable. F, measurements of area under the curve as a percentage of baseline EMG for the experiment shown in (D). The R2 response was significantly facilitated ($P<0.05$). For all panels, black traces refer to measurements before wearable device stimulation, and red after.

Figure 2.2C illustrates the approach taken to this problem. I first measured the background EMG in each of the 60 available sweeps over the 50 ms prior to perturbation onset, for both morning and evening recordings. The interval from the largest to the smallest background measured was divided into 20 equally-spaced bins, and each sweep was allocated to one of these bins. This led to a distribution histogram, as shown in Fig. 2.2C. For each bin, I took the minimum count between the morning and evening recordings. For the session which had this minimum, all sweeps falling in that bin were used. For the other session, a number of sweeps equal to the minimum count were chosen at random. Following this procedure for all bins led to selection of a subset of sweeps, with equal numbers of in both morning and evening sessions, and with a very similar mean background EMG level. These sweeps were averaged together, generating traces as in Fig. 2.2D.

Following previous work (Mortimer et al., 1981, Pruszynski et al., 2011b), responses were categorised by their latency (Fig. 2.2D) as short latency stretch reflex (R1; 20-50 ms), long-latency stretch reflex (R2; 50-100 ms) and voluntary (>100 ms). Within the R1 and R2 windows, the percentage increase of the area under the curve relative to the background was used as a measure of reflex amplitude. The significance of changes in reflex amplitude were assessed using t-tests on the single sweep measures of area under the curve above background, with a threshold of $P<0.05$.

## 2.3 Results

Figure 2.2D illustrates an example result from one subject, in which the wearable device was programmed to deliver electrical stimuli to the biceps muscle 10 ms before a click (protocol of Fig. 2.1C). The background level of EMG was very similar for recordings made before and after the wearable device intervention (red *vs* black traces in Fig. 2.2D), confirming the efficiency of the method of sweep selection described in Methods (Fig. 2.2C). Although very similar for the R1 component, the long-latency stretch reflex (R2) was noticeably enhanced in the later recording. Figure 2.2F presents measures of area

under the curve for each response window; there was a significant increase in the R2 response.

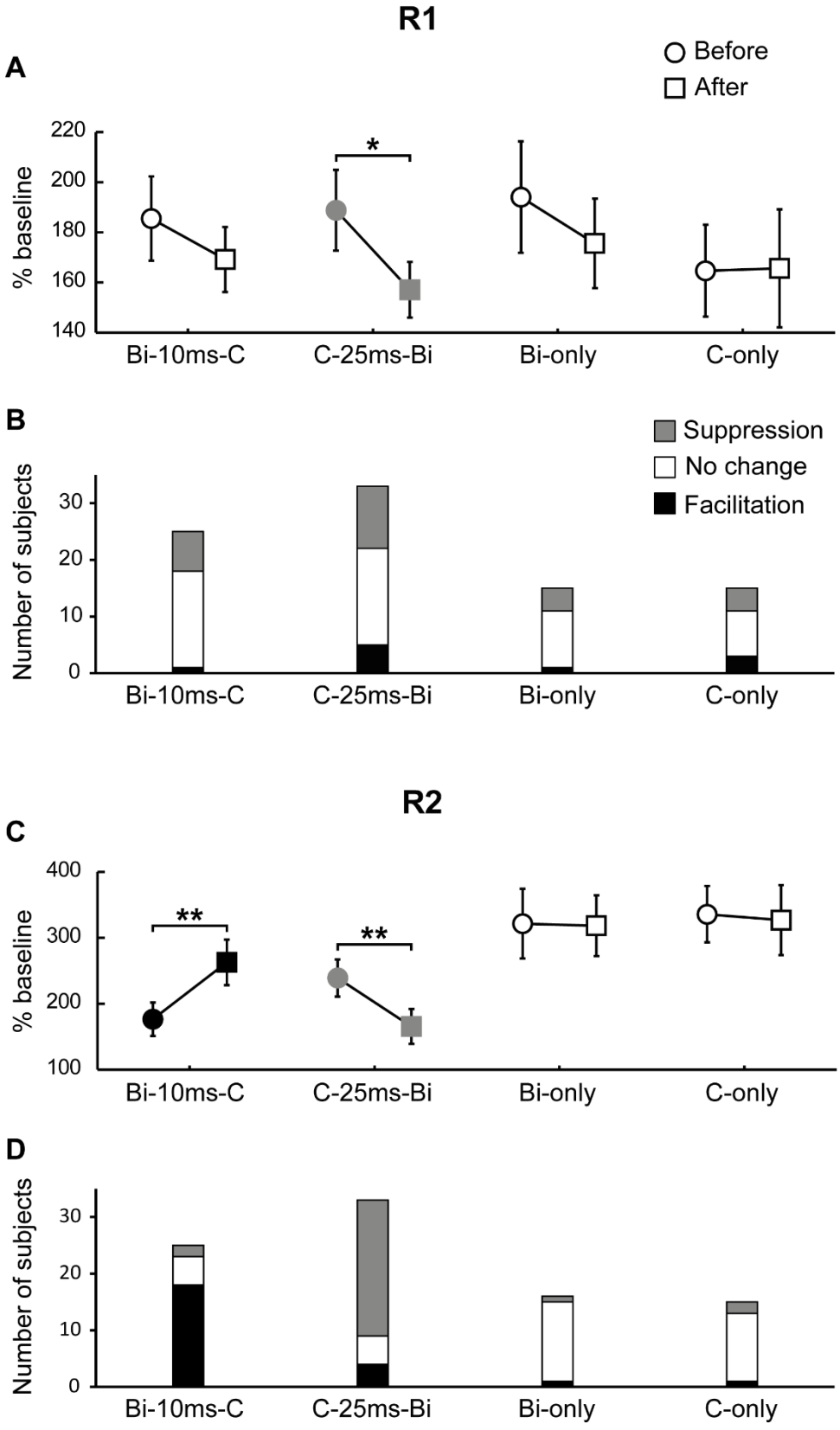


***Figure 2.3. Group results in the biceps muscle.***

A&C, comparison of mean reflex size (expressed as a percentage of baseline EMG activity) before (circles) and after (squares) wearable device stimulation, for the four stimulation protocols illustrated in Fig. 2.1C-F. *, P<0.05; **, P<0.005. B&D, stacked bar plots showing the number of subjects with significant (P<0.05) reflex facilitation (black), suppression (grey) or no change (white). A&B relate to the short latency (R1) reflex component, C&D to the long latency (R2) component.

Figure 2.3 presents group results for recordings from the biceps muscle. These have been separated into R1 (Fig. 2.3AB) and R2 (Fig. 2.3CD) components. Figure 2.3AC shows mean reflex amplitudes, calculated across all subjects participating in a given wearable device protocol (as defined in Fig. 2.1C-F). The R2 component showed a significant increase (P<0.0002, paired t-test), on average from 176% to 263% of baseline (an increase of 49%) for the condition where the electrical stimulus preceded the click by 10 ms. There was a significant decrease (P<0.005, paired t-test), on average from 239% to 165% of baseline (a decrease of 31%) when the click preceded the electrical stimulus by 25 ms (Fig. 2.3C). The direction of these effects was as predicted on the basis of spike-timing dependent plasticity (Fig. 2.1CD). There was no significant difference in the size of the control reflex (before wearable device stimulation) between these two experiments. A small but significant decrease (by 17%; P<0.02, paired t-test) was also seen in the R1 reflex for the second protocol. The control protocols, where either electrical or auditory stimuli were given alone, produced no significant changes (P>0.05, paired t-test).

It is now well recognized that plasticity protocols often lead to substantial heterogeneity in response across subjects (Wiethoff et al., 2014); some of this may be determined by genetic factors (Cheeran et al., 2008). Accordingly, Fig. 2.3BD examines at the single subject level how many showed significant changes. Each bar shows the number of subjects with significant increases, significant decreases or no significant change for a given protocol and response window. For protocols with no significant change in average response, the pattern across subjects tended to be inconsistent, with some increases and decreases seen. However, for R2 reflex following paired stimulation, a clear pattern emerged which supported the group-averaged data. When the electrical stimuli preceded the click 15/25 subjects showed a significant rise in R2, compared with only 2/25 a decrease. By contrast, when the click preceded the electrical stimulus, 24/33 showed a drop in R2 amplitude but only 5/33 a rise. To see counts as extreme as 15/25

and 24/33 is highly unlikely by chance ($P<10^{-17}$, based on the binomial distribution, with n=25 and 33 respectively and P(hit)=0.025). Similar results were obtained if I considered simply whether reflexes increased or decreased, irrespective of whether these changes were significant for an individual subject (electrical stimulus before click, 23/25 subjects R2 increased; click before electrical stimulus, 26/33 subjects R2 decreased; both $P<0.002$ based on binomial distribution with P(hit)=0.5).

The wearable device protocols examined involved electrical stimulation of the biceps muscle; however, stretch reflex recordings were made from both biceps and brachioradialis, which are both elbow flexors in the arm posture tested. This allowed me to examine the extent to which changes in reflex amplitude were specific to the stimulated muscle, or might spread to anatomical agonists.

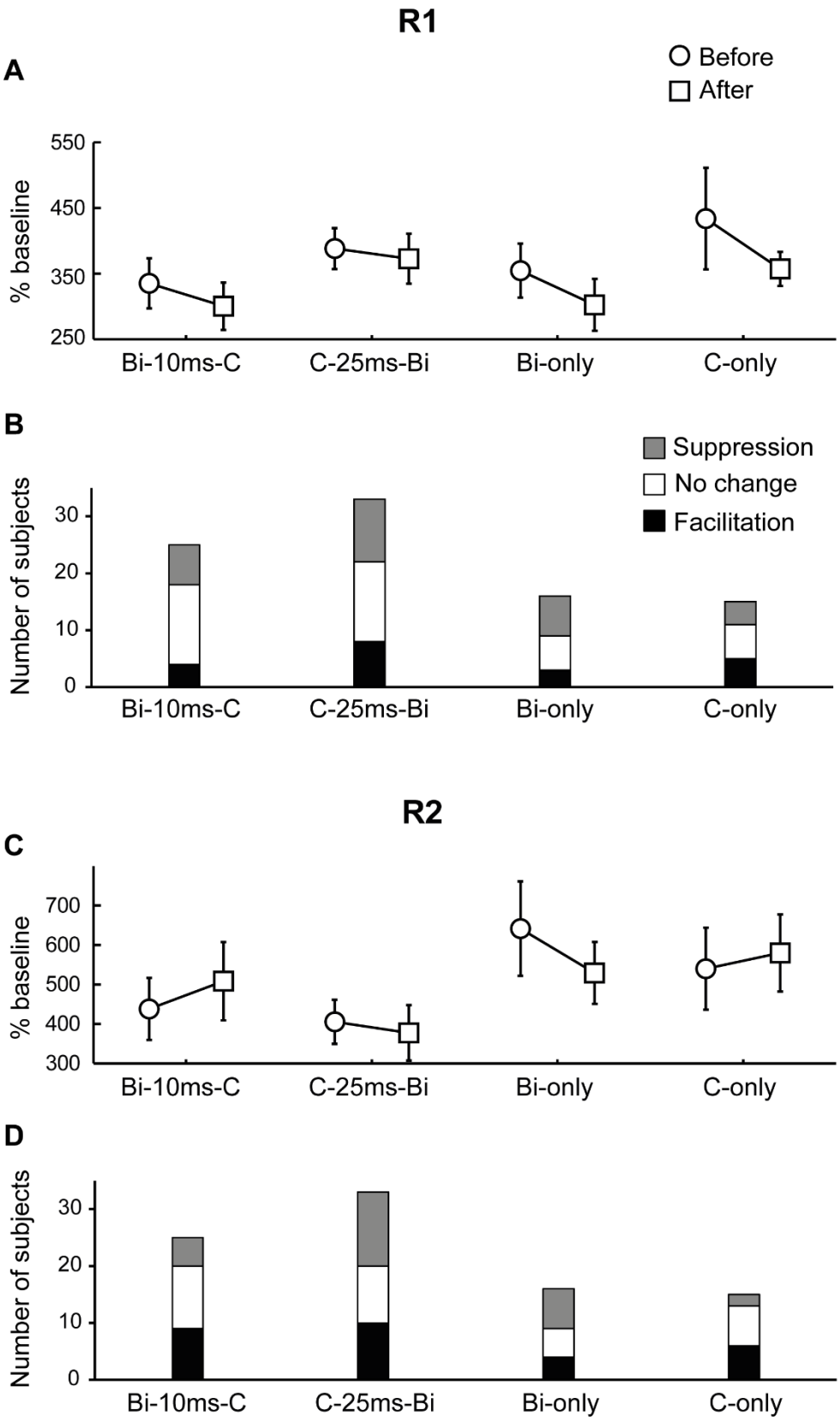


***Figure 2.4. Group results in the brachioradialis muscle.***
A&C, comparison of mean reflex size (expressed as a percentage of baseline EMG activity) before (circles) and after (squares) wearable device stimulation, for the four stimulation protocols illustrated in Fig. 2.1C-F. There were no significant differences

(P>0.05). B&D, stacked bar plots showing the number of subjects with significant (P<0.05) reflex facilitation (black), suppression (grey) or no change (white). A&B relate to the short latency (R1) reflex component, C&D to the long latency (R2) component.

Figure 2.4 presents the results for the brachioradialis muscle, in a similar format to those of Fig. 2.3. No significant changes were seen in any of the group averages (Fig. 2.4AC). At the single subject level, similar numbers of subjects in a given protocol showed significant increases or decreases (Fig. 2.4BD), suggesting that this reflected noise fluctuations in amplitude measurement rather than consistent plastic changes.

# 2.4 Discussion

## 2.4.1 Pathways Contributing to the Long Latency Stretch Reflex

Following the discovery of the LLSR (Hammond, 1954), considerable research focussed on the pathways responsible. This reached a consensus by the early 1990s that the LLSR in muscles acting on the digits or wrist was mediated largely by Group Ia muscle afferents traversing a transcortical pathway (Matthews, 1991b). However, this did not exclude other contributions. For example, some continued to argue that cutaneous afferents play a dominant role (Corden et al., 2000); earlier studies suggested that the LLSR was a spinal reflex mediated by slower conducting Group II afferents (Matthews, 1984), although subsequent results did not support this (Matthews, 1989). For muscles acting around the elbow or shoulder, motor cortical recordings in monkey do reveal evidence for some transcortical contribution (Pruszynski et al., 2011a, Evarts and Tanji, 1976). Transcranial magnetic brain stimuli delivered over motor cortex and timed to coincide with the LLSR are facilitated, also suggesting a transcortical contribution (Pruszynski et al., 2011a). However, evidence from patients with motor disorders suggests that the transcortical route for the LLSR may be less important in elbow muscles compared to the hand (Fellows et al., 1996, Thilmann et al., 1991b). The tonic vibration reflex, which may relate to the LLSR, relies on the brainstem reticular formation (Gillies et al., 1971). Even for finger perturbations, it has been recently shown that the reticular formation probably contributes to the LLSR (Soteropoulos et al., 2012). It therefore seems probable that the reticular formation contributes to LLSR in more

proximal muscles as well (Kurtzer, 2014), and could even be the dominant pathway. Such evidence caused me to measure changes in the LLSR following elbow perturbations after a paired stimulus protocol designed to target reticulospinal output. The finding that the LLSR exhibits plastic changes, in a manner consistent with the predictions of how paired stimuli should modify the reticulospinal output, is consistent with a reticulospinal role in elbow muscle LLSR. For instance, my results show that facilitation occurred in the LLSR in biceps after a time-adjusted paired stimuli paradigm designed to affect the reticular formation within the calculated (discussed below) STDP window. Considering the afferent nerve conduction speed, the onset of the stretch reflex and timing of the sensory input, it is likely that these paradigms are capable of changing excitability in the reticular formation, further reflecting its contribution to the LLSR.

