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Investigating Plasticity Differences in the Motor Area in Tourette's Syndrome, using Non-Invasive Brain Stimulation

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Thesis Abstract

Tourette's syndrome (TS) is a neurodevelopmental disorder characterised by motor and phonic tics, which have been linked to over excitability in the motor areas within the brain. The primary aim of this thesis was to investigate those with a diagnosis of TS and explore the use of non-invasive brain stimulation techniques in these relevant motor areas. It is hypothesised that those with this developmental condition may have an altered pattern of plasticity in the brain areas related to motor movements, making this a key target in TS research using transcranial magnetic stimulation (TMS). TMS is a widely used non-invasive brain stimulation technique that can be used to investigate and modulate cortical excitability. In doing so, it can provide interesting insights about brain plasticity.

Previous work has examined the motor area in TS, but often with small sample sizes or alternative stimulation techniques. Three experimental designs were used in the course of this thesis, all with the aim of further understanding plasticity in the motor area and develop robust experimental designs that can be used in future work when larger sample sizes are accessible. In Chapter 3 and 4 a behavioural task, called the serial reaction time task, is introduced to examine habit learning, whilst concurrently taking measurements of excitability using TMS. This was followed by theta burst stimulation, a particular pattern of TMS, used as an extensive within-subjects investigation. Finally cortical motor mapping was used as another method of assessing plasticity changes. Chapter 4 was the first incorporation of a clinical sample. However, due to the unforeseen lockdowns caused by the Covid-19 pandemic, this was the only instance where those with TS were able to participate. Consequently, the intended outcomes of this thesis were compromised as it prevented us from being able to establish any new conclusions about this population group. All subsequent experiments were also impacted by this, and instead these had to be treated as pilot studies, a means of testing the

legitimacy of the experimental designs, or the control sample for the clinical population data that will be collected at a later date.

Finally, the conclusion summarises the data presented, discusses the potential of this research if it was possible to conducted in full, and looks ahead to what may be achieved in the future with the use of these experimental designs.

Thesis Research Questions

This thesis aims to explore plasticity in the motor area with the following main research questions:

- *Is there an observable change in the pattern of cortical excitability during a motor learning task?*

- *Is this observable pattern altered in those with a diagnosis of TS?*

- *Can we replicate the findings of previous research that has investigated the use of theta burst stimulation?*

- *Are there improvements we can make to this theta burst stimulation protocol?*

- *Does the application of theta burst stimulation alter the cortical motor representations of the hand area?*

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Firstly, I would like to express my gratitude to my supervisors, Stephen and Georgina Jackson, for the opportunity to pursue this PhD.

During my time in the office with fellow PhD candidates and others within this research team, I had support and advice from many, stretching from my first undergraduate degree to the completion of this huge achievement. That long term commitment to my academic career did not go unnoticed. I appreciate the endless hours of help working with Matlab for analysis and experimental scripts, company and assistance during those long stretches in the TMS lab, MR Centre or the office, and everything else the past few years have entailed. Thank you to each of you.

To my family, I cannot overstate my thanks for your support as I fulfilled this life goal. Whilst I know you didn't doubt me once, I can't believe we finally got there – before you Dad! An extra massive shout out to Mum for selflessly taking on the role of Chief Proof-Reader. I'd also like to acknowledge my friends for being such wonderful and enthusiastic cheerleaders, it really meant the world. Finally, a huge thank you to Aaron, for his unwavering patience and endless encouragement for so many years.

Covid-19 Impact Statement

From the 16th of March 2020, all experimental testing was halted in the University of Nottingham Psychology faculty, and further data collection could not go ahead. TMS testing requires extended periods of time in the company of the subjects, as the equipment is required to be held by a researcher against their head. Consequently, close proximity is maintained for over two hours at a time. Following risk assessment it was deemed inappropriate to test from this date. The department was closed from the 19th of March 2020, and we were not invited back until the 19th of October 2020. At this time only limited access to office space was allowed, and lab access was discouraged, meaning there was a considerable disruption to the access of the facilities. Though constantly reviewed, from the 22nd October 2020 a decision was made to halt data collection. Risk assessments from the university were not able to prioritise this in person testing, nor was it felt that subjects would be confident and willing to consent to what was already a very unknown experience for them, let alone with added health complications. No clear deadline was in sight for when testing would restart, and therefore the write up period was instigated with significantly less data than originally planned. This has proved disappointing, as a significant part of the remaining data collection would have involved patient work, thus enabling conclusions about the patient population. In addition to the missed data collection, the impact on the write up process should not be underestimated. Not being present in the research and office space disrupted the ability to have spontaneous collaborative discussions with peers and supervisors, the surrounding environment was considerably less stimulating, and motivation was subsequently impacted.

Additional planned activities for the thesis prior to the announcement of homeworking included:

- Further data collection in the studies detailed in Chapter 5 & 6
    - Aimed for at least another 5 participants
- Testing with TS patients in SRTT experiment detailed in Chapter 4
    - This was already a slow recruitment process and was exacerbated by uncertainty before the pandemic
- Matched controls were also beginning to be recruited for the experiment detailed in Chapter 4
- Pilot experiments detailed in Chapter 5 & 6 with one or two individuals from patient groups
- More participants for the TMS mapping study in Chapter 7 to mitigate having to exclude some participants and to solidify the work
- New TMS mapping study with 36 subjects
    - To further the work discussed in Chapter 7. This had been organised, recruitment had started, but subsequent data collection was suspended

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Chapter 1 : Introduction

Keywords: *Plasticity, Brain stimulation, Long term potentiation (LTP), Long term depression (LTD), Tourette's syndrome (TS), Transcranial magnetic stimulation (TMS), Cortical excitability, Facilitation, Inhibition, M1, Theta burst stimulation (TBS), Synaptic plasticity, Cortico-striatal-thalamo-cortical circuits (CSTC), Hebbian, Non-Hebbian, Bienenstock-Cooper-Munro theory (BCM), γ-aminobutyric acid (GABA)*

This chapter describes and discusses studies relating to the neuroplasticity of the motor area of neurologically typical individuals and individuals with a diagnosis of Tourette's Syndrome. There is a particular focus on non-invasive brain stimulation techniques as a method of observing and modulating the plasticity in these areas.

1.1 A brief introduction to neuroplasticity

Maintaining a degree of flexibility is an intrinsic part of human life. It allows adaptation and development in changing circumstances. Initially, the pervasive theory was that a brain was 'fixed' when it reached maturity (Duffau, 2006). This has since been overturned in the face of ever-growing evidence and understanding that shows the brain is continuously changing as a result of our actions and experiences. Without these capabilities, it would be impossible to master a new skill, or to recover from a neurological injury. Plasticity, a term that has now been used in brain science for over a century, broadly refers to this continuous change. More specifically, it describes the potential of the brain to change in neural organisation, resulting in both short and long-lasting modulations in behaviour. This includes during the maturation of an individual throughout their lifespan, not just until adulthood, adaptation to the environment,

both internal and external or in response to a loss in function perhaps following damage (Kadosh, Roi, 2014).

Hebb's (Hebb, 1949) influential postulate on the mechanism of synaptic plasticity, or connectivity between neurons, states the following: 'When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic changes takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased'. More simply put by Shatz (1992), 'cells that fire together, wire together'. Synapses are highly specialised junctions between cells in the brain, which enable fast communication between the neurons and the central nervous system via chemical transmission. The phrase 'wire together' is referring to a strengthing of efficiency of that connection, physiologically meaning there is a change in the amount of neurotransmitter being released, and the number of pre- and postsynaptic receptors (Caverzasio et al., 2018). There are two categoies of synpatic modulation, the one described above is called "Hebbian" plasticity, or spike timing dependent plasticity. It occurs over a shorter timescale, and, as the specific wording in Shatz's summary suggests, the relative timing between the presynaptic input and the resulting post-synaptic depolarisation were key, meaning coordinated co-activation is necessary for the synapse specific change in strength (Caverzasio et al., 2018). Simultaneous and coordinated firing suggests there is a common cause for those synapses to be firing. However, if one fires slightly before the other, then causation could be implied. Synaptic strengthening occurs in instances where there is a close temporal connection and weakened when this is reversed. This is now referred to as spike-timing dependent plasticity (Suppa et al., 2017) or Hebbian plasticity, and is considered to be the mechanism responsible for the coding and retention of information in the brain (Fox & Stryker, 2017). Long-term potentiation (LTP) and long-term depression (LTD) are both particular phenomena within Hebbian plasticity; LTP refers to activity dependent long lasting enhancement of synaptic transmission, and LTD is

defined as the opposite, with a reduction in synaptic transmission following a low frequency of stimulation. The timing of pre-synaptic spikes is important in determining whether LTP or LTD occurs; if the presynaptic spike arrives before the postsynaptic spike, approximately 20ms, then LTP will occur. LTD occurs when the presynaptic spike occurs after the postsynaptic spike (Caverzasio et al., 2018). The mechanisms underlying these phenomena have been found to involve N-Methyl-D-Aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, with changes observed in the densities of both of these receptors at synapses throughout these processes (Thickbroom, 2007).