### 2.4.2 Spike Timing Dependent Plasticity

Several features of my results suggest that it was possible to induce spike-timing dependent plastic changes. Firstly, the control conditions which delivered either electrical stimulation of biceps alone or clicks alone failed to generate consistent changes in the reflex measures. This not only controls for the effects of the unpaired stimuli, but also provides confidence that there were no consistent changes in the reflexes between the morning and evening assessments, caused for example by diurnal rhythms or fatigue. Secondly, it is striking that shifting the relative timing of the two stimuli by only 35 ms should have had such a profound effect, reversing an average facilitation in biceps R2 reflex to a suppression. Finally, plastic changes were only seen in the biceps muscle which was stimulated, and not in the closely related agonist brachioradialis. This seems to fulfil the 'specificity' criterion of long-term potentiation whereby effects are limited to the stimulated site, although I cannot rule out the alternative possibility that only pathways targeting the biceps are capable of showing these changes.

The stimulus timings were chosen based on the expected delays to generate activity within the reticular formation. In monkey, it is known that reticular responses to clicks have onset latency around 7 ms (Fisher et al., 2012). Although the human head is larger, most of this delay relates to central processing rather than axonal conduction, so that it is likely to be only slightly longer in man. Electrical stimulation of the human median

nerve at the wrist produces EEG responses attributed to the medial lemniscus with latency around 14 ms (Taylor and Black, 1984); effects should reach the reticular formation shortly afterwards. I estimate the distance between the biceps motor point and the wrist as 350-400 mm; using a fast afferent conduction velocity of 85 m/s, this would imply a reduction of 4-5 ms in latencies to account for the more proximal stimulus site. Synaptic potentials in the reticular formation should thus start around 10 ms after the biceps electrical stimulus. Stimulating the biceps motor point 10 ms before the click should therefore place the earliest synaptic potentials from afferent input around 7 ms before the action potential burst generated by the click. This timing is therefore appropriate to generate potentiation of the synaptic inputs.

When reversing the timings, the duration of the action potential burst must be considered; neural firing can continue for up to 25 ms after the click (Fisher et al., 2012). Placing the biceps stimulus 25 ms after the click therefore should position the synaptic potentials from afferent input around 10 ms after the end of the action potential burst; this should be appropriate to depress the synaptic inputs.

It is impossible to be certain of the site of the plastic changes which was measured in the LLSR, but the success of the chosen timings argues that some modification of synapses may have occurred within the brainstem itself. Other possibilities are within spinal cord interneurons, or within the cortex, but the anatomy of the conduction delays conspires to make these less likely. If a spinal cord interneuron discharged following a click-elicited reticulospinal burst, this would be at least 3-4 ms *later* than the burst onset in the brainstem. This estimate is based on the fact that central motor conduction time from M1 to cervical enlargement in human is around 7 ms (Jaiser et al., 2015). The brainstem is approximately halfway along this path, and fast reticulospinal and corticospinal fibres have similar conduction velocity (Riddle et al., 2009). By contrast, an afferent volley following the biceps stimulus would arrive at the cervical enlargement 3-4 ms *earlier* than at the brainstem. The interval between the responses then increases from the estimate of 7 ms at the brainstem estimated above, to around 14 ms at the spinal cord; this is less likely to drive plastic changes. For the cortex, the responses to biceps stimulation will be delayed by around 6 ms relative to the brainstem, but those following the auditory click by substantially more: the earliest cortical auditory evoked potential occurs with 50 ms latency (Farrell et al., 1980). The responses in the brainstem appear

best timed to generate spike-timing dependent changes, although it is impossible to rule out a contribution from other centres.

A small suppression was seen in the R1 reflex when the biceps stimulus followed the click (Fig. 2.3A), indicating that changes within spinal circuits may also have played a role in my results. Human subjects, monkeys and rats can all learn to increase or decrease the size of an H reflex (the electrical analogue of the R1 reflex) if appropriately rewarded (Thompson and Wolpaw, 2014). In monkeys subjected to repeated reflex testing, but with no attempt at up- or down-conditioning, there is a progressive reduction of the R1 reflex (Meyer-Lohmann et al., 1986). In rat, reflex conditioning depends on the corticospinal tract and sensorimotor cortex, but not other descending pathways (Chen et al., 2006b, Chen et al., 2006a, Chen and Wolpaw, 1997, Chen and Wolpaw, 2002). Down-conditioning leads to increases in identifiable GABAergic terminals in the spinal cord (Wang et al., 2006), but is dependent on an intact cerebellum (Chen and Wolpaw, 2005); spinal plasticity therefore seems to be guided and maintained by supra-spinal pathways. It is likely that conceptually similar processes are occurring here, although whether the same central structures and descending pathways which contribute to reflex conditioning in rat are responsible in this case remains to be determined.

The LLSR is known to change depending on the behavioural context; this appears flexibly to integrate the known biomechanics of the limb (Kurtzer et al., 2008). It is unclear how the plastic changes which I have seen would interact with these task dependent changes. It is also unclear for how long plastic changes would last, and whether they could be prolonged by applying the stimulus pairing for longer than the ~7 hours which was tested in this report. All of these questions remain to be addressed in future studies. However, this report marks the first demonstration of plasticity in the LLSR induced with paired stimulus paradigms, and may indicate that brainstem as well as corticospinal descending systems can undergo plastic changes. Previous work has shown that rehabilitation after spinal cord injury can be enhanced by up- or down-conditioning of spinal reflexes (Chen et al., 2006c, Thompson et al., 2013). I hope that the novel protocol introduced here may open up new possibilities for enhancing rehabilitation during recovery from stroke or spinal cord injury, in which I have shown that brainstem pathways play an important role.

# Chapter III

# A HIERARCHY OF PLASTICITY IN HUMAN HAND AND FOREARM MUSCLES

## 3.1 Introduction

Reorganization of neural connections or structures in the central nervous system, also known as plasticity, generally shapes the outcome of learning, behaviour and motor reflex in healthy individuals (Classen et al., 1998, Cohen et al., 1997) and plays a critical role during recovery after brain lesions (Thulborn et al., 1999, Lemon, 1981, Murray and Coulter, 1981, Ridding and Rothwell, 1995, Di Lazzaro et al., 2009, Baker et al., 2015, Benecke et al., 1991). To investigate the underlying physiological mechanisms and potential therapeutic role of neurological adaptations, artificial induction of plastic changes in the motor cortex or subcortical level utilizing Transcranial Magnetic Stimulation (TMS) alone (Huang et al., 2005) or in conjunction with other interventions (Stefan et al., 2000) have been studied previously. Stefan et al. (2000), in their seminal study, successfully manipulated motor cortical plasticity with paired associative stimulation (PAS) using synchronous TMS and median nerve stimulation, resulting in an increased Motor Evoked Potential (MEP) at rest. A number of other PAS protocols are known to affect cortical and subcortical excitability leading to rapid reorganization in the corticomotor neural pathways; for example electrical stimulation of peripheral nerves (Ridding et al., 2001, Conforto et al., 2002, McKay et al., 2002a) or motor point stimulation of muscle (Ridding and Uy, 2003, McKay et al., 2002b) can initiate development of transient or persistent plasticity in the motor cortical output. While the capability of specifically designed afferent input or cortical stimulation in reorganizing cortical or subcortical neural pool has been documented in many previous studies, there is still a lack of data whether and how this kind of modulation affects different groups of muscles differently. Especially, how this modulatory mechanism affects the functional outcome in agonist-antagonist muscles was largely overlooked.

The scopes for synaptic reorganization and remodelling for different groups of upper limb muscles may inherently differ from each other. Characteristic facilitation in flexor was evidenced by Vallence et al. (2012) in their study where MEP in the forearm flexor

muscle increased after ischaemic blocking of afferent inputs but not in the extensor. They suggested that the forearm flexors have a greater potential for plasticity than the forearm extensors. Task-dependent and muscle-specific variation in the motor cortical excitability for flexor vs. extensor muscle in the upper limb was noticeable when Godfrey et al. (2013) found that a repetitive flexion task increased cortical excitability in the finger flexors but with no such effect for finger extensors. However, it is yet to establish whether these changes in the cortical excitability for different muscles are influenced by afferent inputs or mostly depend on reorganization in the motor cortex irrespective of sensory feedback. Ridding and Rothwell (1995) showed that reorganization in corticospinal projections took place in the absence of sensory awareness or feedback. In another experiment, Ziemann et al. (1998a) found that ischaemic nerve block could induce a deafferentation-mediated reorganization in the motor cortex which resulted in further plastic changes mediated by subthreshold stimulation of motor cortex using repetitive Transcranial Magnetic Stimulation (rTMS).

As evidenced by some of those previous studies, flexor and extensor muscles in the forearm differ in terms of modulating cortical excitability, but up to my current knowledge, no previous study showed any direct comparison modulating flexor vs. extensor motor outputs by non-invasive protocols that either stimulate motor cortex alone or in conjunction with another afferent input. To test and compare any potential variations in the induced plasticity in distal hand and flexor-extensor group of muscles, here I took a modified approach. Instead of stimulating any peripheral nerve, a common wrist flexor or extensor muscle, close to its motor point, was directly stimulated via transcutaneous stimulation to send an afferent volley. It has been documented that muscle belly or motor point stimulation can recruit multiple types of afferent nerves including Ia, Ib and cutaneous afferents (Bergquist et al., 2012, Dideriksen et al., 2015).

Muir and Lemon (1983) also showed that electrical stimulation of the muscle belly can effectively excite a substantial proportion of alpha-motoneurons of the targeted muscle. In line with previous evidence (Bergquist et al., 2011a), my understanding was by stimulating the muscle belly close to the motor point it could be possible to pass enough afferent drive towards higher cortical motor neuron pool controlling excitability and motor output. When paired with a time adjusted TMS, this afferent input from motor point could induce plasticity in a manner consistent with spike-timing dependent plasticity protocols. Similar techniques to stimulate motor point directly with

subcutaneous electrical stimulation were utilized previously in PAS protocols by other researchers successfully, where they were able to change the MEP in the targeted muscles (McKay et al., 2002b, Ridding and Uy, 2003).

In a separate set of experiments, I tested rTMS in the form of intermittent theta burst stimulation (tTMS) (Huang et al., 2005) and measured the output in the same muscles tested before with my PAS protocol to compare any changes in cortical excitability. In recent years, rTMS has increasingly been utilized as a treatment option for a variety of neurological disorders including Parkinson's disease and other psychiatric conditions like depression with some success (Hausmann et al., 2004, George et al., 2009, Schlaepfer et al., 2010, Wagle Shukla et al., 2016, Martin et al., 2003, George et al., 1995). While high frequency rTMS has a known effect in producing synaptic plasticity in animals (Shang et al., 2016, Yoon et al., 2011, Lenz et al., 2016), relatively moderate to low frequency rTMS, within its safety limit (Huang and Rothwell, 2004), can also change motor cortical excitability in human (Avenanti et al., 2012, Chou et al., 2015, Todd et al., 2010).

I, therefore, set three aims for this study: i. utilizing the PAS protocols as a convenient and translational method by stimulating muscle directly to investigate brain plasticity in human, ii. To compare relative changes in flexor vs extensor and distal hand muscle outputs after interventions for a better understanding of the complex cortical and spinal circuitry controlling hand muscles differently, and iii. To compare and test the contribution of afferent input in changing relative plasticity in flexor vs. extensor. The acquired knowledge might be useful in implementing a successful therapeutic and rehabilitation strategy to reduce flexor spasticity and to recover extensor weakness following stroke or spinal cord injury.

## 3.2 Materials and Methods

### 3.2.1 Subjects

Results were obtained from 23 healthy volunteers (8 male; age range 19 – 50 years) in 30 sessions of the PAS experiment. 9 healthy volunteers (4 male; age range 19 – 32 years) were tested for the rTMS experiments in 16 sessions. All procedures were approved by the local ethical committee of Newcastle University Medical School. Prior

to each experiment, full written consent was obtained from each participant after explaining each procedure in detail.

### 3.2.2 Electromyographic (EMG) recording

In all experiments, Electromyogram (EMG) was recorded unilaterally from three different groups of muscles- Flexor Digitorum Superficialis (FDS), Extensor Digitorum Communis (EDC) and two distal hand muscles Abductor Pollicis Brevis (APB) and First Dorsal Interosseous (1DI). Adhesive surface electrodes (model H59P, Kendall) were placed directly over the muscle belly approximated to the motor point with an interelectrode spacing of 2 cm for FDS and EDC muscles. Another pair of similar electrodes were placed over the muscle belly of the left APB and 1DI, 1 cm apart from each other. Electrodes were connected to a Digitimer D360 amplifier (gain, 1000; bandpass filter, 30 Hz to 2 kHz) and EMG signals were stored in a laboratory computer system for analysis after those were digitized using an analogue-digital converter (model 1401, Cambridge Electronics Design, Cambridge, UK).

### 3.2.3 Transcranial Magnetic Stimulation (TMS)

For PAS experiments, a figure of eight shaped magnetic coil (model D70$^2$) fitted with a tracker and connected with a Magstim (BiStim2, Magstim Ltd UK) stimulator was used to provide focal TMS stimulation to the contralateral hemisphere. Following earlier studies (Stefan et al., 2000, Ridding and Uy, 2003), the coil was positioned tangentially over the skull with the handle pointing backward and laterally producing 45$^o$ angle to the sagittal plane for all subjects. A second tracker, delivering input to an overhead camera, was fitted to a headband and was positioned on the subject's forehead. A Brainsight navigation system (Rogue Resolutions Ltd, Cardiff) was used to track and monitor the relative coil position in all the experiments.

A separate figure of eight shaped magnetic coil connected with a Magstim Rapid was used for the rTMS experiments. It also contained a tracker for the Brainsight navigation. Similar techniques as previously were used for positioning of the coil, tracking, defining and targeting the optimal site for stimulating the motor cortex during the rTMS study.

### 3.2.4 Electrical Stimulation at motor point:

Electrical stimulation was delivered from the same bipolar surface electrodes (Silver-Silver Chloride) that were already placed directly on the skin overlying the stimulated muscle belly. About 15-25 mm distance between the active and passive node was maintained. Two different protocols were used where either the FDS or EDC muscle's motor point was stimulated 18ms prior to TMS. A single square pulse electrical stimulation with a width of 500μs was delivered to the targeted muscle belly close to the motor point from a Digitimer constant current stimulator (DS7A). The perceptual threshold was measured by visual feedback individually. To ensure a clearly visible twitch at the targeted muscle, 2x to 3x of the perceptual threshold was used as the intensity of the electrical stimulation that was delivered during the final test. Either the FDS or the EDC alone was stimulated during each session of the experiment.

## 3.3 Experimental procedures:

Subjects were seated in a comfortable and slightly reclined height adjusted chair and placed their right forearm on the adjacent table. They were instructed to stay completely relaxed during the entire experiment and complete muscle relaxation was ensured by EMG signal live monitoring and visual feedback. Motor Evoked Potentials (MEP) was elicited with suprathreshold stimulator intensity by placing the coil over the left hemisphere. MEPs from the FDS and EDC muscles were assessed to locate the optimal site which showed the maximum response from both muscles. It was then marked as the hotspot in the Brainsight navigation system. Like many other previous studies (Rossini et al., 1994, Stefan et al., 2000) , the minimum stimulator intensity required to evoke at least 50μV response in both relaxed muscles in at least 5 out of 10 repeated trials was obtained and determined as the Resting Motor Threshold (RMT). Throughout the experiment, 1.2xRMT was used to record MEP amplitude from all four muscles. For the rTMS protocol, active motor threshold (AMT) was measured using a second coil. AMT was determined as the minimum intensity required to evoke a MEP amplitude measuring 0.1mv peak to peak, which occurred at least 5 times in a total number the 10 trials, during a sustained voluntary contraction of the targeted muscle active with 20%

of maximal contraction (Rossini et al., 2015, Rossini et al., 1994). A steady contraction level was achieved and maintained by real-time visual feedback from a display.

### 3.3.1 PAS Protocol

To record the resting MEPs from all four muscles, a set of 20 TMS at a rate of 0.1Hz was delivered at the optimum location on the scalp before the intervention (Fig. 3.1A). In most cases, another set of 20 TMS was delivered to get consistent baseline MEPs from the averaged data. For the PAS protocol (intervention), either the FDS or EDC muscle received a square pulse electrical stimulation 18ms before delivering single pulse TMS stimulation at an intensity of 1.2x RMT. A total number of 90 such paired stimuli was delivered over 30 minutes at a fixed rate of 0.05 Hz (pairing duration 30 minutes). Immediately after paired stimulation protocol, two sets of 20 resting MEPs were recorded again delivering TMS at same intensity and rate that was used for recording the baseline MEPs. In all cases, only one muscle was stimulated each time in each session of experiment, i.e., muscle stimulation for intervention was given to either FDS or EDC muscle.

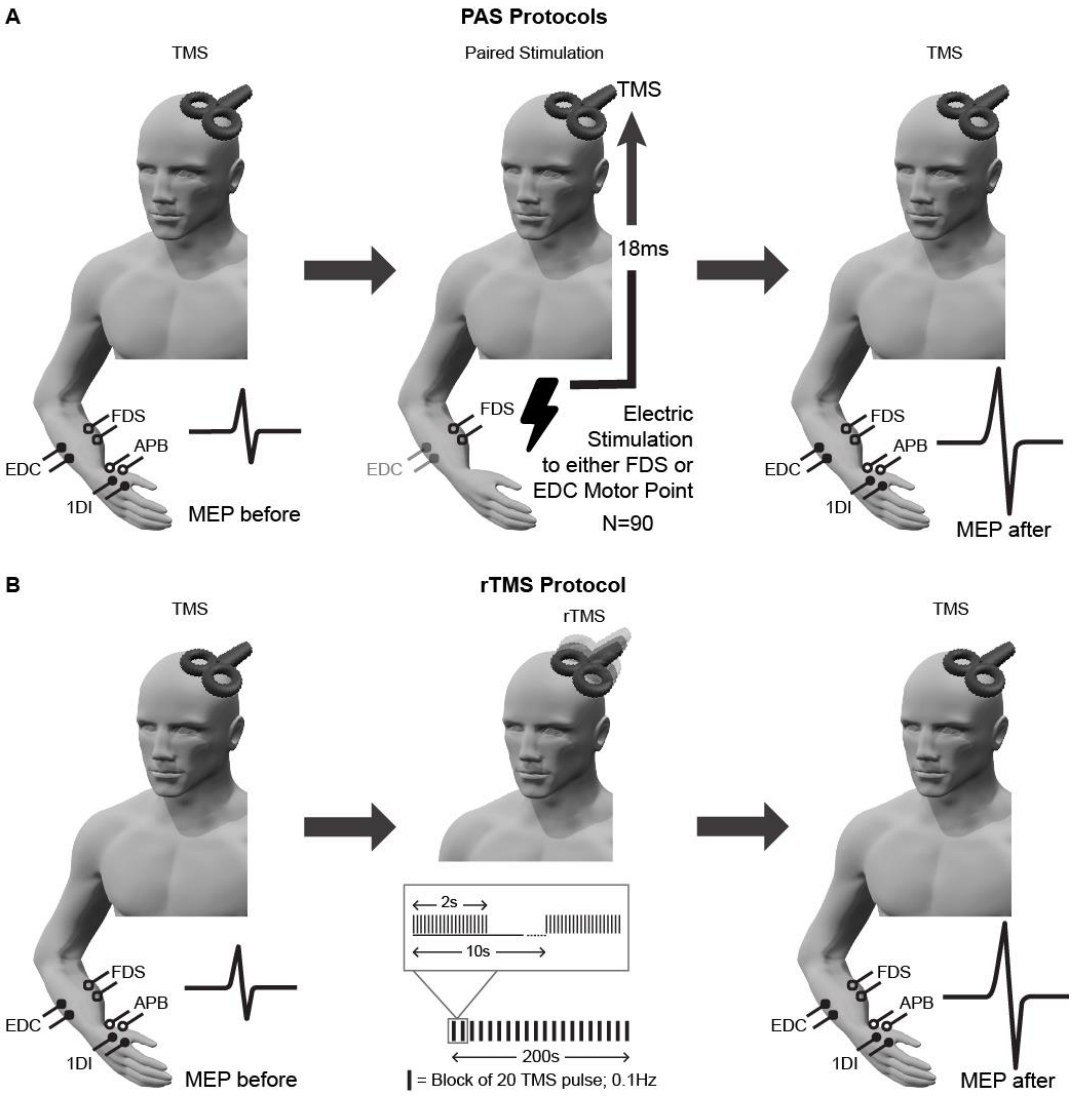


***Figure 3.1. Schematic diagram showing experimental setup and protocols.***
In all protocols, TMS was delivered to motor cortex before and after the test and MEPs were recorded from four muscles – FDS, EDC, APB and 1DI. A, PAS protocols, where electrical stimulation was applied to muscle motor point 18ms before the TMS. Either FDS or EDC muscle was stimulated in each session of the experiment. Total N=90 paired stimulation was delivered. B, Intermittent rTMS protocol, where 2 pulses of TMS were delivered at 50Hz frequency as a block and each block was delivered at 0.1Hz frequency for 190s.

## 3.3.2 rTMS protocol

A Magstim Rapid stimulator was used to measure the active motor threshold (AMT), defined as the minimum intensity required to evoke a MEP with 100 µV peak-to-peak amplitude in at least 5 trials out of 10 during a steady voluntary contraction (20% of

maximal contraction) of 1DI. A Magstim 2002 stimulator was used to determine the RMT, as described previously. Before delivering the rTMS protocol, baseline MEPs were recorded from each subject with 30 single pulse TMS applied at a rate of 0.2Hz with an intensity of 1.2xRMT (Fig. 3.1B). For the intervention, a modified intermittent Theta Burst Stimulation (iTBS) paradigm (Huang et al., 2005) was used. Pairs of stimuli (intensity 0.9xAMT) with 20 ms spacing were delivered every 200ms for 2s; such stimulus trains were given at a frequency of 0.1 Hz for 190s, amounting to a total of 400 pulses during a single intervention session. Occasionally, there has been a brief pause during rTMS intervention, allowing the coil to cool off. MEP measurements (n=40) were taken again immediately following iTBS using the same stimulation parameters as at baseline.

### 3.3.3 Data analysis

Evoked MEP amplitudes for all four muscles were rectified and averaged separately. In all experiments, the MEP window under curve was measured manually from the onset of the response to the point where it returned to the background level usually 40~60ms after the onset of TMS. The mean value from MEP area was calculated from a maximum number of 40 responses before and after the intervention for each subject for both PAS and rTMS protocols. The mean values from before and after data set were compared for each subject in all four muscles. T-test, paired t-test or ANOVA were employed, and results were considered significant only if $p < 0.05$.

Any change in the MEP amplitude after the intervention was calculated as a percentage of increase or decrease considering the mean response from before as a baseline. Any significant changes ($p<0.05$) in the response area after the intervention protocols was calculated and tested using t tests.

## 3.4 Results

Figure 3.2A shows an example of the rectified averaged responses from all four muscles before and after the PAS intervention from single subject who received motor point

stimulation at FDS. Post intervention MEP was increased significantly for flexor and more distal finger abductors but not for the extensor. 1DI showed maximum facilitation and MEP amplitude increased 129% ($P<0.0001$) for 1DI, 125% for APB ($P<0.0001$) and 85% for FDS ($P<0.0001$). EDC showed an opposite behaviour and the MEP decreased by 20% compared to the baseline measurements, which was a significant suppression ($P<0.04$).

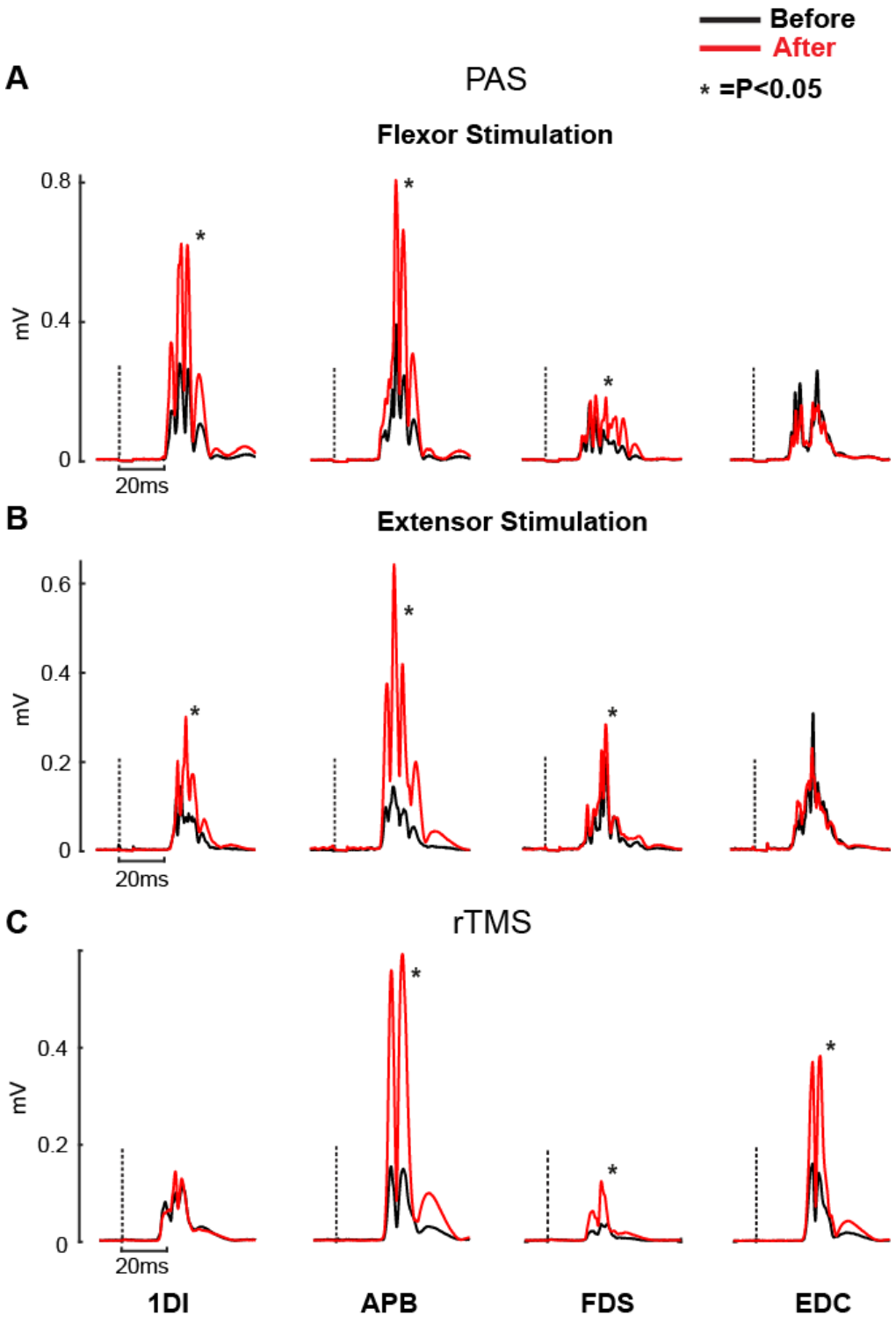


***Figure 3.2. Example result in single subject after testing with PAS and intermittent rTMS protocols.***

Dotted line indicates the TMS onset. Black trace=MEP before, red trace=MEP after. *, P<0.05. A, Average rectified MEP traces recorded from 1DI, APB, FDS and EDC muscles before and after running PAS protocol where Flexor (FDS) received stimulation. MEP increased significantly in all muscles except for EDC. B, Similar traces as in (A), but Extensor (EDC) received stimulation during the intervention. MEP increased significantly for all muscles except for EDC. C, Average rectified MEP traces

recorded from similar muscles before and after running the intermittent rTMS intervention protocol. Here, MEP increased significantly in all muscles except for 1DI.

A similar result was observed when the extensor muscle was stimulated and paired with the TMS, shown in the example traces in Figure 3.2B. Again, the responses in 1DI, APB and FDS were significantly ($P<0.005$) greater after paired stimulation, but there was no change in the EDC measurements. The noticeable characteristics of much bigger facilitation in the distal muscles than the FDS persisted, where increases in 1DI, APB and FDS were 97%, 254% and 49% respectively.

Figure 3.2C shows similar example of averaged sweeps of rectified MEP from a single subject before and after testing rTMS protocol. In contrast to PAS, rTMS appeared able to induce plastic changes also in the EDC muscle (Fig. 3.2C). In this subject, the MEPs in the APB, FDS and EDC muscles were all significantly increased (increases relative to baseline of 223%, 131% and 121% respectively, all $P<0.05$). Here, the MEP in the 1DI muscle was not significantly changed ($P>0.05$).

All the differences in after responses as a percentage of before measured from individual subjects were compiled, analyzed, plotted and compared against each muscle. Figure 3.3 illustrates the group results from both the PAS and rTMS protocols for all four muscles. All the differences in after responses as a percentage of before measured from individual subjects were plotted and compared against each muscle. The PAS results have been further separated into flexor (Fig. 3.3A) and extensor stimulation protocols (Fig. 3.3C). For the flexor stimulation protocol, the mean response from 1DI for all subject showed the maximum change, with an increase of 182% in the MEP amplitude ($P<0.001$). The second highest change was seen in APB, MEP of which increased 154% ($P<0.003$) from before measurements. After responses in FDS also increased significantly (133%, $P<0.03$) in the group result. MEP amplitudes in the extensor muscle (EDC) failed to manifest any changes in the accumulated result after PAS intervention with flexor stimulation.

Results gathered for extensor stimulation protocol from all subjects almost mirrored the result found from earlier protocol. Both distal finger muscles showed maximum increase in MEP after the test, where the % increase for 1DI and APB were 169% ($P<0.006$) and

216% respectively (P<0.001). Relatively smaller but significant increase was observed in FDS (129% P<0.04) and after measurements in EDC remained unchanged.

Figure 3.3E shows mean percentage changes in the MEPs after testing rTMS protocol from all subjects. Significant increase in MEP in all four muscles were observed and the changes were almost similar in 1DI (141%, P<0.01), APB (141%, P<0.02), FDS (130%, P<0.01) and EDC (130%, P<0.02).