Despite the importance of these plastic traits and ability to change, the brain also requires a way to maintain a relatively stable equilibrium of activity over time. Hebb's rule could only be partly responsible for a full explanation of synaptic modification as this would lead to the equilibrium becoming unstable. Mechanisms are in place to ensure there is not an excessive change in activity such as runaway potentiation, and it is kept within a sensible range (Karabanov et al., 2015). The plasticity mechanisms that maintain this equilibrium are referred to as "Non-Hebbian" plasticity, the second category of synaptic modulation. It is a homeostatic occurrence and is therefore also known as homeostatic plasticity, to maintain the balance within the network over a longer period of time (Caverzasio et al., 2018; Karabanov et al., 2015). Many models predict that without such a mechanism, the brain would rapidly reach the extremities of the range and therefore limit the formation and storage of information (Keck et al., 2017), or could result in excitotoxic damage or a comatose state, therefore, these two forms of plasticity are often working in opposite directions (Fox & Stryker, 2017). The Bienenstock-Cooper-Munro theory (BCM) for cortical synapse modification was first described in 1982 as one possible explanation of the responses that occur in the visual cortex in different visual environments (Cooper & Bear, 2012). They proposed that these forms of plasticity work opposingly as

there is a sliding threshold for LTP and LTD plasticity. Decreases of activity result in a reduced threshold for the induction of LTP, making it easier to encourage a strengthening of synapses and reducing the likelihood of further LTD. If this situation was reversed, this would shift the threshold so that LTD was more likely (Keck et al., 2017). Synaptic scaling theories have also been proposed separate from the BCM theory. Instead, these describe the synaptic changes through synaptic scaling and shifts in the excitation and inhibition balance. On further inspection and reflection, they have been found to be highly similar in their properties (Keck et al., 2017). Synaptic plasticity is also influenced by a higher order of plasticity. Metaplasticity is a term used to describe the concept of the plasticity of synaptic plasticity (Karabanov et al., 2015; Thickbroom, 2007). First introduced by Abraham & Bear, (1996) this refers to synaptic activity that primes the ability of future synaptic plasticity, such as LTP or LTD described above. Whilst metaplasticity can be homeostatic or non-homeostatic, the exact nature of these mechanisms is beyond the scope of this thesis but is none the less a key component to be considered in future research.

Neuroplasticity is a necessity throughout the brain in many different areas and for various functions. For example, it has been demonstrated that the acquisition of a new motor skill requires plasticity in the structural and functional organisation within the M1 (Dayan & Cohen, 2011). These theories and mechanisms form the foundations of our understanding of plasticity, upon which this thesis is developing, focusing on the motor area in particular, whilst investigating the relation between plasticity patterns, motor skill learning, and changes following a brain stimulation protocol that is thought to modulate the plasticity of that area.

1.2 A brief introduction to brain stimulation

There are references to the concept of using external stimuli as a way to manipulate the brain and treat neurological disorders throughout history. The physician of the Roman Emperor Claudius described one of these early encounters in his medical texts, explaining the way to harness the natural electricity of certain fish species as a way to remedy headaches (Sironi, 2011). As understanding of electrical currents developed, so too did the ways in which humans experimented with it and used it to study the nervous system of both animals and humans. Following the application of currents directly onto the brain, the locations of specific functions could be highlighted. This began as very invasive surgery, with human subjects often having undergone a traumatic brain injury, and therefore necessitating a surgical procedure, prior to taking part in the studies before alternative, less risky methods were developed. A form of brain stimulation that frequently comes to mind for many is electro-convulsive therapy (ECT), a procedure where a seizure is induced in the patient following the administration of electrical currents. This was an extreme form of brain stimulation initially used to treat a large range of mental health conditions, until better care and understanding of neurological conditions reduced the need for its use, and more targeted pharmacotherapy was introduced (Leiknes et al., 2012). Contemporary use of ECT is more often reserved for medication resistant conditions, most commonly to treat severe depression. However, due to safety and efficacy concerns of using these methods merely for research there was a need to develop alternative stimulation methods for situations that do not necessitate the risks associated. Nowadays there are ways of inducing a current in the brain without the need of applying it directly to the brain tissue, or if necessary, employing stringently developed procedures, such as deep brain stimulation (DBS) that require the surgical implantation of microelectrodes in precise areas.

Less invasive techniques have since been developed that enable the study and treatment of many brain areas and processes with minimal risk to research subjects, patients receiving this treatment therapeutically, and practitioners. These are pain free and have transient effects on the individual and can be applied outside of a medical environment. Primarily, the studies discussed within this thesis use transcranial magnetic stimulation (TMS), a method that eliminates the need for application of an electrical current against the skin. There are significant advantages to this technique, as it can be applied whilst the recipient is conscious, rather than examining the brain under anaesthetic or as part of a post-mortem investigation. For a non-invasive stimulation technique it can also be fairly precise, with a limited spread of excitability in comparison to other forms of stimulation. TMS can be applied prior to brain tumour surgery located in the motor cortex, to distinguish excitable and non-excitable areas to an accuracy of approximately 5mm, a much better method than mapping directly onto exposed cortex as mentioned (Priori et al., 2009). This also makes TMS a key method for virtual lesion research, as it can temporarily produce transient, reversible functional lesions, and the behavioural consequences can be observed (Siebner & Rothwell, 2003). Different protocols can also provide insight into axonal excitability, the GABAergic system (an important component of motor research), temporal accuracy (necessary for more physiological measures to be determined) and the time course of cognitive processing (Priori et al., 2009). Such a versatile piece of equipment allows researchers to both observe and modulate cortical excitability. Chapter 2 describes the principles behind the workings of this technique in more detail, along with some of the more common TMS protocols, including those used in this thesis.

1.3 Tourette's Syndrome

1.3.1 What is it?

In recognition of the first detailed classification and thorough observation of multiple patients with compulsive tic syndrome, as it was first known, the French neurologist Jean-Martin Charcot renamed it Gilles de la Tourette after a member of his staff who worked extensively on the assigned project (Teive et al., 2008). More widely referred to now as Tourette's syndrome (TS), it is a term used to describe this multifaceted developmental condition that has motor, behavioural and cognitive symptoms. It falls into the categorisation of a hyperkinetic movement disorder, and is most strongly associated with chronic motor tics, which are rapid stereotyped, repetitive movements. According to the Diagnostic and Statistical Manual (DSM-5) a diagnosis of TS is given when an individual presents with multiple motor tics and at least one vocal tic for the duration of a year before the age of 18 years old. These symptoms are not induced from an underlying condition or from the use of medication (APA, 2013). These criteria differentiate TS from other tic disorders such as chronic motor tic disorder and chronic vocal tic disorders which do not feature both types of tic, and transient tic disorder which is present for less than 12 months (M. M. Robertson, 2008). This thesis will focus on TS specifically.

1.3.2 Tics

Whilst TS can be complex in its presentations and co-occurring conditions, meaning a 'typical' presentation is difficult to identify, the defining characteristics of this condition are the tic behaviours. These are motor and vocal, are repetitive in nature and occur outside of the correct context. All human movement can appear as a motor tic, and most human noises as phonic ones (Ganos et al., 2013). Within these categories, tics can also be described as simple or complex. For those in a motor category,

a simple tic would maybe only involve one muscle and may look like a twitch. Examples include an eye blink or eye roll, and head or hand jerks. A simple phonic tic might be a grunt, cough, or clearing of the throat. A complex motor tic requires more coordination, for example reaching for something, hitting or jumping, or giving the impression of being a more goal directed behaviour. A complex phonic tic may be the use of words or phrases, with unusual patterns of speech with varied rate, volume or rhythms (Leckman et al., 2006). In this complex tic category, there is also echo phenomena, repeating words and phrases, called echolalia, when repeating the words of others, and palilalia, when the individual is repeating one's own words, and echopraxia, where an individual will imitate gestures and movements. Coprophenomena can also be observed, including coprolalia, the involuntary use of inappropriate or obscene words or phrases outside of the usual context, and copropraxia, the inappropriate use of gestures or touching. Coprolalia is commonly presented as being a key symptom of TS when depicted in the media, however, the prevalence rates are observed to vary considerably and in much lower numbers than many realise, reportedly between 7% and 43% within the TS population (Eapen & Robertson, 2015).

Whilst there are a few tics that may be lifelong, such as eye blinking, the tics that an individual experiences rarely stay the same, especially during childhood. Tics vary and change over both shorter and longer periods of time. The frequency will increase and decrease over months, and the repertoire will evolve. Periods of stress, anxiety and excitement will also alter the frequency, or if an individual is feeling tired. But during times that require more focus and fine motor control, tics can be less forceful and frequent (Leckman et al., 2006). For some, their tics can be repressed for brief periods of time, although this varies between patients for how long it is possible to supress their tics. Patients can do this because the large majority (~90%) report that prior to wanting to make the movement or vocalisation there is a strong sensation, or drive to perform that tic behaviour (Cohen et al., 2013). This is referred to as a

premonitory urge. Patients may become aware of these urges around the age of 10 years old (Leckman et al., 2006), with approximately 90% of TS patients reporting this symptom (Eapen & Robertson, 2015). These have been described as feeling in a specific place on the body, for example on the hands, or a particular place on the back of the neck, which would precede a neck jerk tic. These urges have also been described more generally as a feeling of tension (Leckman et al., 2006). Completing a tic behaviour creates a brief sense of relief for the patients from the premonitory sensory phenomena.

1.3.3 Co-occuring and coexistent pathologies

TS is a heterogeneous condition, with extremely varied expressions depending on the diagnosed individual. Current evidence suggests that neurodevelopmental coexisting conditions are common and are often the root cause of distress and impairment as opposed to the individual's tics. In one large clinical study, diagnostic interviews were employed to measure the lifetime prevalence of individuals with TS and other co-occurring conditions. Their results suggested that 85.7% of individuals were diagnosed with at least one other co-occurring psychiatric disorder and 57.7% had two or more additional psychiatric disorders (Hirschtritt et al., 2015). The most commonly present coexisting conditions are comprised of attention deficit-hyperactivity disorder (ADHD) (60% of the TS population) and obsessive-compulsive disorder (OCD) (30-50% of the TS population) (Eapen & Robertson, 2015) and to a lesser extent autism spectrum disorder (ASD), but the list also includes anxiety, learning disabilities, depression, behavioural problems, self-injurious behaviour amongst others (Hashemiyoon et al., 2017), although exact estimates vary considerably depending on the samples. It is estimated that the incidence of "pure" or "uncomplicated" TS is present in only approximately 10% of the TS population (Eapen & Robertson, 2015).

Throughout all the studies in this thesis that required recruitment of TS individuals, screening for symptoms of these commonly coexisting conditions was conducted. The Conners-3 self-report measure (Conners, 2008) was used for ADHD; the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) and the Yale-Brown Obsessive Compulsive Scale (YBOCS) was used for obsessive-compulsive behaviours (Scahill et al., 1997) and ASD was screened for using the Social Communication Questionnaire (SCQ) (Berument et al., 1999). Additionally, vocabulary and matrix reasoning tests were used from the Wechsler's Abbreviated Scale of Intelligence (WASI) to record IQ estimates from participants (Wechsler, 1999). As these coexisting conditions are a common occurrence within this patient population, it was important that these measures were taken. In larger studies when more subjects can be recruited, this may be an important factor for analysis to compare between different expressions of the condition.

1.3.4 Prevalence and prognosis

Initially TS was considered to be a rare disorder, but it is now estimated there is an overall prevalence of approximately 1% of school aged children. This is a conservative estimate due to many experts agreeing that TS is underdiagnosed, but one that is still 10 times more than the accepted rate for a rare disease (M. M. Robertson, 2008). It is reported in all cultures, and is often cited as being more common in males, although the M/F ratio cited can vary between 1.6:1 to 9:1 (Cravedi et al., 2017) with the gender ratio becoming more comparable as individual's near adulthood (Yang et al., 2016). The initial prognosis is often uncertain for many when they are first diagnosed, as their experience of TS can change through their adolescence as their tics wax and wane and the many factors that influence their experience and quality of life with the condition. The importance of thorough follow ups for patients has been highlighted so that therapeutic approaches can be tailored to the patient

as needed, and any early indicators of emerging co-occurring conditions can be quickly managed (Hassana & Cavanna, 2012).

Tics change during the developmental course of a child. They begin in early childhood, initially as simple motor tics; the mean tic onset has been reported to be at 5.6 years of age. Tic severity then increases, and individuals can develop vocal tics, which may or may not become more complex. This severity peaks between the ages of 8 and 12 years old, with the reported average at 10 years of age. In most cases it is then followed by a decline in severity, meaning a large number reach adulthood tic free (Leckman et al., 1998). For the majority, they continue to develop into relatively tic free adults with less than 20% experiencing clinically impairing tics into adulthood. In one study 90% reported a significant improvement in their tics, with the remaining ones being either mild or not present (Leckman et al., 1998). Whilst there does seem to be a significant association between a higher tic severity in childhood and an increased tic severity when followed up in adulthood (Bloch et al., 2006), individual prognosis can be variable. Previous studies have documented that 44% of recruited patients considered themselves as symptom free when followed up during early adulthood, but that more than 10% experience worse tic severity at follow up (Burd et al., 2001). However, this particular area is lacking in thorough longitudinal studies, and is often reliant on self-report measures. This may reduce the accuracy as recall may not be perfect, and for many, tics may seem less obtrusive as they reduce their awareness of their own tics as time passes. Currently there is no definitive way of predicting which individuals will show improvements, experience complete tic remissions, or those who remain or get worse as they progress into adulthood. Longitudinal studies with a quantitative approach are necessary to further our understanding of how TS changes over time before it is possible to predict likely outcomes.

There is a variety of treatment options to target the individual needs of the patients. For example there are a variety of behavioural therapies such as habit reversal training, brain stimulation treatments, oral medications such as alpha agonists, dopamine depletors, anti-psychotics and anti-epileptics, and even botulinum toxin injections have been effective for some who experience focal tics (Jankovic, 2020). For those that experience a low quality of life but their TS seems to be treatment resistant, then in these extreme cases invasive brain surgery to facilitate deep brain stimulation may be considered. Options for non-invasive therapies with fewer possible negative consequences would be welcomed and more applicable to a larger cohort of individuals. The treatments currently available all have some form of limitation; the medications can cause unpleasant side effects, or it is difficult to access behavioural therapies. With further knowledge more options and effective treatment combinations can be made available to families, alongside a better understanding of how and why they work.

1.3.5 Pathophysiology of TS

In order to develop more targeted and successful treatments for TS, a thorough understanding of the pathophysiology is necessary. The exact aetiology and neurobiological underpinnings of TS are not fully understood, perhaps because of the often varied and complicated presentations within individuals, denying the possibility of researchers finding a single explanation. This has contributed to the speculation that there may be a spectrum with distinct phenotypes (Cavanna & Rickards, 2013), although the specific bio-markers for these have not yet been identified.

Genetics

There is evidence for genetic risk genes and factors that could be responsible for a variety of effects on brain functionality and the development of the condition (Domènech et al., 2021). Familial studies have demonstrated that there are significant increases in the incidence of TS or related tic disorders amongst first degree relatives of diagnosed individuals. However, the exact genes that are implicated in this remain elusive (Felling & Singer, 2011). When present in conjunction with environmental factors, such as infections, perinatal issues and auto-immune function, the result is dysfunction in brain circuitry (Cravedi et al., 2017).

Neurobiology

The dysfunction of the cortico-striatal-thalamo-cortical (CSTC) circuits, which links regions of the frontal cortex to subcortical structures, have been implicated in the development of TS and its coexisting conditions. The associated regions, including the caudate nucleus, putamen, thalamus, globus pallidus, supplementary motor area (SMA) and the basal ganglia, are where changes are evident in the grey and white matter structures (Worbe et al., 2015), resulting in small observable differences in volumetric MRI studies (Felling & Singer, 2011). This may be a contributing cause of the functional changes that result in tics and other symptoms experienced by patients. As there is not one particular area implemented, and the clinical manifestation of TS can present in so many ways, it is also difficult to identify a single neurotransmitter that may be responsible. Dopamine, glutamate, γ-aminobutyric acid (GABA), serotonin, acetylcholine, norepinephrine and opiates are all integral to the functioning of the CSTC circuits, and each have been suggested as a potentially responsible neurotransmitter (Singer & Minzer, 2003). There are key findings suggesting a hyperactivity of the dopamine system for diagnosed individuals (Kleimaker et al., 2020). Rodent models examining

this system utilise dopaminergic enhancing medications, which subsequently exacerbate tics, movements and stereotypic behaviours (Felling & Singer, 2011). TS is subsequently alleviated for many people with the use of antipsychotics, which are dopamine antagonists. There is also good evidence to suggest that dopamine plays a substantial role in this complex condition (Buse et al., 2013). The involvement of glutamate is supported by evidence following post-mortem investigations of reduced glutamate in those regions connected to the CSTC circuits, and the established interaction between dopamine and glutamate, which also interacts with GABA (Mahone et al., 2018). Further evidence for the involvement of GABA in TS, as evidenced with TMS measures, is described in Chapter 2.