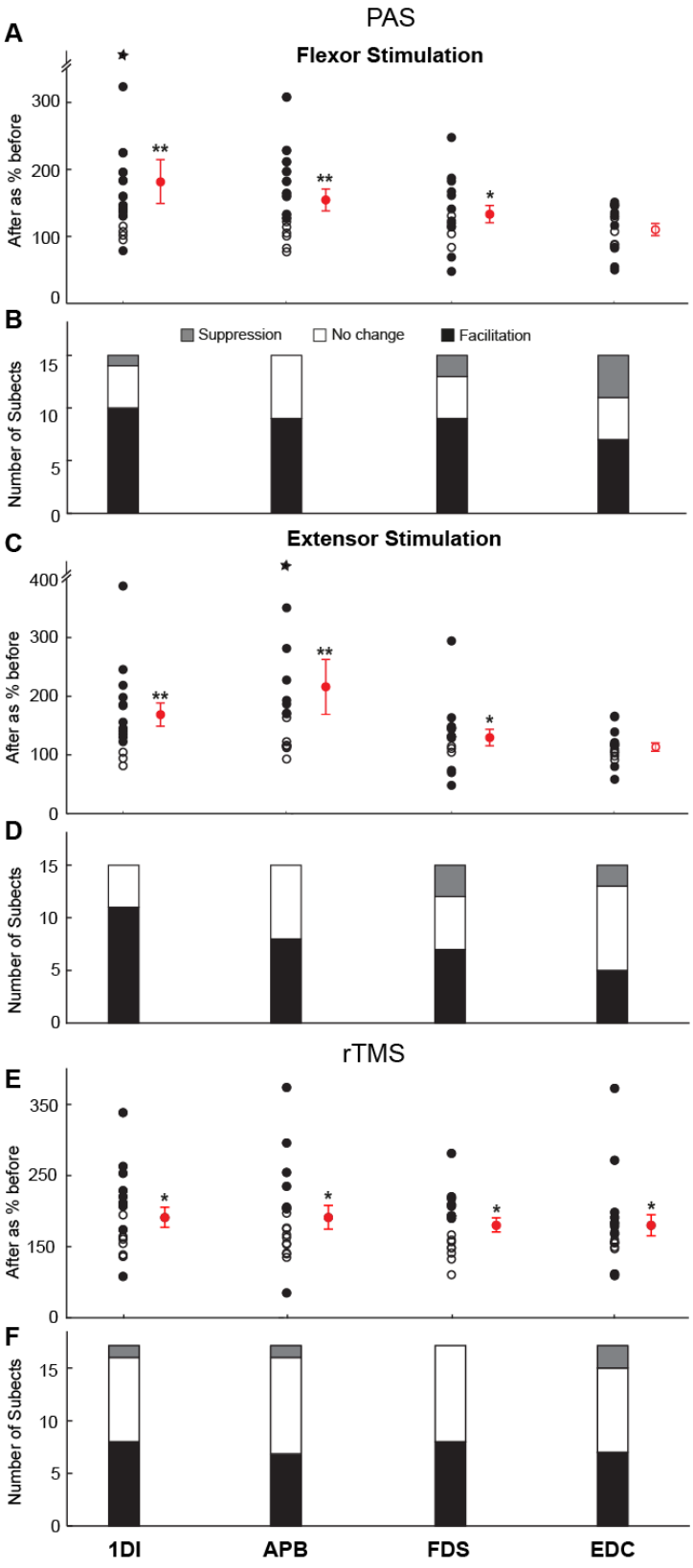


***Figure 3.3. Individual and group results in four muscles.***
A-F, Plots showing mean changes (expressed as percentage of baseline MEP) in MEP after running a protocol for all subjects. Bar plots are showing number of subjects with observed effects. Black=significant facilitation, grey=significant suppression, white=no change. *, P<0.05; **, P<0.005. A,B, Results after running PAS protocol where flexor

stimulation was paired with TMS. Consistent significant facilitation occurred in all muscles except EDC. C,D, Results after running PAS protocol where extensor stimulation was paired with TMS. Consistent significant facilitation occurred in all muscles except EDC. E,F, Results after running intermittent rTMS protocol. Significant facilitation was observed in all four muscles in this group data.

Figure 3.3 (B, D) examines the number of individual subjects who showed significant facilitation, significant suppression or no change for the PAS flexor and extensor stimulation protocols. Almost 2/3$^{rd}$ of the subjects showed a facilitatory effect in the after MEPs in flexor and distal hand muscles. In contrast, less than half of the subjects showed facilitation in the extensor muscle. Interestingly, 4 subjects ended up with suppression in EDC output (Fig. 3.3B). Stimulating extensor showed a similar trend and over 50% of the subjects showed increased MEP response in distal hand muscles, almost 50% subjects showed increased MEP in flexor, whereas less than 50% subjects showed increased MEP in extensor muscle (Fig. 3.3D).

For the rTMS protocol, as illustrated in Figure 3.3F, almost 50% subjects presented with an increase in the after-MEP response in all four muscles and did not show any trend for any single or group of muscles.

Plotted data in Figure 3.4 illustrates the overall comparison of changes for each muscle and protocol. Figure 3.4 (A-C) represents the scatterplot for PAS results. FDS vs EDC plot shows a trend where more changes were found in FDS, making it more susceptible to changes with PAS protocols. Also, both 1DI and APB received more facilitation than EDC or FDS. A three factor ANOVA analysis showed that the ‘after responses’ in 1DI, APB and FDS were significantly different than that of the EDC ($p<0.05$) in this case.

Figure 4

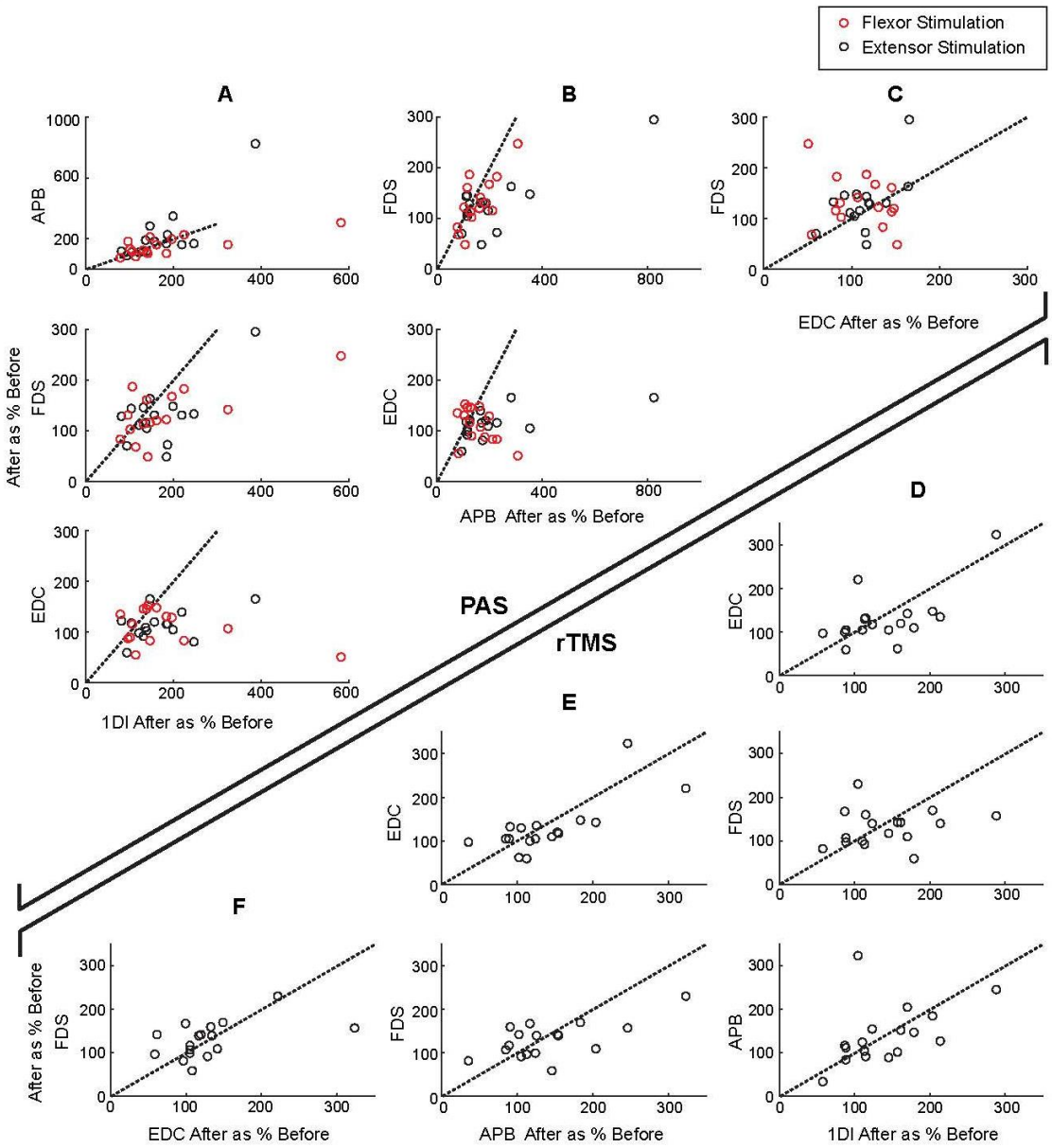


***Figure 3.4. Scatter plots illustrating changes in muscles with relation to each other.*** Results are expressed as percentage of baseline MEP, data from all subjects after tested with protocols. A-C, Relation and trend of changes in each muscle against others after running the PAS protocols; Red circle, when flexor stimulation was paired with TMS; black circle, when extensor stimulation was paired with TMS. Following hierarchy was observed: 1DI>APB>FDS>EDC. D-F, Similar scatter plots from changes in MEP after intermittent rTMS protocol. No consistent trend was noticed.

Figure 3.4 (D-F) shows similar scatterplots representing all after changes as percentage of before in all four muscles for the rTMS protocol. In contrast with the PAS, no

consistent change was observed, and ANOVA test did not reveal any significant changes in any pair.

Summing up the results finds that more distal hand muscles showed most pronounced facilitatory effect after paired cortical and motor point stimulation irrespective of the muscle stimulated. The flexor muscle also showed significant facilitation after paired stimulation, which was greater than the extensor muscle. In the contrary, a generalized facilitation was observed in all muscles after rTMS.

## 3.5 Discussion

### 3.5.1 Cortical stimulation paired with time adjusted motor point stimulation induces plasticity

Widely accepted protocols of PAS involve precisely time adjusted stimulation of a peripheral nerve, most often electrical stimulation of median, ulnar or radial nerve, and a cortical stimulation capable of activating the targeted muscles. The peripheral nerve stimulation sends mixed afferent input through the muscle spindle or mechanoreceptors via Ia or II afferent fibres which carries an orthodromic volley to the supraspinal or cortical level. The functional nature and areas of activation in the primary motor cortex after cutaneous or deep stimulation in primates was known from some early seminal works (Strick and Preston, 1978, Lemon, 1981). Before reaching the primary motor cortex, this mixed afferent input along with a direct thalamic-M1 input (Hooks et al., 2013), activates regions in the primary or secondary somatosensory areas, which were previously reported by several studies (Pons and Kaas, 1986, Allison et al., 1989, Forss et al., 1994). Advancement in functional and magnetic brain imaging techniques has allowed researches to find more complex areas which are activated by this input in both ipsilateral and contralateral cortex with an activation pattern relying on temporal distribution and onset latencies. These bilateral areas of activation roughly include: primary sensorimotor cortex, secondary somatosensory cortex, posterior parietal (Brodmann's Areas) and anterior operculum, supplementary motor area, few areas in the frontal cortex (Boakye et al., 2000, Korvenoja et al., 1999). Overall, the contralateral areas seem to be activated before the ipsilateral sites (Korvenoja et al., 1999). A

cutaneous afferent input, for example, tactile stimulation to digit, showed a similar tendency in activating the brain areas (Boakye et al., 2000). The presence of cerebello-thalamo-cortical (Asanuma and Hunsperger, 1975, Hamada et al., 2012) or thalamo-cortical projections (Home and Tracey, 1979), which may act as a relay station for the afferent input before reaching to somatosensory or motor cortex is also undeniable (Stefan et al., 2000, Carson and Kennedy, 2013).

My modified PAS protocol implementing a direct stimulation of muscle at 3x perceptual threshold close to motor point, instead of a median nerve stimulation, was able to modulate the MEP amplitudes. This result is not unsurprising because the central contribution of motor point stimulation achieved via stimulation over muscle belly is comparable with the central contribution evoked by direct stimulation of a peripheral nerve (Bergquist et al., 2011b, Bergquist et al., 2012). Besides, it has been shown earlier that peripheral electrical stimulation sufficient to evoke contraction can co-modulate the excitability of primary sensory and motor cortical areas (Schabrun et al., 2012, Koželj and Baker, 2014, Ridding et al., 2000). Therefore, the motor point stimulation over motor threshold instead of a median nerve stimulation was able to send the required afferent volley upward for to generate a pre-synaptic potential in the motor cortex, the first component of PAS. The effect of this modified PAS protocol was mostly facilitatory. This observation supports spike-timing dependent plasticity like mechanism since an EPSP evoked by TMS at motor cortex was preceded by the afferent input within 25ms window. Multiple pathways and structures in the cortical connectivity might be involved inducing STDP (Caporale and Dan, 2008) mediated activation of LTP (Baranyi et al., 1991, Hess and Donoghue, 1994, Stefan et al., 2000).

### 3.5.2 The bias in plasticity mirrors changes during recovery after corticospinal lesions

One of the most debilitating residual impairments of stroke or spinal cord lesion is reduced hand function caused by the development of spasticity. Often, asymmetrical extensor weakness in the hemiplegic upper limb becomes prominent during recovery after such lesions. This produces a stereotypical flexor-extensor bias in the form of asymmetrical weakness affecting functionality. My results from PAS protocol showed

striking similarity with the outcome of some of those changes where flexor muscle strengthens specifically during recovery. Several secondary changes are believed to be the cause of this phenomenon which includes reduced stretch reflex threshold (Powers et al., 1989), increased reflex gain (Thilmann et al., 1991a), reduced reciprocal inhibition (Artieda et al., 1991, Baykousheva-Mateva and Mandaliev, 1993) and uncoordinated coactivation of muscles (Baykousheva-Mateva and Mandaliev, 1993, Beer et al., 2000, Dewald et al., 1995). Asymmetric or reduced cortical drive triggering imbalanced reciprocal inhibition from flexor to extensor and vice versa may also be a factor for the development of flexor spasticity and hypertonia (Lackmy-Vallee et al., 2014). However, studies in primate implicates that strengthening of reticulospinal tract during recovery after stroke or corticospinal tract lesion can produce significant bias in flexor extensor remodelling, where connections towards flexor becomes stronger (Zaaimi et al., 2012). This pronounced bias in different agonist and antagonist muscle groups reflects their functional and organizational differences substantially. Numerous evidences suggest that the flexor and extensor muscles of upper limb are differently controlled at various levels of the nervous system. Higher cortical drive is required for the activation and precision of extensor muscles in the upper limb, which suggests the presence of differently organized pathways for flexor-extensor muscles (Divekar and John, 2013, Yue et al., 2000, Martin et al., 2006). A stimulus triggered averaging study by Belhaj-Saif et al. (1998) showed that flexor muscles are more likely to be inhibited from the connections derived from magnocellular red nucleus and primary motor cortex (Park et al., 2004). They also found a biased strengthening of the distal forelimb muscles than the proximal ones, mediated by the red nucleus and cortical connections.