1.4 Motor Learning

Tourette Syndrome

Tics and habits share some similarities, with tics having been described by some as voluntary movements that are completed in an automatic or habitual way, that may be misplaced in timing and context following premonitory urges (Delorme et al., 2016). In comparison, habits are key adaptive behaviours, consisting of assembled routines linking sensory cues with a motor action, allowing organisms to direct cognitive resources towards more demanding tasks. In environments that do not present a lot of variety, then energy can be conserved by relying on habitual behaviour. However, when circumstances change, behaviour needs to be adapted in order to accommodate this and avoid inappropriate behaviours that may outwardly resemble tics for some people (Leckman & Riddle, 2000; Foerde, 2018). These shared features of tics and habits both seem to involve the CSTC, in particular the basal ganglia, with a role in nonconscious acquisition of skills and habit formation, which has previously been a research focus for TS (Graybiel, 2008).

Behavioural therapies, such as habit reversal training, have been developed which rely on these theories and are designed to replace tics with an alternative response. When described, this type of therapy consists of three components; education about TS for the individual and subsequent social support, awareness training for tics and competing for response training to implement behaviours other than tics. The combination of these approaches should counter the learnt associations and subsequent automatic tic responses reinforced in TS by relief from unpleasant premonitory sensations when the individual completes a tic action (Hwang et al., 2012). This has proven significant outcomes of tic reduction (Liang et al., 2021) which is both promising for those with TS and for researchers as this contributes to theories that tic behaviours and habit behaviours are intrinsically linked. This similarity and link to similar structures within the brain is a useful characteristic for creating relevant paradigms to use in a laboratory setting and enabling the use of control groups. In doing so, researchers are better equipped to draw conclusions about behavioural and learning differences between a number of patient groups and typically developing individuals from a variety of experimental designs. There is a growing body of evidence to support these methods and the conclusions that there are striatal learning dysfunctions in adults and children (Marsh et al., 2004) and that tics are maladaptive habits (Scholl et al., 2021). As tics most commonly present as motor movements, motor learning tasks are most often used for these investigations. The relevance of these to TS will be further elaborated on in Chapter 4.

Motor Learning

This term describes the process of procedural learning following repetitive training, as a way that we can adapt our interactions with our surroundings. It is often used to study the mechanisms of cortical

plasticity, as plastic changes are associated with skill acquisition (Kolasinski et al., 2019). Motor skill learning refers to the process by which we are able to refine our movements with practice, meaning they can be executed quicker and with a higher accuracy. This skill has been shown to alter the brain (Ostry & Gribble, 2016), including topographic reorganisation, synaptogenesis in the M1 and myelination changes in white matter. These are just some of the structural and functional changes observed in the M1 which supports motor learning (Kolasinski et al., 2019). Motor learning paradigms include associating a stimulus with a motor reflex response, learning to improve a reaction time, learning a finger tapping sequence or adjusting movements to an external perturbation (Luft & Buitrago, 2005). Pascual-Leone, Grafman, & Hallett, (1994) utilised one of these motor learning tasks, now a standard paradigm for the assessment of sequence learning (Werheid et al., 2003). They opted for the serial reaction time task, originally developed by Nissen & Bullemer, (1987), which consisted of a visual cue that can appear in one of four horizontal positions. When the visual cue appears, the participant is required to make a response, which ends that trial, and the next visual cue is revealed. The response time is the primary measure of the task and enables the researcher to assess motor learning by the changes in those reaction times. The visual cue presentation is given in a sequence pattern that repeats over the course of the experiment. Measures of skill learning can be deduced from the reduction of time it takes for participants to makes responses across trials as they become more proficient at learning the presented sequences and visuomotor associations between the given cue and necessary response (E. M. Robertson, 2007). Pseudosequence trials, which do not conform to the sequence can also be inserted into the procedure, which can then be contrasted with the sequence trials. This will show a better, more sensitive, measure of skill acquisition as the response timings can be better compared, and also it will control for confounding factors such as fatigue and motivation (E. M. Robertson, 2007). To participants this set up appears straightforward and easy to understand, but there are many measures and insights about human behaviours that can be deduced from

its application. For the participant to complete the SRTT task, they must first learn the associations between the visual stimuli on the screen and the motor responses, in a process termed visuomotor learning, followed by rule learning. In the SRTT, these processes are evident as the reaction times reduce during both these processes. The addition of pseudosequence trials, and the typically observed RT increase, reflects the rule learning that is underway either implicitly or explicitly, as, if it were purely a visuomotor learning exercise then no change would be observed in the RTs (Werheid et al., 2003). These two subprocesses of sequence learning involves striatal, left dorsal premotor cortex, supplementary motor cortex, M1, primary somatosensory cortex, thalamus, putamen and cerebellum activations (Werheid et al., 2003; Hardwick, Rottschy, Miall, & Eickhoff, 2013), aswell as strong evidence for the involvement of the basal ganglia which appears to be relied upon during sequence learning (Janacsek et al., 2020).

This thesis will specifically be focusing on the paradigm that requires a participant to learn a finger tapping sequence, also called the serial reaction time task (SRTT). Compared to other sensorimotor tasks which require greater motor demands and the learning of a novel motor movement, the SRTT variants have more minimal motor demands, instead focusing on learning sequential motor behaviour (Hardwick et al., 2013).

1.5 Summary and Research Aims

The primary aim of this research is to improve the understanding of motor learning in the human motor cortex and how that affects cortical excitability. Non-invasive brain stimulation techniques will be used to obtain snapshots of how the motor cortex changes during the learning process or following the application of a plasticity protocol. During this process the aim is to gain insight into the patterns of excitability through examining motor skill learning in a healthy population, and to be able to contrast them with the TS population. The experiments documented in this thesis all seek to contribute to a better understanding of plasticity differences in the M1 area specifically as this is of particular interest in the study of TS because of the prevalence of motor tics. The long-term aim is to better understand tic formation and to ultimately contribute to a better method of treatment, preferably using non-invasive brain stimulation and therefore reducing the need for pharmacological interventions.

The initial study described in Chapter 3 investigates the use of motor learning in typically developing individuals and is used to inform the development of the subsequent experiment for TS groups, which is described in Chapter 4. The focus of the third study, in Chapter 5, was to develop an in-depth experiment that could examine the cortical excitability changes following the application of theta burst stimulation (TBS) protocols. This experiment was then expanded in Chapter 6 to include priming TBS, to broaden the understanding of plasticity and the underlying mechanisms of metaplasticity in a small sample of healthy individuals. Finally, Chapter 7 and 8 utilise the same TBS protocol to manipulate the cortical excitability of the M1 with the additional measure of cortical motor mapping. This experimental method was developed with the intention to perform comparisons between TS patients to investigate if there was a correlation between tic severity and task performance, and

to understand the differences between those typically developing individuals and those with a diagnosis.

1.6 Patient and Public Involvement in this research

My initial personal contact with PPI was through attendance to a department meeting to highlight the need for this in our research. The contacts available to us were introduced, and examples of how their involvement can further our work was explained and discussed in broad terms. This gave us an opportunity to think about how it could be applied to our own research.

Additionally, I also attended a masterclass: *'Involving children and young people in health services and research'* in September 2019. This was highly relevant as the subjects recruited in this work are often within the age range described as 'children and young people'. The aim was to improve subsequent engagement in the studies and to improve my communication skills with this group during all aspects of contact; from approaching them to participate, relaying information during the study, and any debrief or follow up they may require.

Recruitment of the patient groups was conducted through the lab group's pre-existing database of individual's who were happy to be approached with regards to taking part in studies. Additional advertising was conducted through the charity Tourette's Action. This charity also gave me the opportunity to participate in a residential weekend specifically organised for children and adolescents with the condition, and their families. My role required me to be available throughout the weekend for young people and parents to approach me at any time to ask questions about current research and how they may be able to get involved in the future. I was a friendly face that encouraged engagement and involvement in the world of research in a gentle manner when it suited the families.

Chapter 2 : Investigating the human motor cortex using non-invasive brain stimulation

Key words: *Transcranial magnetic stimulation (TMS), Non-invasive brain stimulation (NIBS), Primary motor cortex (M1) Motor evoked potential (MEP), Electromyography (EMG), Motor threshold (MT), Short interval intracortical inhibition (SICI), Interstimulus interval (ISI), Repetitive transcranial magnetic stimulation (rTMS), Brain derived neurotrophic factor (BDNF), Gamma aminobutyric acid (GABA), Paired associative stimulation (PAS), Motor Threshold (MT), Centre of gravity (CoG), Theta burst stimulation (TBS), Continuous theta burst stimulation (cTBS), Intermittent theta burst stimulation (iTBS), cortical-striatal-thalamic-cortical circuits (CSTC), input output curves (IO), Supplementary motor area (SMA)*

Electrical stimulation has been used extensively throughout the investigation of the brain. One of the most popular methods for investigating the human motor cortex is transcranial magnetic stimulation (TMS), due to it being a non-invasive method of investigating and altering cortical excitability. It is well tolerated amongst human subjects, with minimal discomfort, proving itself to be a useful tool for the study of plasticity, as it can quickly measure changes in cortical excitability. It is particularly suited to investigations within the motor area as it is possible to measure this directly by recording the size of muscle twitches elicited from the applied TMS.

2.1 Transcranial Magnetic Stimulation

TMS is a type of non-invasive brain stimulation (NIBS) which uses the principles of electromagnetic induction as opposed to the direct electrical stimulation applied in other techniques, which is often associated with uncomfortable sensations. It is a highly useful tool as it enables both

insights into plasticity through observation and manipulation. When an electrical current is passed through a coil which contains windings of copper wire, a rapidly changing and brief magnetic field of approximately 1-2 Tesla, perpendicular to that wire is created (Rossini et al., 2015). If a second wire is placed within this magnetic field, then an electrical current can be induced along it. In this case, the coil is held against the scalp and the magnetic field passes through the skin and skull, inducing an electrical current in the cortex, which is acting as that second wire, without having to directly apply electrical stimulation to the skin (O'Shea & Walsh, 2007; Hallett, 2007). The most typical shapes of coil used are either single round coils or figure of eight coils. Each of these are suited to particular experiments as the shape influences the magnetic field generated when in use. This subsequently alters the strength and localisation of the induced electrical current. In the figure of eight coil the current converges in the centre, where the loops intersect, creating a more focal point (L. G. Cohen et al., 1990). As a result, this is more commonly used for experiments that requires more precision, and the circular coil is more often deployed in circumstances where deeper regions are required to be stimulated, but are less focal (Rossi et al., 2009). The research associated with the topic of this thesis will most frequently have opted to use coils that are a figure of eight shape with the electrical current flowing in opposite directions around the windings and then converging in the middle. This creates a focal point for the stimulation to target the intended brain area, as shown in Figure 2.1, with directional arrows on the coil depicted over the axial view of a brain scan indicating the flow of current. Other possible differences within TMS experimental equipment that need to be considered to ensure consistency within experiments, includes not only the coil type, but also the coil size, the particular TMS stimulator models, current direction, and pulse waveform. All can influence the size and shape of the magnetic fields, and therefore alter induced currents (Kammer et al., 2001). TMS is regarded as a safe technique, with minimal reports of adverse events or discomfort (Rossi et al., 2009) within both adult, adolescent and child populations if the safety protocols and guidelines are adhered to (Allen et al., 2017).

2.2 TMS and the Human Motor Cortex

TMS can be a versatile tool in experimental procedures; it can be used to investigate neurophysiology, to observe the cortical excitability at that time using single pulse protocols, or as a method of modulation. To observe changes in cortical excitability, motor evoked potentials (MEPs) can be elicited by TMS, recorded and then compared. These movements begin in the motor cortex as a result of the TMS discharge in either the pyramidal cells or intracortical interneurons involved with them, or a combination of the two. These lead to a descending volley down the pyramidal tracts in the brain, spinal cord and into the peripheral nerve. This nerve is in contact with a muscle and results in a muscle twitch which is recorded as an MEP (Orth & Münchau, 2013). An increase in cortical excitability, or facilitation, means these MEPs would become larger, and

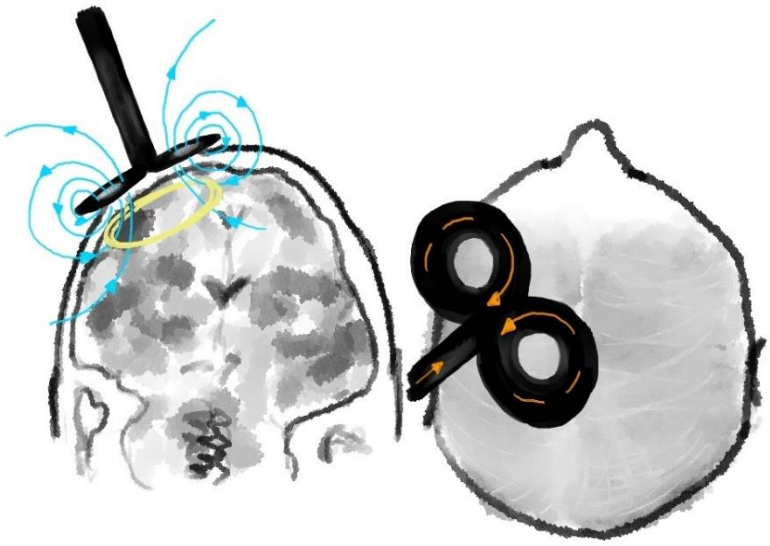

Figure 2.1 - Illustrations of TMS coil placement for targeting the motor area from a coronal and an axial viewpoint. The orange lines show the direction of the current in a figure of eight coil, the blue lines depict the magnetic field, and the yellow line indicates where an electrical current is induced

the opposite for inhibition, or a reduction in excitability (Di Lazzaro et al., 2008). As well as insights into cortical excitability changes by examining the amplitudes, the latency of the MEP is a measure of the conduction time of the neural impulses from the cortex to the peripheral muscles and can therefore provide an insight into physiology.

Whilst TMS can produce twitches in various individual or groups of muscles, dependent on where the current is induced, most commonly the literature cited in this thesis, reviews recordings of MEPs taken from the first dorsal interosseous (FDI), a muscle in the hand. This is partly due to ease of measuring or observing with a visible twitch within this muscle without too much discomfort to participants, and the location of the relevant representation of this area being close to the surface of the cerebrum, and therefore easily accessed by TMS (Karabanov et al., 2015). The exact mechanisms that are triggered following the application of TMS are complex and not yet fully understood. To briefly explain the process, following a pulse of TMS, a short-lived electrical current is induced in the cortical tissue below the skull, prompting an action potential to travel along the neuronal axons within the area of stimulation, where neurotransmitters such as GABA and glutamate are released into the postsynaptic neuron to continue the descending volley down the corticospinal tract. If the TMS pulse is of a suitable stimulation intensity, and in the correct position, then these corticospinal action potentials will reach the spinal cord, where they activate motor neurons, which then elicits a muscle response in the contralateral muscle ~20ms after the application of the TMS pulse. This muscle response is an MEP (Huerta & Volpe, 2009), and is recorded using surface electromyography (EMG). The effect of TMS pulses depends on where the coil is placed. Most of our knowledge of how TMS physiologically interacts with cortical processing is from investigations particularly targeting the hand region within the M1, as depicted in Figure 2.1, and it remains a popular area to target due to its relatively superficial location near the surface of the brain, and the improved comfort for the participants. Motor map locations that are at a

greater depth within the brain are unable to be targeted so easily as the electrical current disperses over the surface, meaning higher intensities of stimulation may be required to target the intended region, which can be less comfortable for subjects. It is also easy to quantitively measure the output from the hand area, as the size of muscle twitch correlates with the excitability of that area. In the studies described in this thesis, the FDI muscle is the most commonly targeted muscle. The hand motor area can be identified on an MRI scan as the sigmoidal hook sign, or the omega sign because of the shape, marked in red on Figure 2.2. As the hand area is identifiable and close to the cortical surface, it is possible to precisely target specific muscles in the hand with TMS, which can result in clearer data as the specific muscle in the participant's hand is targeted, but also in experiments such as those described in Chapter 7 and 8, it means that other hand area muscles can also be specifically targeted for detailed mapping data. All studies described in this thesis use posterior-anterior induced current, which describes the direction of the current in the brain. This is important to highlight as different orientations of the TMS coil will activate different elements or neuronal populations, even if the location is kept the same (Di Lazzaro et al., 2008) and will change the threshold for direct waves (D-wave) or indirect waves (I-wave) of activation of axons (Rossini et al., 2015).