The imbalanced strengthening of different muscle connections during recovery in the corticospinal tract lesioned macaque was previously reported by Zaaimi et al. (2012). Of course, the role of reticulospinal tract during recovery after unilateral pyramidal tract lesion was evident from this experiment, yet another striking feature of this reticulospinal tract mediated recovery was also prominent- the motor input connections towards the flexor and intrinsic hand muscle was significantly strengthened, whereas the input towards extensor showed no change. These findings were quite similar with the biased flexor facilitation and extensor weakness after corticospinal tract lesion in monkey reported by Belhaj-Saïf and Cheney (2000). In this case, however, the plasticity

was observed in the rubrospinal tract mediated output, which is mostly absent in human. Since my observed effect of biased plasticity after paired cortical and afferent input is most likely to be originated from cortex, it would be unlikely to have any indirect and passive reticulospinal tract mediated changes but not impossible. There might be several explanations that can speculate biased changes in flexor vs. extensor. Some of the previous studies emphasized the differences and distinct pattern in the cortical projection or input towards distal, flexor and extensor muscle groups in the upper limb in human and primate (Palmer and Ashby, 1992, Phillips and Porter, 1964, Clough et al., 1968, Lemon et al., 1986). While testing short latency facilitation with TMS, Palmer and Ashby (1992) found a hierarchical monosynaptic facilitation which is greater to motoneurons of distal hand muscles and flexors than more proximal and extensors muscle groups and argued that there are more cortical projections present towards distal hand and flexor muscles than extensors.

A striking feature of my findings was the hierarchy in the plasticity towards distal hand muscle, flexor and extensor, which was evident in the PAS protocol where motor cortex was stimulated synchronously paired with an afferent stimulation, but not in rTMS protocol, where only the motor cortex received stimuli. It is possible that the functional organization in cortical and subcortical level of sensorimotor system inherently possess a subtle but highly characteristic blueprint that favours adaptations towards distal and flexor muscles during task specific learning, acquiring new skills or regaining functions, which all requires active or functional afferent input. Contribution of afferent and sensory input in changing cortical or subcortical plasticity is undeniable as evidenced by many researchers (Vallence et al., 2012, Hamdy et al., 1998, Martin et al., 2006). Afferent inputs might also be responsible modulating motor plasticity during practice (Godfrey et al., 2013, Divekar and John, 2013), learning (Krutky and Perreault, 2007), active or passive movements (Chye et al., 2010, Lemon et al., 1986).

The Possibility of structural differences at cellular or molecular level in the flexor-extensor motoneuron system cannot be excluded from the factors affecting reorganization of neural network (Massey et al., 2006, Kwok et al., 2011). Particularly, emerging evidence suggests a role of Perineuronal Nets (PNN) in acquisition of plasticity in the central nervous system during learning or recovery, where PNN restricts plasticity (Carulli et al., 2010, Happel et al., 2014, Cabungcal et al., 2013). It has been found that removal of PNN can decrease inhibition and increase gamma activity which

potentially upregulates plasticity (Lensjo et al., 2017) in neural network. Collateral sprouting of axon after damage in sensory afferents is known to affect cortical and subcortical plasticity in human aided by depletion of PNN in the cuneate nucleus (Kaas et al., 2008). The possibility of differential PNN reorganization in cortical or subcortical pathways controlling flexor-extensor bias during motor learning, recovery after lesion or during artificial induction of plasticity with PAS protocols cannot be excluded.

In this study, my results showed that it is possible to induce plasticity in cortical and, arguably in the subcortical level, with simplified PAS techniques where afferent input arrives directly from muscle stimulation. My result also mirrors the biased plasticity in the distal hand muscle and flexor, which strengthens flexor and extensor remains weak during recovery in patients with stroke or spinal cord injury. Direct cortical repetitive stimulation with rTMS could also modulate cortical plasticity with a generalized facilitatory effect without any preference towards any muscle group. This result suggests that presence of an afferent input might be in effect or necessary for the induction of biased plasticity towards functional distal hand muscle and flexor. This interpretation leaves a ground for further investigations into plastic changes in different hand muscle groups in health and disease and will help to design effective and non-invasive translational methods to improve motor performance in upper limb after brain or spinal cord lesions.

# Chapter IV

# INDUCTION OF PLASTICITY IN THE MOTOR OUTPUT WITH PAIRED MOTOR IMAGERY AND TRANSCRANIAL MAGNETIC STIMULATION

## 4.1 Introduction

Performing a mentally simulated movement or Motor Imagery (MI) initiates complex neural mechanisms involving activation of interconnected areas in the central nervous system. The impact of mentally simulated action in large areas of the brain has been studied extensively over the last few decades, including motor cortex. Those related areas in the brain influenced by MI share some of the common functionality of actual movement (Jeannerod, 1995). MI activates cortical and subcortical areas and mirrors some neurophysiological properties seen in actual movement (Ingvar and Philipson, 1977, Roland et al., 1980, Rao et al., 1993). Activation of brain regions during MI is generally considered to represent cognitive motor preparation and programming (Yue and Cole, 1992, Decety et al., 1994). Inconspicuous activation of the motor cortex or specific pools of motoneurons during MI was highly anticipated but debatable until a number of studies conducted with electrophysiological techniques such as Transcranial Magnetic Stimulation (TMS), EEG or ERPs have been able to show direct involvement of the motor cortex with MI (Rossini et al., 1999, Beisteiner et al., 1995, Romero et al., 2000). Direct or indirect activation of motor cortex during mental simulation of movement was also observed in studies that used high-resolution fMRI or PET techniques (Lotze et al., 1999, Dechent et al., 2004). The striking feature of congruent activation of motor cortex during MI continued to influence many researchers to attempt measuring motor cortical output and plastic changes non-invasively. Although the available resources are still limited, Motor Evoked Potential (MEP) by TMS (Rothwell, 1991) has been considered one of the most convenient and non-invasive methods to measure motor cortical output to date. Later, a number of TMS studies concluded with the evidence that MI exerts a direct or indirect effect on motor cortex and modulates corticospinal excitability, which may also have influenced by co-activated supplementary motor area (Abbruzzese et al., 1996, Facchini et al., 2002, Pascual-Leone et al., 1995, Lacourse et al., 2005). This characteristic of MI to induce motor cortical activation led to researchers

utilizing it in conjunction with physical therapy (Warner and McNeill, 1988) or to improve motor learning skills (Yágüez et al., 1998). It has since been regarded as a 'backdoor' to activate the cortico-motoneural components to improve recovery after brain lesions, especially after stroke or spinal cord injury (Page et al., 2005, Liu et al., 2004, Crosbie et al., 2004, Dijkerman et al., 2004, Jackson et al., 2001, Sharma et al., 2006, Grangeon et al., 2010). The summarized outcome of these studies shows lack of efficacy, sensitivity or translational benefit, which necessitates further research to utilize the potential of MI. Previously, during a TMS study, Kasai et al. (1997) found that MI of wrist flexion could activate motor cortex and increased MEP of forearm muscles, but there is still lack of evidence whether MI can be utilized to change the motor cortical excitability for a sustained period to induce plasticity.

In this study, I tested whether MI in combination with TMS can generate a long term potentiation (LTP) and long term depression (LTD) mediated changes in the corticomotoneuronal output to forearm flexor, extensor and hand muscles. Some paired and repetitive stimulation protocols of TMS are already known to change plasticity in the corticospinal connections (Ziemann et al., 1998a, Stefan et al., 2000, Godfrey et al., 2013), but there is still lack of knowledge about how these changes affect different group of muscles in the upper limb, especially with MI.

The working hypothesis of my experiment was to utilize the modulatory effect of MI in conjunction with TMS to induce robust plasticity in the motor output using a novel technique. It was also an intention to observe any hierarchy or flexor-extensor difference in the potential of MI to induce plasticity in corticomotoneuronal projections to different muscles controlling the hand. I previously demonstrated a gradient like plasticity facilitating motor outputs towards intrinsic hand muscles and forearm flexor more than the forearm extensor after delivery of a PAS protocol that relies on integration of sensory and motor input (Chapter III).

In healthy humans, I recorded and compared cortical responses from the forearm and hand muscles before and after delivering paired TMS and MI paradigms. This approach of influencing plasticity may play a role in further understanding corticomotoneuronal connections, designing rehabilitation therapy and finding new ways of learning motor skills.

## 4.2 Materials and Methods

### 4.2.1 Subjects

A total number of 10 healthy volunteers (8 female; age range 19 – 45 years) with no contraindication for TMS, were tested in 60 different sessions of the experiment. All procedures were approved by the local ethical committee of Newcastle University Medical School (ethical approval number 000023/2008) and were in accordance with the Declaration of Helsinki. Prior to each experiment, full written consent was obtained from each participant after a brief explanation of the intent and procedure of the experiments.

### 4.2.2 Electromyographic (EMG) recording

In all experiments, Electromyogram (EMG) was recorded from four contralateral muscles- Flexor Digitorum Superficialis (FDS), Extensor Digitorum Communis (EDC), Abductor Pollicis Brevis (APB) and First Dorsal Interosseous (1DI). A pair of adhesive surface electrodes (model H59P, Kendall) were placed directly over individual muscle belly with an inter-electrode spacing of 2-3 cm for FDS/EDC, and around 1cm for APB/1DI. Electrodes were connected to a Digitimer D360 amplifier (gain, 1000; bandpass filter, 30 Hz to 2 kHz) and EMG signals were stored in a laboratory computer system for analysis after digitized using an analogue-digital converter (micro1401, Cambridge Electronics Design, Cambridge, UK).

### 4.2.3 Transcranial Magnetic Stimulation (TMS)

A figure of eight shaped magnetic coil (model D70$^2$) fitted with an optical position tracker and connected to a Magstim (BiStim2, Magstim Ltd UK) stimulator was used to provide focal TMS stimulation to the contralateral hemisphere. Following earlier studies (Rossini et al., 1994, Stefan et al., 2000), the coil was positioned tangentially over the skull with the handle pointing backward and laterally producing 45$^o$ angle to the sagittal plane for all experiments. A second position tracker attached to a headband was placed over the subject's forehead. A Brainsight TMS Navigation system (Rogue Resolutions Ltd, Cardiff) was used to track and monitor the relative coil position in real time during each session to provide TMS precisely on the defined spot.

### 4.2.4 Experimental procedures

Subjects were seated in a comfortable and slightly reclined height adjusted chair and placed their right forearm on an adjacent table. They were instructed to stay completely relaxed all times unless instructed. Complete muscle relaxation was ensured by live monitoring of the EMG signal on a monitor. MEPs from the FDS and EDC muscles were assessed with suprathreshold TMS to locate the optimal site that generated best responses from the both muscles. This site was then marked as the 'hotspot' in the Brainsight TMS Navigation to use throughout the experiment. Like many other previous studies (Rossini et al., 1994, Stefan et al., 2000), resting motor threshold (RMT) was obtained and recorded. It is the minimum stimulator intensity required to evoke at least 50μV response in both relaxed muscles in at least 5 out of 10 repeated.

Prior to intervention, each participant was trained to perform MI where they were instructed to imagine a specific wrist movement, either flexion or extension. During each training session, a few cortical stimuli were applied while the participant practiced MI. Peak to peak increase in the MEP amplitude during MI without any noticeable background EMG activity was regarded as a successful outcome of such training. For example, while the subject was imagining wrist flexion, a test TMS (intensity 1.2xRMT) would be delivered to the contralateral motor cortex. An increase in the MEP amplitude of the FDS muscle would be observed if the subject could have imagined the wrist flexion successfully (Fadiga et al., 1998). Most of the time it was difficult and challenging for the subjects to stay concentrated and imagine a task repeatedly for a while, but the aim was to achieve a good level of MI to be paired with TMS. I found that if the participants were asked to perform the actual movement, wrist flexion for instance, prior to MI, it improved their concentration, efficacy, and timing of imagination. Therefore, I adopted an approach where subjects were asked to repeat actual movement three times at the start of each MI cycle. The actual wrist flexion or extension had to be finished at least 4-5s before the delivery of TMS. This was confirmed by careful monitoring of the resting baseline EMG throughout the experiment. Since I delivered TMS at a rate of 0.1Hz during intervention, a subject would get a 5s window to repeat 3 actual flexion or extension, depending on the protocol, before starting MI during each cycle.

To start the experiment, baseline MEPs were recorded from all four muscles (FDS, EDC, ABP and 1DI), while TMS was given at a rate of 0.1Hz and with an intensity of 1.2xRMT (Fig. 4.1A). Two different intervention protocol were used in separate sessions of experiment. As mentioned earlier, an intensity of 0.9xRTM and a rate of 0.1Hz was used the intervention. In the first protocol (TMS+Flexion Imagination, Fig. 4.1Ai), TMS at a rate of 0.1Hz (0.9xRMT) was delivered to the contralateral motor cortex while participants actively imagined the wrist flexion. In total, 90 TMS were delivered simultaneously with flexion MI over a duration of 15 minutes. As discussed earlier, during each TMS+MI cycle and before starting flexion MI, subjects were doing 3 consequtive wrist flexion physically before coming to a complete rest prior to the MI+TMS event. Subjects were receiving direct visual feedback from the monitor placed in front of them showing live feed of the EMG recording. Occasionally, there was a brief break (1-2 minutes) during the event if the subjects were losing concentration required for the MI. Immediately after the intervention, MEP measurements were recorded again using the same TMS parameters (1.2xRTM, 0.1Hz) and experimental conditions as in baseline (Fig. 4.1C). For consistency, a total number of 60 MEPs was recorded after the tests, which were averaged later to compare with the baseline MEPs.

For the second protocol (TMS+Extension imagination, Fig. 4.1Aii), all the condition remained same, except for the imagination part. Base line MEPs were recorded as before from all four muscles. During intervention, subjects were asked to perform extension imagination of the wrist. After completion of 90 TMS+MI, after-MEPs (N=60) were recorded using same conditions as before.

All ten subjects were also tested with the four control protocols. Baseline MEPs and after-MEPs were recorded for each experiment like before. In the third (Flexion Imagination) and fourth (Extension Imagination) protocols, either flexion (Fig. 4.1Aiii) or extension (Fig. 4.1Aiv) MI was performed respectively by the subjects in a similar manner described above, but without any TMS. Subjects could still hear the click sound coming from the TMS coil placed at a distance so that they get the cue to start the next cycle of MI.

In the fifth (TMS-following-Flexion, Fig. 4.1Av) and the sixth (TMS-following-Extension, Fig. 4.1Avi) protocols, no MI was performed. Subjects were only receiving

TMS, and all other conditions remained same. For each subject, experiments (6 sessions in total) were performed in separate visits in a random order at least one week apart.

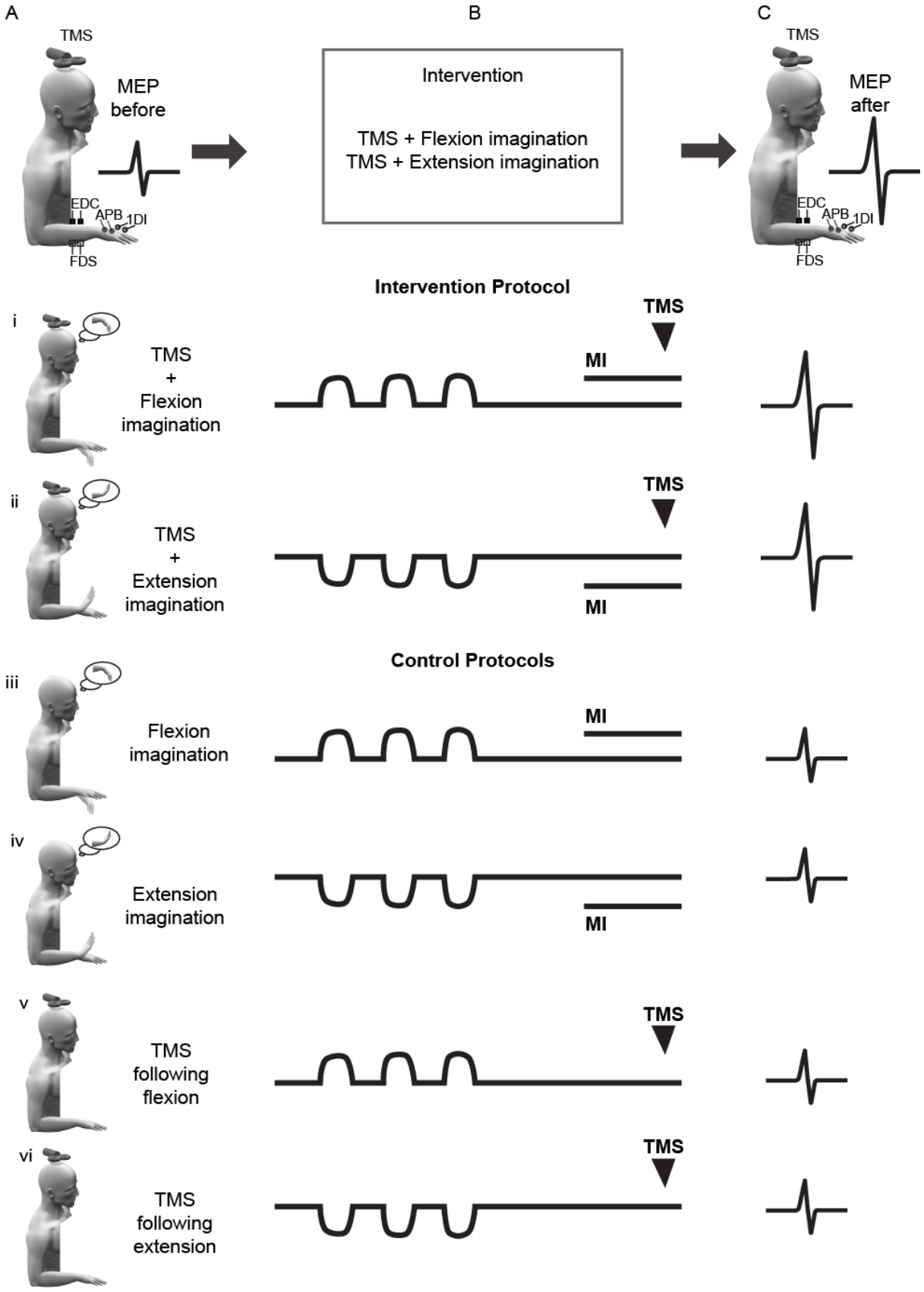


***Figure 4.1. Schematic illustration of Motor Imagery (MI) protocols.***
A, Recording and measurement of MEP from four muscles- 1DI, APB, FDS and EDC before any intervention or control protocols, B, Two intervention protocols and four

control protocols. C, Recording and measurement of MEP from those muscle after each protocol. i, Intervention protocol where Flexion imagination was paired with TMS following three successive flexion movement of the wrist ii, Intervention protocol where extension imagination was paired with TMS following three successive wrist flexion. iii, Control protocol where only Flexion imagination was performed after three successive wrist extension. iv, Control protocol where only Extension imagination was performed after three successive wrist extension. v, Control protocol where only TMS was delivered after three successive wrist flexion. vi, Control protocol where only TMS was delivered after three successive wrist extension.

### 4.2.5 Data analysis

Evoked EMGs for all four muscles were rectified and averaged separately. The area under curve was measured by determining the MEP onset and offset latencies for individual sweeps. MEP amplitudes were measured from averaged area under the curve for each muscle. Significant differences in MEP were assessed by performing t-tests from the mean area under the curve above baseline for individual sweeps. An ANOVA with factors recorded muscle and protocols was performed to assess changes at the population level, with subsequent pairwise testing as required (t-tests). Changes after the intervention were expressed as a percentage of the measurement before and the averaged response from all subjects were plotted and compared for all six protocols (two intervention and four control). The number of subjects showing significant facilitation, suppression or no change were also plotted. Changes were considered significant with a threshold of $P<0.05$. All analyses were performed using custom scripts in the MATLAB environment (Mathworks Inc, Natick, USA).

## 4.3 Results

### 4.3.1 Flexion imagination with simultaneous TMS increased MEP amplitude

Figure 4.2(A-B) shows examples of rectified averaged responses before and after each protocol in a single subject for four muscles that I recorded; FDS, EDC, APB and 1DI.

The black traces represent the average response of 20 basline MEP traces recorded before and red traces represents the average response of 60 after-MEP traces recorded after each test.

The MEP in FDS increased significantly after paired flexion imagination and TMS in the first protocol (Fig. 4.2A). Example of averaged data from before and after recordings in a single subject showed a significant increase in the MEP area (151%, $P<0.01$, Fig. 4.2A, TMS+Flexion imagination) in FDS when the subject was asked to imagine wrist flexion and received TMS simultaneously. For the same test, similar facilitatory effect was found in APB and 1DI with an increase of 104% ($P<0.03$) and 129% ($P<0.03$) respectively, both of which were less than the increase found in FDS (151%). The facilitation was smaller in EDC than all other muscles and accounted for only 37.01% ($P<0.02$) increase.

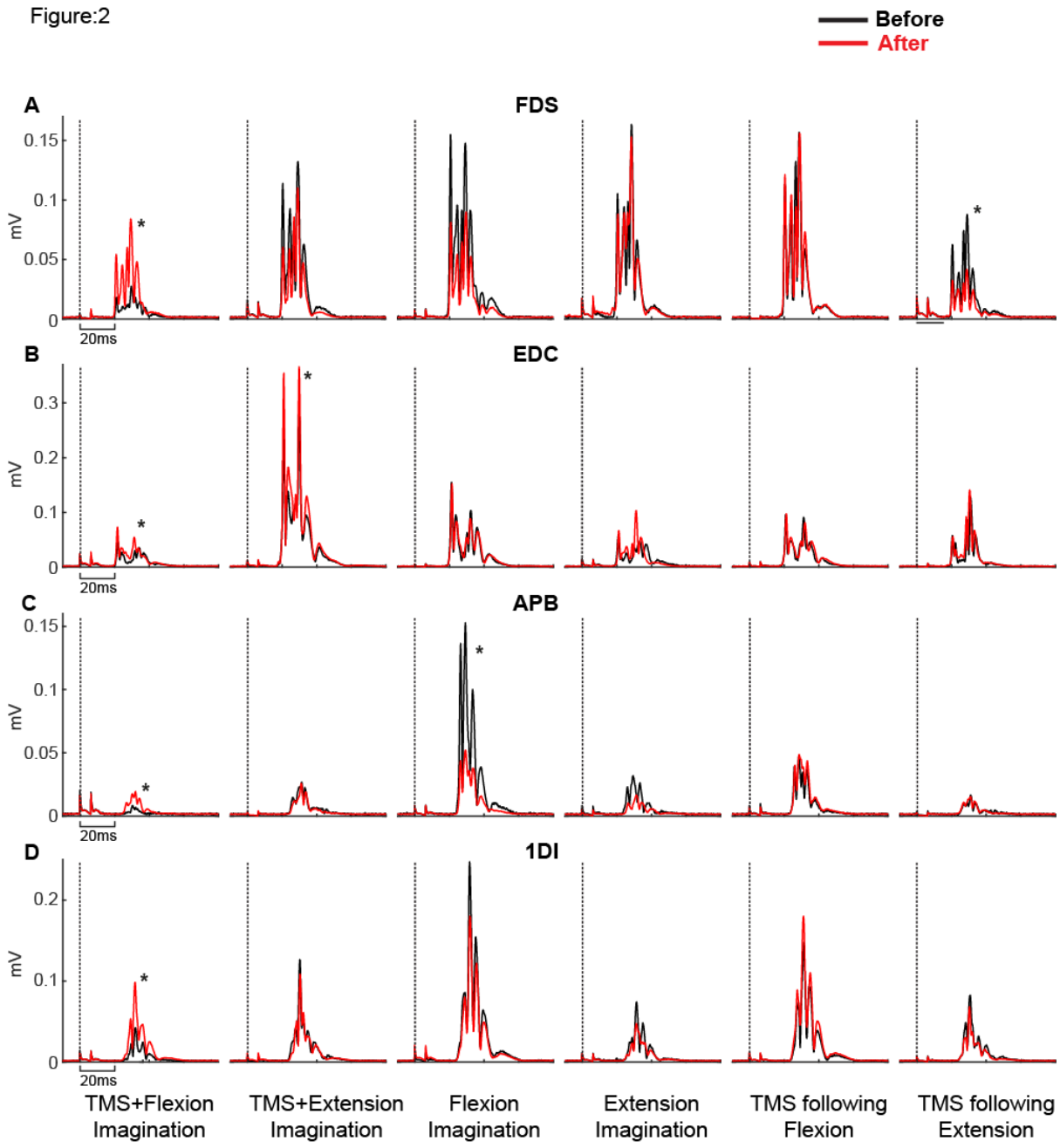


***Figure 4.2. Example result in single subject after testing six separate protocols.***
The dotted line indicates the TMS onset. Black trace=MEP before, red trace=MEP after. *, P<0.05. A, Average rectified MEP traces recorded from FDS muscle. MEP increased significantly after testing TMS+Flexion Imagination protocol. Significant suppression was noticed in this subject after TMS following extension protocol. B, Similar traces as in (A), but for EDC muscle. MEP increased significantly after testing both TMS+Flexion and TMS+Extension Imagination protocols. C, Similar traces for APB muscle. MEP increased significantly after TMS+Flexion Imagination protocol, and decreased significantly after Flexion imagination protocol. D, Similar traces for 1DI. MEP increased significantly after TMS+Flexion Imagination protocol.

The accumulated result from all subjects for the protocol showed that 90 paired TMS with flexion MI (TMS+flexion MI) sufficient to increase the MEPs in the FDS muscle significantly ($P<0.002$) with an average 189% rise (Fig. 4.3A). For this protocol, a significant facilitatory effect was seen in eight out of ten participants.

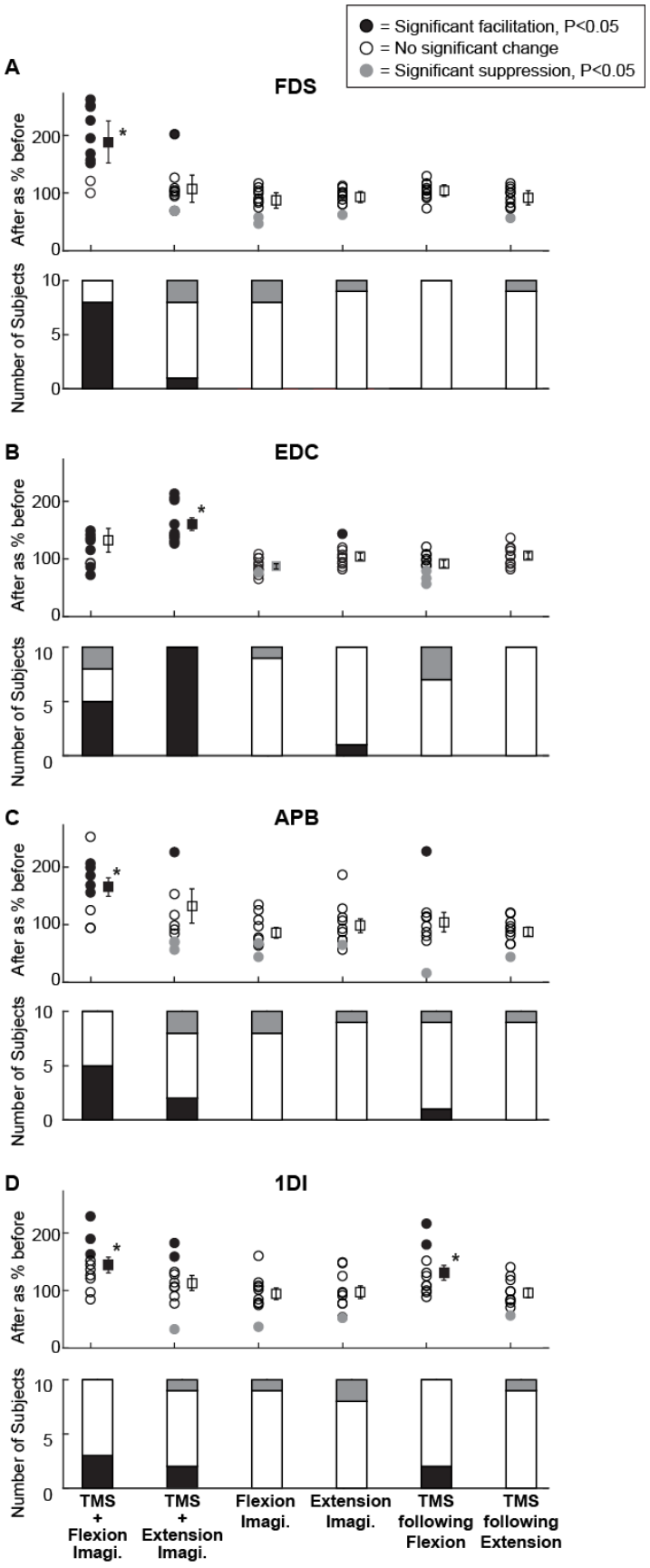


***Figure 4.3. Group results for all muscles after delivery of each protocol.***

A-D, Mean changes (expressed as percentage of baseline MEP) in the MEP were plotted in the top bars. Bottom staked bar plots show number of subjects with observed effects. Black=significant facilitation, grey=significant suppression, white=no change. *, P<0.05; A, Results for FDS after running all six protocols. Significant facilitation occurred in the TMS+Flexion imagination protocol. B, Results for EDC after running all six protocols. Significant facilitation occurred in the TMS+Extension imagination protocol. C,D, Results for APB and 1DI respectively; significant facilitation occurred only in the TMS+Flexion imagination protocol in both of the muscles.

Averaged data from all ten subjects for the other three muscles reflected the over picture seen in the example tracings (Figure 4.2). I found significant increase in MEP area both in the APB (P<0.01) and 1DI (P<0.01) muscles, with an overall increase of 165.49% and 144.40% respectively, producing facilitation smaller than that was observed in FDS. For APB, nine subjects out of ten showed some degree of facilitation, in which five subjects accounted for significant increase in MEPs. Eight subjects out of ten exhibited a tendency of facilitation in 1DI using the same paradigm, three of them were with significant changes.

A mixed pattern of changes was observed in the antagonist muscle EDC after simultaneous flexion imagination and activation of motor cortex with TMS. Although six subjects showed facilitation, five of which were significant, the other four subjects showed a decrease in the MEP, two of which were significant. In this case, when all the data from 10 subjects were pulled, the overall change in MEP was not significant (P>0.1) in EDC.