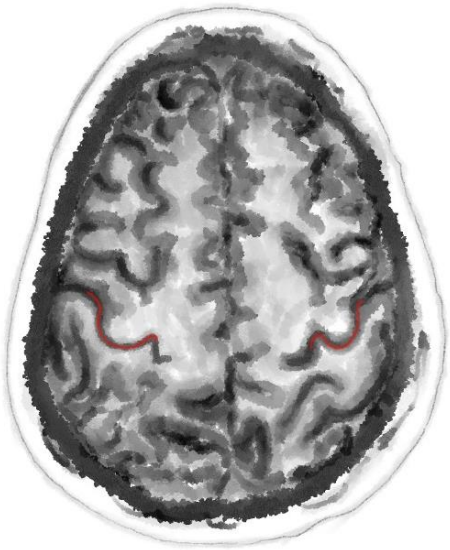

Figure 2.2 - Illustration of an average MRI scan from an axial viewpoint. The red lines highlight the omega shape often used to target the hand area in the M1

As previous studies have demonstrated that levels of M1 excitability change following participation in a task involving learning (Smyth, Summers & Garry 2010), this indicates that the M1 is engaged during rapid motor learning in these sorts of tasks, to improve performance in that specific motor behaviour. Muellbacher, Ziemann, Boroojerdi, Cohen, & Hallett, (2000) tested this using a ballistic or ramp pinch task to a specified metronome beat. Subjects were required to complete ballistic and a ramp pinch task, during which they were learning the optimum force and acceleration. For the ballistic task, they were required to make a pinch movement of a short duration after a beat of a metronome, and then relax. The ramp pinch task instructions were to slowly increase the force of the pinch over a set time. The EMG recording was used to provide visual feedback to the subjects during the task. TMS was used to target the flexor pollocis brevis (FPB) which was involved in the task, and abductor digiti minimi (ADM) muscle, which was a control. Some subjects underwent TMS of the descending corticospinal pathways, meaning brainstem stimulation was used, and these TMS results could be

compared with the excitability measures taken from the M1. The researchers observed an increase in the MEP amplitudes elicited by TMS in the muscles directly involved in the ballistic practice, but not in the muscles unrelated to the task. This was only observed in the TMS over the M1 site and not following stimulation of the brainstem, indicating, the researchers suggest, that the changes in the M1 excitability are undergoing reorganisation processes during motor learning.

2.2.1 Single Pulse

Single pulse TMS is a method of application where a TMS pulse is described as being given in isolation. Despite there being more than one pulse given in this protocol, the length of time between each one is purposely selected to avoid any carry over effects from one pulse to the next. This format of TMS application provides insight into the state changes of the M1. During data collection, fluctuations of neural excitability can be responsible for highly variable MEP amplitudes even when the target muscle is at rest and therefore it is advised to use a mean MEP amplitude as a marker of cortico-motor excitability (Rossini et al., 2015). Repeats of TMS pulses at the same intensity are necessary to obtain a representative estimate of MEP size. The amplitude of the MEP is measured from peak-to-peak. An illustrated depicted of a single pulse MEP is depicted on the left on Figure 2.3.

Motor threshold

The motor threshold (MT) is the amount of stimulation needed to consistently generate a MEP of a predetermined size. In many protocols, first the hotspot is identified. There are a number of methods for this, including using brain scans as a guide, using scalp measurements to estimate the hand location, and also systematically moving the TMS coil

over the approximate scalp area to find the point of best response. Once identified, this location can be marked directly on the scalp or a target is created within neuronavigational software to ensure consistent targeting of the hotspot. The next step is selecting the stimulus intensity used in a TMS protocol, these are often expressed as a percentage of that individual's motor threshold. You can use active motor threshold (AMT), where the muscle is voluntarily contracting at a percentage of their maximum, or resting motor threshold (RMT), which is identified when the target muscle is at rest with no visible artefacts on the EMG recording. AMT threshold is typically lower, as the threshold for muscle contraction is lowered with it already being in use and active. In both instances, the intensity is then adjusted in a step-wise fashion until the minimum intensity is found that produces an MEP 50% of the time (Rothwell et al., 1999). This is a hugely advantageous technique as between individuals there can be a large degree of variability as to their threshold level, but within that individual it remains consistent. Therefore, the use of this technique means that TMS protocols can be tailored to the participant. The variability may be partly a result of biological differences such as the shape and thickness of the skull (Klöppel et al., 2008), or the composition and organisation of brain matter in that area (McConnell et al., 2001). For some individual's their hand area representation on the cortex may be further away from the scalp, and therefore a higher TMS intensity is required for muscle contraction (Cukic et al., 2008). There is evidence that handedness also has an effect on MT as this reflects motor cortical organisation. Specifically this relates to whether you are applying TMS to stimulate their dominant or non-dominant hand, as the dominant hand has a higher degree of excitability and therefore the MT would be lower (Nicolini et al., 2019).

Input output curves

The higher the TMS intensity the larger the resulting MEP, and vice versa. Typically, following stimulation at a range of stimulus intensities, this

relationship can be demonstrated with the MEP sizes recorded as a sigmoidal curve called an "input-output curve", "stimulus-response curve" or "recruitment curve". These recruitment patterns differ amongst individuals, and can change if the muscle is active or at rest, after motor skill training, application of a NIBS plasticity protocol, CNS-active drugs and in neurological conditions (Rossini et al., 2015). The curve is determined partly by the number of recruited corticospinal fibres during the stimulation, which increases with higher intensities as the stimulus would be able to spread and activate additional fibres, depending on the individual's unique placement and strength of corticospinal pathways (Chen, 2000); (Siebner & Rothwell, 2003). The area of linear increase on the sigmoidal shape typically corresponds to TMS intensities between 120% and 140% of RMT (Han et al., 2001) before a final plateau where the MEP size reaches a ceiling in its maximum output.

Cortical Mapping

This technique can be used to demonstrate the somatotopy of the motor homunculus (Siebner & Rothwell, 2003). A fixed intensity is selected as a percentage of the MT, and this is applied over the selected area of the scalp. A map of the sites and the response from the muscles can then be obtained. The resulting data of muscle representations will also provide a number of measures with which to examine this data. A maximum value, also termed the hotspot can be computed. A centre of gravity (COG) can be identified, which is the amplitude-weighted centre of the map, rather than just being the site of the biggest MEP response. The dice similarity coefficient, which indicates the amount of overlap of different muscle representations, is another measure. The Euclidean distance between muscles can be measured and compared, and so too can the surface area or surface volume of the map representations. This technique can be used in the experimental protocols to compare cortical maps between groups of individuals, or before and after the application of an intervention, such as a rTMS protocol used to induce plasticity.

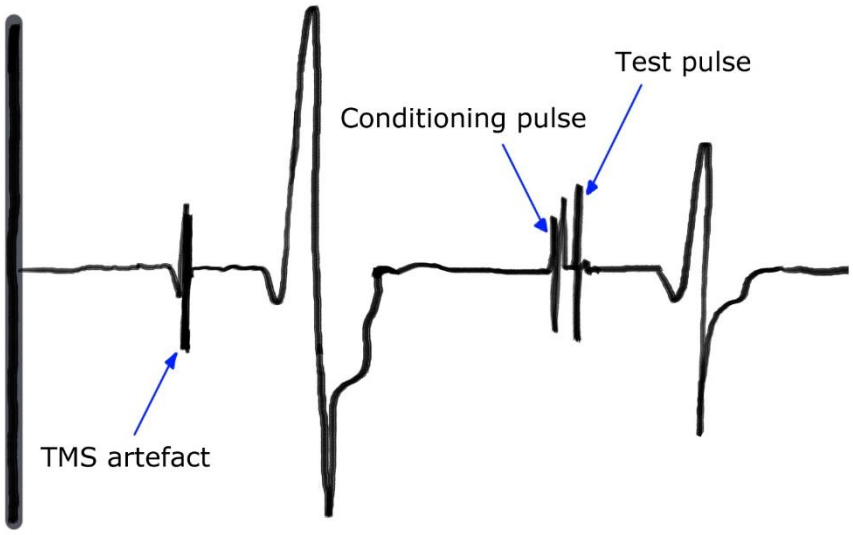

Figure 2.3 - A depiction of a single pulse MEP (left) and a paired pulse MEP (right) that illustrates the effects of inhibition

2.2.2 Paired Pulse

Paired pulse TMS is a method of assessing intra-cortical inhibition and facilitation, using two pulses of stimulation where the first pulse modulates the second. The first pulse is referred to as a conditioning pulse (CP), and the second pulse is referred to as the test pulse (TP). Both of these are depicted in Figure 2.3, alongside an illustration of an MEP following a single pulse for comparison. When examining the data, the single pulse TMS will be used as a baseline from which to compare the effect of the CP on the size of the MEP amplitude.

Short interval intracortical inhibition (SICI) protocols are the main focus of the research within this thesis, as it is a well-established measure for exploring intracortical excitability and inhibition within the motor cortex, mediated by the $GABA_A$ receptor. There are other protocols including long interval intracortical inhibition, intracortical facilitation, interhemispheric inhibition and short interval intracortical facilitation to name some.

However, of most interest in relation to TS in this research is SICI due to alterations in GABA and physiological inhibition in TS (Jackson, Draper, Dyke, Pépés, & Jackson, 2015). SICI is elicited when a subthreshold CP, at an intensity lower than necessary to cause a muscle twitch, is followed by a suprathreshold TP at an interstimulus interval (ISI) of 1-6ms (Kujirai et al., 1993). An example of a SICI protocol with the resulting MEP is illustrated on the right hand side of the Figure 2.3. There are two phases of SICI, depending on the length of the ISI. The first is observed between approximately 1ms and 2.5ms and is thought to be partly due to a combination of a neuronal refractory period and synaptic inhibition (Roshan et al., 2003). SICI phase at 2.5-6ms is likely representative of post-synaptic inhibition, which is intrinsically linked to $GABA_A$ receptors (Rossini et al., 2015). The relationship between SICI and the CP intensity is a U-shaped curve (Ilić et al., 2002). By increasing the CS intensity, the curve dips as the SICI effect becomes greater before eventual facilitation. The CS intensities that produce the largest inhibitor effects in individuals is reportedly between 60-75% RMT (Kossev et al., 2003) and therefore studies often target this range of CS intensities.

2.2.3 Repetitive TMS

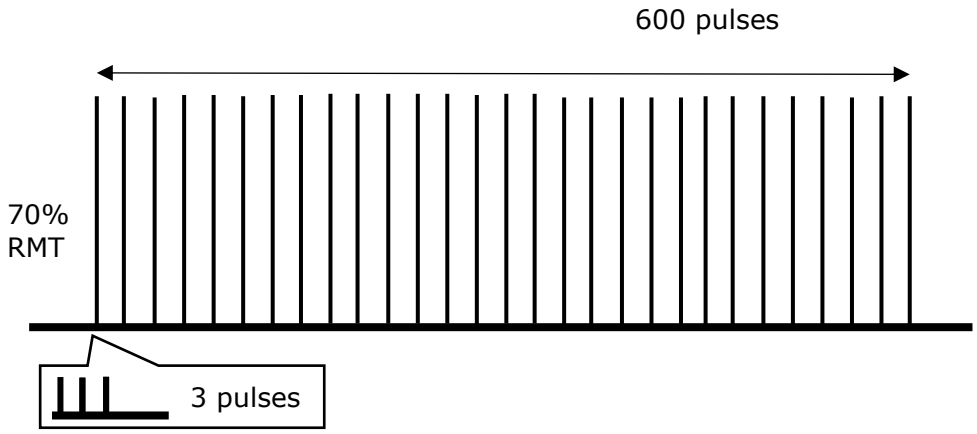

Figure 2.4 - Visual representation of the stimulation pattern of cTBS

Repetitive transcranial magnetic stimulation (rTMS) is an established investigative technique used for inducing plasticity, using trains of TMS pulses. The pattern of stimulation can be altered depending on the desired changes in cortical excitability. Regular trains of rTMS, at a high frequency of 5Hz or higher, have been shown to increase cortico-motor excitability in the M1 area at the site of stimulation (Ziemann & Siebner, 2008). Low frequency rTMS at a frequency of approximately 1 Hz have been shown to do the opposite and decrease excitability (Karabanov et al., 2015). There are a variety of patterned rTMS protocols which feature short high-frequency bursts in amongst longer intervals. Two patterns in particular have become established methods, quadripulse stimulation and theta burst stimulation (TBS). Quadripulse stimulation requires an application of four-pulse bursts at a low repetition rate of 0.2Hz. When applied over the M1 at short inter-stimulus intervals of between 1.5-10ms the mean MEP amplitude has been shown to increase, and at longer intervals greater than 30ms then MEP amplitudes have been demonstrated to decrease (Hamada et al., 2008). Theta burst stimulation was first

developed for human application by Huang, Edwards, Rounis, Bhatia, & Rothwell, (2005), with the subsequent protocol consisting of short bursts of three subthreshold TMS stimuli at 50 Hz bursts at a repetition rate of 5 Hz. Continuous theta burst stimulation (cTBS), depicted in Figure 2.4, involves continuous application of this pattern, often resulting in an inhibitory effect on MEP amplitude, whereas intermittent theta burst stimulation (iTBS), depicted in Figure 2.5, has a facilitatory effect (Karabanov et al., 2015). These theta parameters were originally developed from studies using both rodent (Diamond et al., 1988) and human brains with evidence that theta rhythms are associated with long term potentiation (Oberman et al., 2011). The majority of studies use 600 pulses, for cTBS this takes approximately 40s and for iTBS 190s (Wischnewski & Schutter, 2015). A benefit of this type of stimulation, compared to other rTMS protocols, is that it can be applied rapidly, taking just seconds or minutes, making it much more tolerable for subjects compared to the much longer stimulation durations typical in other protocols. Shorter versions of this paradigm have been investigated, using just 300 pulses, however, the excitability changes have been shown to not last for as long (Wischnewski & Schutter, 2015). When applying TBS using 600 pulses, the subsequent effects have been observed for up to 30 minutes in iTBS and 60 minutes in cTBS, after stimulation, which

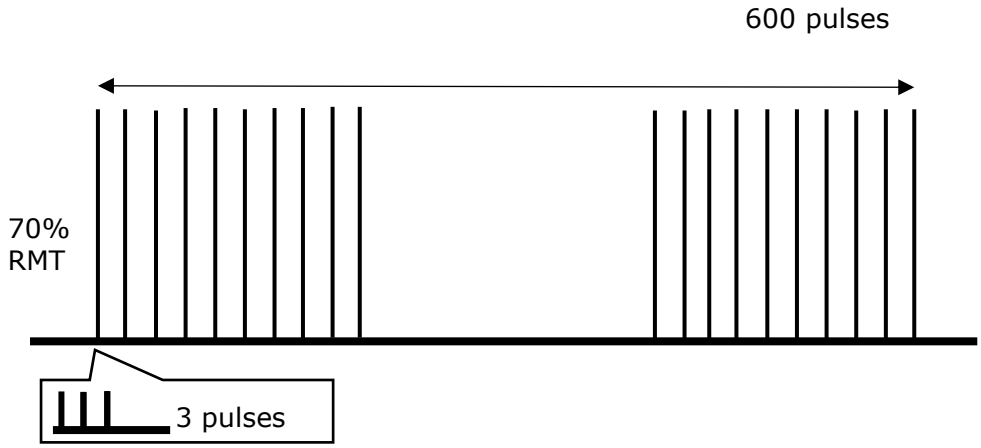

Figure 2.5 - Visual representation of the stimulation pattern of iTBS

suggests this is one of the most powerful non-invasive brain stimulation modulatory techniques as it can induce long lasting change (Chung et al., 2016). Additionally, results show more consistency when applying TBS compared to simpler rTMS protocols (Hoogendam et al., 2010). Studies have shown that rTMS can affect molecular pathways involving BDNF, dopamine, GABA, glutamate, serotonin, cortisol or endogenous opioids, all of which may be contributing to the subsequent changes in cortical excitability (Cirillo et al., 2017), whilst a recent review supported this by discussing a number of studies that demonstrated evidence for GABA as a molecular target of rTMS (Kim et al., 2021). The cortical effects following the application of iTBS and cTBS does seem to reflect similar processes to LTP and LTD (Wischnewski & Schutter, 2015), although this explanation is insufficient in explaining the complex changes that take place, nor is the information yet available to model the full effects of stimulation (Cirillo et al., 2017).

Previous systematic reviews have been limited by a large variability in the parameters used, and a limited number of controlled studies, as discussed by Kim et al., (2021). Their body of work aims to contribute to a growing pool of TMS data by taking care to use replicable parameters and add controls where possible. Initially, the experimental designs discussed in this thesis focus on the use of single pulse and paired pulse measures to make observations. In Chapters 5, 6 & 7 this develops into an increasingly combined approach, using single and paired pulse measures to examine the differences in plasticity following the application of TBS protocols to induce neuromodulation.