### 4.3.2 Extension Imagination with simultaneous TMS increased MEP amplitude in the extensor muscle

For the second sets of experiments (TMS+ Extension Imagination), averaged example tracings from a single subject showed a significant 26% increase in MEP in EDC when TMS was paired with MI (P<0.02, Fig 4.2B). Contrary to the first intervention protocol (TMS+Flexion Imagination), the other muscles here did not follow the similar pattern of facilitation in this case, rather MEP in FDS went down to 44.62% in the after the

intervention which was a significant (P<0.02) decrease. ABP and 1D1 did not show any significant changes in this case.

In the accumulated data for TMS+Extension Imagination, all ten participants showed significant facilitation in the EDC muscle both individually and combinedly, with an average160% increase (P<0.002, Fig. 4.3B). Averaged data showed that there was no significant change in any of the other three muscles. A few indivuals showed significant facilitation or suppression in FDS, APB or 1DI randomly, which was unremarkable.

### 4.3.3 Motor Imagery or TMS alone caused no consistent changes

In my third (Flexion Imagination) and fourth (Extension Imagination) protocols, subjects performed only MI of wrist flexion and extension without TMS. The before and after MEP responses for all four muscles were compared as shown in the example recording from a single subject in Figure 4.2 (A-D).

In the example subject, the first trace (Fig. 4.2A) for Flexion Imagination shows a significant decrease in the MEP amplitude in FDS (P<0.0001), which was a 72.22% reduction, but this subject was one of the two subjects who showed a reduced response after Flexion Imagination only. Recordings from EDC and 1DI showed no changes. APB tracing of MEP showed a significant decrease (P<.002) of 125.19%. Again, this subject was among the two subjects who showed suppression in APB muscle response in this test.

Example traces from the same subject after extension imagination protocol did not show changes in MEP amplitude in any muscle.

The overall result for all ten subjects are plotted in Figure 4.3 (A-D) for Flexion Imagination and Extension Imagination; both which were control protocols. For Flexion Imagination, there was no significant increase in MEP in any of the ten subjects. No consisted changes were found for Extension Imagination protocol either.

I tested the effect of TMS alone in the TMS-following-Flexion and TMS-following-Extension protocols. For TMS-following-Flexion, example recordings from a single subject (Fig. 4.2 A-D) tracings showed no changes in any of the four muscles. The same

subject showed 77.23% decrease (P<0.01) in averaged MEP amplitude in FDS after 90 TMS-following-extension input and was the only subject showing such changes.

Figure 4.3 (A-D) shows the overall result of ten subjects for TMS-following-flexion and TMS-following-extension control protocols. No consistent changes were found in those two control protocols, except for 1DI, where two subjects showed significant facilitation. I assume this is spurious due to multiple comparisons.

## 4.4 Discussion

### 4.4.1 Motor imagery with synchronized TMS induced plasticity

The present data demonstrate that MI significantly influences excitability in the motor cortex, which is consistent with many previous observations (Fadiga et al., 1998, Kasai et al., 1997, Rossini et al., 1999). It is possible that movement specific MI activates related areas in the premotor and motor cortex and the increases overall cortical drive resulting in a 'subthreshold' state. This can increase the overall corticospinal excitability without necessarily firing the alpha motor neuron and the related muscle does not become functional until a motor command is executed (Yahagi and Kasai, 1998). Pre-activation of areas in the motor and premotor cortex in the presence of mental representation of action was found from works in monkey and human. The premotor cortex (F5) is known for imagery representation (Murata et al., 1997) and the mirror neurons can be triggered by visual action recognition (Gallese et al., 1996). Automatic retrieval of motor action by observation was previously seen in human where the excitability of the motor system was increased simply by observing a similar action performed by another person (Fadiga et al., 1995, Rizzolatti et al., 1996). An altered state of excitability or subliminal activation of the motor cortex induced by mental simulation or MI has been also documented by measuring MEP in many previous and recent studies (Rozand et al., 2014, Braun et al., 2003, Li et al., 2009). This natural phenomenon of covert activation of the cortical and subcortical network at a subthreshold state by MI was utilized in this study as a key concept to induce a lasting effect in the corticospinal output in conjunction with another non-invasive stimulation.

It is now well established that Paired Associative Stimulation (PAS) and several Repetitive Transcranial Stimulations protocols can effectively induce plasticity at cortical, and arguably at subcortical level, which affects final motor output once EPSP is generated at pyramidal cells in the motor cortex. In standard PAS protocol, an afferent input is delivered before the TMS at motor cortex within a favourable window of time to modulate of LTP or LTD in a manner consistent with STDP mechanism (Stefan et al., 2000). Conversely, in an ideal rTMS protocol, intermittent theta burst stimulation is utilized to modulate cortical excitability (Huang et al., 2005). Intermittent rTMS is also known to induce LTP/LTD mediated plasticity in the motor cortex. As per recent evidence, both PAS and rTMS modulations are regulated by NMDA receptors/$Ca^{2+}$ influx at synaptic level and can cause persistent changes in synaptic excitability in the pyramidal cells, especially in the dendritic projections (Huang et al., 2017, Sjöström et al., 2008). Since MI is capable of activating several interconnected areas in the premotor and motor cortex, it is possible that onset of MI induced sub-threshold activation of motor cortex allows convergence of stimulus input at the cortex once paired with TMS. This can induce synaptic changes consistent with LTP mechanism. In this case, instead of an afferent input, MI served functionally comparable purpose (presynaptic input for LTP) and the readiness of the motor cortex for the burst of action potentials for an anticipated movement was synchronized with TMS input. These results showing modulation of cortical excitability after paired MI and TMS might share a comparable mechanism of inducing plasticity explained in the PAS techniques.

Some might argue that the repetition of wrist flexion or extension prior to the intervention (MI+TMS) might have induced some plasticity in the motor cortex as evidenced by (Classen et al., 1998). If this was the case, similar changes would be observed in the MEP after delivery of the control protocols which either used MI or TMS only following similar repetitive flexion or extension. On the other hand it was quite important to achieve a consistent level of MI performance among subjects to quantify any changes after paired MI+TMS paradigms (Lebon et al., 2012, Helm et al., 2015).

### 4.4.2 Task specificity and biased plasticity in the flexor and intrinsic hand muscles vs extensor

TMS during flexion imagination was able to increase the drive in the motor-cortical output observed as an increase in the MEP amplitudes of all three flexor muscles. Interestingly, TMS with extension imagination increased responses specifically towards the extensor muscle, but not in the flexor muscles I tested. The flexion MI of wrist increased MEP in the FDS muscle the most and to some extent in other muscles and caused facilitation in the EDC muscle only in few subjects with no significance in the accumulated result. Conversely, wrist extension imagination significantly facilitated the response specifically towards the EDC muscle in all subjects, but not in the other muscles. Task-related variability in corticospinal excitability was known from previous works in monkeys (Baker et al., 1995, Lemon et al., 1986). In a recent study, Zaaimi et al. (2018) found that with task-specific direct cortical stimulation most sites in the motor cortex activate either elbow flexors or extensors, not both. Therefore, task-specific MI would be expected to recruit non-overlapping sets of corticospinal cells. During TMS, the activation of movement specific subpopulation is higher than the diffused recruitment. MI induced enhancement is also known to show specific representation at least in the contralateral motor cortex (Li, 2007, Facchini et al., 2002). My findings are in agreement with the previous findings where MI modulated corticospinal excitability was more noticeable in the target muscle than non-targeted muscles (Li, 2007). However, it cannot be rejected that wrist MI can also produce a diffuse effect and co-activates other projection areas representing distal hand muscles (Porter and Lemon, 1993). The somatotopic gradient of the motor cortex and the subliminal co-activation of other muscles during a specific task related imagination (flexion MI in FDS) can explain the diffused facilitation found in other muscles (APB, 1DI and EDC) in this case. Although the 'enslaving effect' (Danion et al., 2000) can explain the occurrence of low to moderate plastic changes in the other muscles during the flexion imagination task, it was not quite the observed effect seen in the extension imagination protocol. Only significant enhancement was noticed in the muscle of target, EDC in this case. I argue that the hierarchy of plasticity in human forearm and hand muscles play a role here. In another study (Ref: Chapter Two), I have found that LTP and LTD mediated plasticity is more prominent in APB, 1DI, and FDS than in EDC in certain conditions; which is consistent with the results observed from this study. The biased tendency of plasticity in the forearm flexor and hand muscle outputs is also seen in post-stroke patients where spasticity develops specifically in the flexor and hand muscles accompanied by extensor weakness.

This result emphasizes that there is a substrate for lasting plastic changes somewhere in the cortical, subcortical or spinal level which shows a tendency to favour the forearm flexor and hand muscle more than the extensor.

### 4.4.3 The site of Plastic changes

My results show that change in the excitability in the motor cortex or corticospinal tract output can be initiated by MI. The observed changes in the MEP reflect synaptic reorganization or changes in excitability at the cortical or subcortical level after repeated application of paired MI and TMS at motor cortex. This finding is consistent with several previous TMS and fMRI studies that found that the motor cortex can be activated by MI (Lotze et al., 1999, Kraus et al., 2018). However, controversy exists regarding the relative contribution of TMS and MI in the various levels of cortical and subcortical excitability. MEP responses evoked by TMS only do not provide a clear view about the relative contributions of the motor cortex, brainstem, spinal pools of motor and interneurons in inducing plasticity. Li et al. (2004) observed that MI has a role in modulating spinal stretch reflex pathways. They found that MI increased the EMG response during the event of muscle stretch reflex and suggested that short latency response in these muscles were greatly facilitated by MI (Li et al., 2004). It is possible that MI that activates motor cortex also sends descending inputs to spinal reflex pathways (Pierrot-Deseilligny and Lacert, 1973, Gandevia et al., 1997). Augmentation of H-reflex by mental rehearsal without any change in the fusimotor drive supports the idea that facilitation of excitability at subcortical or spinal level could be an outcome of MI (Gandevia et al., 1997, Bonnet et al., 1997).

On the contrary, (Hashimoto and Rothwell, 1999) suggested a dynamic effect of MI in the motor cortex similar to those found during actual movement as no MI-related changes in the H-reflex were found in their study. Others reported a reduction in the spinal excitability evidenced by reduced H-reflex during mental movement simulation found in human (Oishi et al., 1994, Yahagi et al., 1996). During MI, the presence of an inhibitory influence operating in the spinal level preventing depolarization of motor neurons cannot be excluded. A dual mechanism might co-exist affecting the spinal

excitability: a subthreshold drive to move in the presence of increased corticospinal tract output and a subsequent inhibitory influence preventing an actual motor discharge (Jeannerod, 2001). It can be argued that if plastic changes occur in the corticospinal connection or motor cortex, the drive towards the spinal level can also be stronger to eventually overcome any inhibitory effect. This also explains the reduction of motor threshold and increased the amplitude of MEP during MI in TMS. It is not impossible that the activation of the motor cortex, altered corticospinal excitability, the inhibitory effect of spinal interneurons and subliminal activation of spinal motoneurons contributed together and resulted in metaplastic changes after combined MI and TMS.

### 4.4.4 LTP/LDP mediated plasticity change:

My proposed hypothesis to induce plastic changes utilizing MI and combined TMS is based on the classic phenomenon seen in Paired Associative Stimulation protocols, where TMS is paired with an afferent electrical stimulation to modify plasticity in a manner consistent with LTP and LTD (Stefan et al., 2000). I speculate that the plasticity induced by simultaneous TMS and MI may have observed a similar effect. In this case though, instead of an afferent input, MI served functionaly comparable purpose (presynaptic input for LTP), where some of the motoneuron pools in the motor cortex or SMA were pre-activated by the mental simulation of movement. In this case, the functional readiness of the motor cortex for the imminent burst of action potentials for an anticipated movement was synchronized with TMS input and resulted in a corticospinal drive sufficient to activate the spinal motor neurons or sufficient to overcome the spinal inhibition (which normally prevents any overt movement during MI). My results show a lasting effect of changes in the corticospinal tract excitability after the paired intervention and those changes were mostly task-specific.

### 4.4.5 Motor imagery and rehabilitation:

The ability successfully to imagine a movement relies on the internal action representation of the brain. In the past, the question emerged of whether patients with

significant brain damage preserve this internal representation for successful MI, or if the brain loses the ability to imagine a specified task to a significant extent. Since intact parietal cortex is important for generating MI representations, it was found that patients with lesioned parietal cortex struggled to predict the time properly for differentiated finger movement tasks (Sirigu et al., 1996). Later on, it was found that acute or chronic hemiplegic patients were able to use motor imagery to activate partially damaged motor networks (Johnson, 2000, Johnson et al., 2002), suggesting the ability of the brain to retain the motor representations in the event of loss of function. In recent years, with the notion that central processing of imagined movement involves interconnected regions of the brain including the motor cortex, SMA or frontal cortices, MI alone or in conjunction with other measures has increasingly been used to help motor recovery in patients with neurologic disorders. Particularly, the feasibility of using MI as a tool to recover limb functions after brain lesion has been studied extensively. A number of pilot studies and patient trials reported a positive outcomes of MI implementations (Page et al., 2005, Liu et al., 2004, Crosbie et al., 2004, Page et al., 2001, Dijkerman et al., 2004, Jackson et al., Mulder, 2007, Sharma et al., 2006). More recently, MI has been reported to improved motor performance in spinal cord injury patients with quadriplegia (Grangeon et al., 2010, Mateo et al., 2015). One crucial aspect of rehabilitation is to reorganize the motor system network (Jones, 2000) by inducing activity dependent plasticity in the motor cortex (Jones, 1993, Florence et al., 1998), brainstem (Fisher et al., 2012, Foysal et al., 2016) or spinal cord (Wolpaw and Tennissen, 2001, Dunlop, 2008). Until now, MI has only been used as a passive way to activate motor connections in the absence of an actual movement or in conjunction with other physical training, physiotherapy or behavioural therapy. My result suggests that robust plastic changes in corticospinal tract excitability could be induced using specially designed protocols of MI in conjunction with TMS to aid in motor recovery. The most interesting and crucial finding of my study was the occurrence of task-dependent plastic changes, where extension imagination task significantly increased plasticity specifically towards the extensor muscle. Although I have seen a diffuse pattern of change during the flexion imagination task, where significant plasticity changes were also observed in other muscles, a flexor-extensor bias might be an inherent nature of plasticity development. Since most post-stroke patients develop spasticity in the upper limb flexor muscles and weakness in the extensors, a targeted synchronized TMS+MI protocol designed to strengthen the weak muscles might prove useful in regaining hand functions.

# Chapter V

# CONCLUSION

It is now well established that the adaptability and plasticity at various levels of motor control contribute to shaping motor behaviour, skill acquisition and recovery after neurological lesions. The idea of changing plasticity by strengthening of existing connections through cascades of metabolic process or growth was first introduced by Ramón y Cajal (1935) and later theoretically proposed by Donald Hebb (Hebb, 1949) in his postulate. His basic concept about plasticity was "cells that fire together wire together", which has been later supported by a vast amount of scientific experiments. The adaptability of motor nervous system in humans is not confined to motor cortex but also extends to the brainstem, subcortical projections, interneuron connections and extracellular space.

There are multiple factors affecting motor plasticity, and it relies on feedforward inputs from central cortex or periphery. Example of such factors includes sensory input deprivation, functional deprivation, usage and correlation, motor learning and practice, compensatory mechanism after a lesion and even artificial stimulations triggering STDP type response.

STDP mechanism has Hebbian-like (Kempter et al., 1999, Turrigiano and Nelson, 2000) computational properties that allow predicting direction and level of synaptic plasticity induced by stimulation protocols. Evidence from a number of experiments indicate that repeated pairing of precisely timed pre- and postsynaptic action potentials can lead to lasting changes in synaptic remodelling, the efficacy of which depends on magnitude, frequency and timing of the pre-synaptic potential and EPSP (Debanne et al., 1998, Bi and Poo, 1998, Feldman, 2000, Egger et al., 1999, Zhang et al., 1998).

The outcome of remapping and reorganization in synaptic space by exploiting STDP like protocols is also influenced by its temporal symmetry/asymmetry that governs the strengthening or weakening of the connections (Levy and Steward, 1983, Sjöström et al., 2008, Kempter et al., 1999). Strengthening can occur by LTP in a paired stimulation protocol when repeatedly occurring presynaptic spikes precedes EPSP within 25ms (symmetric STDP) or 50ms (asymmetric STDP) each time. On the other hand, repeated

EPSP preceding presynaptic spikes within a window relatively longer than the LTP window or similar, causes LTD mediated weakening of the connection (Song and Abbott, 2001). This mechanism is consistent with the results from my study where precisely timed paired stimulation was delivered using a wearable electronic device. When an afferent input by stimulating biceps muscle motor point was delivered 10ms prior click stimulation, it induced a presynaptic potential before click stimulation could evoke an EPSP within the specified window of time. Repeated delivery of this paired stimulation strengthened the output towards biceps muscle significantly as indexed by an increase in the amplitude of the stretch reflex output. Conversely, when EPSP evoked by click preceded the afferent input repeatedly, it resulted in the weakening of the connection towards biceps muscle, which was reflected by a reduction of amplitude in the stretch reflex output. Since the facilitation or suppression was observed mostly in the long latency part of the stretch reflex, and click stimulation is known to activate reticular cells, it is likely that induction of plasticity took place in the reticular area, but excitability changes in the motor cortex cannot be excluded.

The STDP model synaptic plasticity by modulating LTP-LDP supports most aspects of the results observed in my other studies. A number of previous studies used various paired protocols using PAS techniques, where presynaptic potential in motor cortex or in the subcortical space, was generated before a direct cortical stimulation that evoked EPSP (Stefan et al., 2000, Ridding et al., 2000). In a comparable setup, I tested 30 subjects with a motor point stimulation to either flexor or extensor to induce presynaptic action potential prior eliciting EPSP with the application of TMS and this was repeated 90 times, resulting in a strengthening of the motor outputs towards flexor and distal hand muscles.

It remained a question that exactly how this kind of reorganization was achieved. Underlying mechanisms of cortical and subcortical plasticity are widely diversified. Cellular plasticity can be a physiological mechanism where existing neurons and synapses are reorganized to such extent that alters the functional outcome, or it can be a structural mechanism where modification occurs by morphological changes, deletion or formation of new synapses, the formation of dendritic projections or even reorganization in extra-cellular components can occur for rewiring a connection. Well accepted models calls for a cellular mechanism which largely depends of NMDA (*N*-methyl-d-aspartate) receptor or $Ca^{2+}$ channel dependent LTP and LTD, where rapid

influx of $Ca^{2+}$ occurs through NMDA receptors and rises concentration of $Ca^{2+}$ in dendritic spine triggering a cascade of intracellular mechanism (Malenka and Bear, 2004, Malenka et al., 1992, Yasuda et al., 2003). A number of modulators are already known to trigger such intracellular cascades favouring NMDAR-dependent LTP like mechanism, for example calcium/calmodulin-dependent protein kinase II (CaMKII) (Lisman et al., 2002), protein kinase M zeta (PKMξ) (Hrabetova and Sacktor, 1996), mitogen-activated protein kinase (MAPK) (Sweatt, 2004), phosphatidylinositol 3-kinase (PI3 kinase), tyrosine kinase Src and some others (Malenka and Bear, 2004). While comparing distinctive features of LTP induced by STDP, theta-burst and spike pairing techniques in the barrel cortex of calcium/calmodulin-dependent protein kinase II ($\alpha$CaMKII)$^{T286A}$ mutant mice, Hardingham et al. (2003) found that none of those three protocols could trigger LTP in the knocked out mice, whereas all those protocols successfully induced plasticity in wild mice. Their result suggested that STDP or other stimuli-triggered plasticity protocols depend on αCaMKII autophosphorylation for inducing synaptic reorganization at the cellular level. Also, Lu et al. (2007) suggested that activation of mGluR1a receptor and inositol 1,4,5-triphosphate (IP3) receptor-mediated Ca2+ elevation are also required for inducing LTP like plasticity in fast spiking pyramidal cells, where the critical window of time is more supportive of a symmetric model of SPTD than the temporal asymmetry. Neurotrophins, such as BDNF, are also regarded as modulatory factors that can influence synaptic plasticity (Poo, 2001).

On the other hand, the cellular mechanism of LTD is known to be regulated mainly by the presence, quantity, and trafficking of another ligand-gated ion channels AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors in the plasma membrane (Bredt and Nicoll, 2003, Soderling and Derkach, 2000). NMDA-receptor dependent LTD is triggered when $Ca^{2}$+/CaMKII activates dephosphorization of GluR1 subunit on AMPA receptors and initiates delivery of tagged AMPA receptors into synapses (Feldman et al., 1998, Hayashi et al., 2000).

I used a modified PAS technique in my second study. The hypothesis of inducing plasticity by changing cortical excitability using PAS protocols was extensively tested by Stefane et al (2000). In their study, conducted in human, they applied TMS

stimulation to the primary motor cortex (M1) at rest and during sustained voluntary contraction (active state), which was paired with low frequency median nerve stimulation. They recorded MEP generated from abductor pollicis brevis (APB) muscle (Stefan et al., 2000) before and after the test. Evidenced by a number or previous studies (Baranyi and Szente, 1987, Baranyi et al., 1991, Iriki et al., 1989, Hess et al., 1996), they tested the possibility of inducing long term potentiation (LTP) in the cortical horizontal or vertical fibres by delivering low frequency peripheral stimulation eliciting somatosensory afferent volley which was paired with action potential generated by TMS at motor cortex. They delivered 90 pairs of TMS and median nerve stimulation near the wrist joint at a rate of 0.05Hz with a condition where peripheral input preceded TMS by 25ms. To measure and compare any effect of paired stimulation protocol applied at resting state, they delivered 20 TMS stimulation to motor cortex before and after the intervention at a rate of 0.01Hz and simultaneously recorded surface EMG from APB during complete resting condition. Their protocol significantly increased the resting MEP amplitude after the intervention (Stefan et al., 2000). Induced plasticity lasted for about 30 to 60 minutes which proved it was not merely a short term or post tetanic potentiation. This plasticity, which could be generated even with a few paired stimulations, persisted for about 24 hours in a few subjects, which makes it more likely to be LTP mediated. Rapid changes in membrane excitability could also be responsible for the observed effect (Aou et al., 1992, Stefan et al., 2000).

However, they proposed that the induced plasticity using associative long-term potentiation, or any closely related neuronal mechanism reflected changes in the cortical synapses. Their speculation was those changes could occur at cortical level through TMS activation of vertical or horizontal intracortical fibers (Rothwell, 1997) and generation of a subsequent EPSP. In short, the driving part of the Stefan's (2000) PAS technique was to induce LTP or LTD by means STDP mechanism (Song et al., 2000). Synaptic modification by STDP can be stated as a neuronal process where two separate inputs are paired in a way that both arrives at a neuronal circuit within a short space of time in a specific sequence. By this way synaptic connectivity in that circuit can be changed and this modification can sustain for a considerable duration, and this also allows strengthening or weakening the related connections (Wolters et al., 2003, Ridding and Rothwell, 2007, Markram et al., 1997). Depending on protocols, the connection can be potentiated if the second input comes within a few milliseconds after

the first stimulation (excitatory post synaptic potential) or synchronously with the first stimuli. Or, the connection can be weakened if the excitatory post synaptic potential fails to reach within the specific window of times. In Stefan's PAS experiment, the most effective inter-stimulus interval (25ms) was selected in such a manner that the afferent volley from the median nerve stimulation (near wrist joint) would arrive at motor cortex at least a few milliseconds before TMS evoked EPSP (Stefan et al., 2000). Later, some researchers carried out the successful implementation of PAS protocols, but the debate still exists whether it induces plasticity in cortical or subcortical space.

In one of my studies, application of intermittent high frequency rTMS (within safety limit) induced significant changes in cortical excitability and MEP amplitude increased in all four muscles tested. As initially suggested by Huang et al. (2005), and like many other plasticity inducing synaptic remodelling, NMDA receptor/$Ca^{2+}$ dependent LTP-like mechanism explains this facilitatory effect. To confirm this, (Huang et al., 2007) performed a double blind, placebo controlled study where they used NMDA receptor antagonist memantine. After blocking the NMDA receptors, a similar protocol of intermittent rTMS failed to induce changes in MEP amplitudes. This further supports the notion that relatively high frequency intermittent rTMS can modulate cortical excitability in a manner consistent with the LTP-like mechanism. One important difference between PAS and intermittent rTMS induced cortical excitability was that, the former required a precisely timed pre and post synaptic events and followed the principles of STDP, whereas the later delivered only a series of intermittent cortical input at a certain frequency. Recently, it has been found that PAS induced LTP can be blocked by L-type voltage gated Ca2+ channels (VGCC) blocker nimodipine and can be reverted into LTD using T-type VGCC blocker ethosuximide (Weise et al., 2017).

Contrary to this, nimodipine (L-type blocker) turned the facilitation induced by rTMS into depression but ethosuximide (T-type blocker) only blocked the facilitatory effect of rTMS (Wankerl et al., 2010). T-type VGCC are abundant in apical dendrites and spines, whereas L-type VGCC are mostly found near the soma of cortical neurons. Activation of L-type VGCC are known to amplify STDP by enabling backpropagating action potentials in dendrites (Williams and Stuart, 2000), a key condition required for STDP like LTP. It has been further evidenced by Magee and Johnston (1997), that

pairing of subthreshold EPSP with backpropagating action potentials induced strong dendritic action potentials and increased $Ca^{2+}$ influx, a condition specifically favouring STDP. Therefore, blocking L-type receptors can also block the effect of PAS and other similarly paired stimulation protocols. On the other hand, apical dendrites of cortical pyramidal cells are likely to play a crucial role in rTMS mediated LTP, since the blockade of T-type VGCC resulted in lack of facilitation (Weise et al., 2017). A reduction in $Ca^{2+}$ dependent GABAergic synaptic strength and remodelling of postsynaptic gephyrin scaffolds were observed after application of 10Hz repetitive magnetic stimulation in pyramidal neurons during an in vitro study by Lenz et al. (2016). They also noticed a similar reduction of clustered gephyrin in rTMS treated mice. It was suggested that rTMS possibly modulates GABAergic synaptic strengths at dendritic synapses (Lenz and Vlachos, 2016) and repetitive magnetic stimulation strengthens glutamatergic synaptic strength along with the structural reorganization of dendritic tree (Vlachos et al., 2012).

STDP mediated LTP and LTD are also dependent on other factors, including target cell type (Lu et al., 2007), synaptic competition (Song et al., 2000), co-operativity (Sjostrom et al., 2001), coincidence of presynaptic and postsynaptic activity resulting in opposite effect (Holmgren and Zilberter, 2001), activity dependent refinement (Butts et al., 2007) and spike pattern (Markram et al., 1997). Dendritic structure, location and excitability also play a key role in LTP/LTD modulation; and the non-linearity and variable nature of STDP mechanism is also known to be influenced by dendritic projections (Froemke et al., 2005, Kampa et al., 2007, Letzkus et al., 2006, Sjöström et al., 2008). In conclusion, multifactorial properties of STDP mediated LTP/LTD, differences in NMDA receptors affecting cortical excitability induced by stimulation protocols, reliance on feedforward afferent input and non-overlapped activation of selective areas controlling selective muscle groups in the motor cortex might explain, at cellular context, the underlying mechanism of biased facilitation towards flexor and distal hand muscle observed in my experiments. The requirement of the backpropagating dendritic potential for STDP like LTP could also be an alternate explanation of why flexor-extensor differences were seen only in PAS or motor imagery protocols but not in rTMS protocol.

It is worth mentioning that the site of induced plasticity or excitability is also crucial in the observed results in my experiments, which was explained in relevant chapters.

According to my results, the STDP protocols used to provide paired stimulation from a wearable electronic device affected cortical excitability along with subcortical structures, including the reticulospinal connections, whereas the PAS protocols utilizing motor point stimulation as afferent input and TMS as cortical input possibly affected cortical excitability primarily, but a subcortical effect cannot be excluded. As evidenced by (Fisher et al., 2012), TMS at motor cortex is capable of sending a volley towards reticular formation. Hence it can be argued that paired TMS protocols with an afferent input might influence reticular or other subcortical connections parallelly. Since motor imagery cannot be regarded as a direct afferent input, it can be rather explained as a pre-conditioning stimulation where motor cortex awaits in subthreshold level to trigger an EPSP upon complete activation. I also observed a similarly biased facilitation in motor cortical excitability in the forearm flexor and hand muscles using simultaneous motor imagery and TMS. All these observations support the idea that somewhere lies an inherent difference between agonist vs. antagonist muscle groups which also affects the reorganization at cortical and subcortical level. A subtle but crucial influence of reticulospinal connections might amplify those differences to some extent in healthy person but to a great extent during recovery or artificial induction of plasticity.

Finally, much of the above explanations relied on previous evidence but most speculations made in this thesis were based on the observed effects from the studies I conducted. To learn the exact cellular and synaptic mechanism of induced plasticity or change in excitability, or to know the specific cortical and subcortical connections mediating to those changes at the cellular level, further research and exploration are required.

Most of my experiments and protocols were designed to emphasize the translational aspects of neuroscience. My first experiment utilized a wearable electronic device to provide paired pulse stimulation and to test the possibility of strengthening or weakening motor connections to upper limb muscles, especially after corticospinal lesion. It was the first demonstration that the long-latency stretch reflex can be modified by repeated, the precisely-timed pairing of stimuli known to activate brainstem pathways. Furthermore, the pairing was achieved with a portable electronic device capable of delivering many more stimulus repetitions than conventional laboratory

studies. These findings open up new possibilities for basic research into under-investigated pathways, which are important for motor control in healthy individuals. This study can also lead to paradigms capable of enhancing rehabilitation in patients recovering from damage, such as after stroke or spinal cord injury. A similar device and technique were later utilized to deliver a paired stimulation paradigm in a double bind placebo-controlled study at the Institute of Neuroscience Kolkata. A total of 96 stroke patients with corticospinal lesion were divided into three separate groups. The first group received paired stimulation protocol (stimulation to forearm extensor-interval-click) for a prolonged period, the second group received random stimulation for the same duration, and the third group did not receive any stimulation. Only the group receiving paired stimulation showed significant improvement in the Action Research Arm Test (ARAT).

The modified PAS protocol using motor point stimulation and TMS can also be useful in translational, clinical and rehabilitation programmes as delivering stimulation over muscle was proven to be less complicated and practical than providing stimulation at a specific nerve. The motor imagery and TMS protocol also have translation aspect and it was the first demonstration that paired motor imagery and TMS is capable of inducing lasting plasticity at cortical or subcortical level. Overall, these results give insights into the nature of plastic reorganization in the motor system which might help to design appropriate protocols to be utilized in rehabilitation, motor learning, skill training or psychotherapy.

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