2.3 Plasticity and TS

The techniques described in this chapter have all been used to examine the possibility of altered cortical plasticity in TS. Whilst plasticity is a fundamental mechanism in the brain, there is increasing evidence of dysfunction in the patterns of plasticity in a number of neuropsychiatric disorders, including Parkinson's disease, focal dystonia, schizophrenia and major depressive disorder (Karabanov et al., 2015). There are already suggestions as to what form these alterations in plasticity for those diagnosed with TS are, and how they manifest, related to the CSTC brain circuits which are implicated in motor learning and habit formation in TS, as mentioned in Chapter 1. Components of these connected regions and circuits may be responsible for different aspects of TS, and subsequently possible targets for plasticity investigations. This connectivity enables the indirect stimulation of subcortical areas via the relevant cortical regions for that particular aspect of TS. Kleimaker et al., (2020) describe four key networks; Tic generation, Tic inhibition, Urge, and Perception-action network. The M1 is implicated in the Tic generation network, which also consists of the basal ganglia, premotor area and the SMA, and the perception-action network which connects the M1 to the somatosensory cortex, parietal cortex and the SMA. This highlights the versatility of using the M1 as a TMS stimulation site, and why it is the focus of the work in this thesis.

Previously observed functional changes using TMS suggest a reduced synaptic plasticity in TS patients groups compared to healthy controls (Brandt et al., 2014). This has a significant implication in the understanding of the differences in components of motor learning between patient groups and healthy controls. Other studies have similarly found plasticity differences, with a specific focus on their decreased inhibitory response in the M1, meaning that their response to plasticity manipulation TMS protocols seems to be significantly different to that of

control groups (Suppa et al., 2011). This altered pattern of LTP-like and LTD-like plasticity within the M1 intracortical neurons is a promising component in the understanding of motor learning differences. TMS applied over the M1 in TS has demonstrated consistently reduced SICI effects, altered response to TBS protocols and a shallow input-output curve in comparison to the typical population (Ulf Ziemann, Paulus, & Rothenberger, 1997); (Suppa et al., 2011), as opposed to different SICI thresholds (Berardelli et al., 2008). The supplementary motor area (SMA) has also been implicated. This brain area has many connections to areas relating to motor control (Picard & Strick, 2001). Concentrations of GABA within the SMA were significantly higher than the typical population, and positively predicted the severity of motor tics experienced by diagnosed individuals (Draper et al., 2014). TBS protocols over the M1 have also shown clear differences when MEP data is contrasted between health groups and TS groups, showing that TS individuals have a reduced response to both the inhibitory and facilitatory TBS modulations (Marsili et al., 2017).

This evidence implicating the CSTC regions, plasticity, GABA and inhibition alterations in the M1 all involve the same structures that underlie motor learning and habit formation (Leckman & Riddle, 2000) whose descriptions resemble that of tics (Graybiel, 2008). It is hypothesised that individuals with a diagnosis of TS may more readily acquire involuntary habit behaviours such as tics, and subsequently find it harder to un-learn them (Jackson et al., 2015). This is further examined in Chapter 3, where motor learning paradigms are used to probe whether there is altered synaptic plasticity and a subsequent shift in the pattern of motor skill acquisition in TS compared to controls.

Chapter 3 : Investigating Motor Learning using the Serial Reaction Time Task and Transcranial Magnetic Stimulation in healthy individuals

Keywords: *Transcranial Magnetic Stimulation (TMS), Motor learning, Serial reaction time task (SRTT), Reaction time (RT), Sequence, Pseudosequence, First dorsal interosseous (FDI), Primary motor cortex (M1), inter pulse interval (IPI), Resting motor threshold (RMT), Motor evoked potential (MEP), Bayes factors (BFs)*

Motor learning and plasticity are intrinsically linked; learning would not be possible without synaptic modifiability. This chapter discusses an experimental protocol combining brain stimulation measures with a serial reaction time task (SRTT), a well-known method of quantifying skill acquisition. Initially this is piloted within a sample of healthy individuals, prior to its use in TS which is further developed and discussed in Chapter 4.

3.1 Introduction

As outlined in the previous chapter, plasticity is the term used to describe synaptic modifiability in the brain. The size of the evoked muscle response following a TMS pulse reflects the excitability of the corticospinal system and therefore the amplitude is enabling us to have a quantifiable insight into the inhibitory and excitatory mechanisms, or the plastic changes, within that brain area. Changes in cortical excitability, measured by the recording of motor evoked potentials (MEPs) from a single pulse of transcranial magnetic stimulation (TMS) may give us valuable insight into the process behind motor learning. The level of cortical excitability has been shown to alter during the acquisition of a new motor skill, which is

evidence of neuroplasticity (Ostry & Gribble, 2016). This experiment was designed to apply these principles and to establish whether there are changes in motor cortical excitability in healthy individuals, during a motor learning paradigm called the serial reaction time task (SRTT), discussed in Chapter 1.4. In this case, the TMS is being used before, during and after completion of the SRTT. The addition of applying TMS measures whilst participants were undertaking the task is a further development on previous work. This research utilises TMS as a technique to observe and manipulate plasticity in the M1 within healthy subjects, before developing this paradigm to enable a comparison with those individuals with a Tourette syndrome (TS) diagnosis. In selecting and combining these measurements with a SRTT protocol that requires activation of the basal ganglia, an area we know is implicated in TS, and is linked to procedural memory, this should provide us with a clear insight into the differences experienced with those with TS (Janacsek et al., 2020).

Pascual-Leone et al., (1994) used the SRTT task in addition with motor cortical mapping and TMS. They observed that as RTs became shorter, the cortical mapping of the muscles involved in the task increased in size, characterised by amplitude changes of MEPs. After further training, as the sequence became more familiar, subjects began to anticipate the next visual cue. The cortical mapping of those muscles then returned to baseline, with the RTs remaining significantly shorter than the first exposure to the sequence. As TMS was applied between SRTT blocks, the cortical activation pattern may not be totally reflective of the activation during the task. Ambrus et al., (2016) designed their study to include online measures of cortical excitability, meaning that TMS pulses were applied whilst participants were performing the SRTT, and therefore while the muscle was activated. In addition to measuring cortical excitability, Ambrus et al., (2016) also included the use of transcranial direct current stimulation (tDCS) to manipulate the cortical excitability of the subject within the M1 and possibly alter task performance. Their sham stimulation condition, where tDCS was not applied, and therefore the most

comparable to our protocol, found no significant differences between the amplitudes of MEPs recorded during the learning blocks. However, a significant increase in offline before-after SRTT MEP amplitude was observed.

For this study, the use of the SRTT was selected to measure visual spatial finger mapping, in addition to also measuring motor skill acquisition, and to recreate processes needed in the formation of a habit. As the sequence becomes more familiar with the subject and their reaction times decrease, they begin to anticipate the finger movements. This is similar to the acquisition of a habit; a rigid and automatic behaviour that may be triggered regardless of outcome, such as when the pseudo-random sequence trials are inserted and RTs are slowed because the anticipated sequence has been replaced (Yin & Knowlton, 2006). We plan to observe cortical excitability changes, described in Chapter 2.2, as there is evidence to suggest that the excitability changes and influences the MEP amplitude, although it is not necessarily directly related to the motor output (Bestmann & Krakauer, 2015). Our protocol does not include a direct manipulation of cortical excitability, as demonstrated by Ambrus et al., (2016), who applied transcranial direct current stimulation during their experimental design. This will enable us to compare sequence and pseudo sequence blocks more in depth by establishing them as separate conditions. However, similar to their study, there will be the use of both online and offline TMS measures throughout the experiment.

We hypothesised that participants would demonstrate motor learning in both conditions, with faster RTs in both conditions during the initial phase of visuomotor learning. However, in the sequence condition we expected a continued reduction of RTs as participants begin anticipating the sequence. We also expected a difference in motor cortical excitability, evidenced by changes in MEP amplitudes, between the conditions. From the previous work discussed, there is an expectation to observe the MEP

amplitudes to increase as the RT improves in the sequence condition in the offline conditions, similar to the results of both Pascual-Leone et al., (1994) and Ambrus et al., (2016). Whilst Ambrus et al., (2016) found no significant changes during the TMS taken during the SRTT, also referred to as the online measures, we hypothesise we would observe a similar pattern of changes to previous studies for MEP data recorded in offline measures when the participant is at rest.

3.2 Method

3.2.1 Participants

23 participants consented to take part in the experiment (15 female, 8 male). The mean age of participants was 23.3 ± 4.24 years. Participants were screened to ensure they were healthy, free from medication and any counter indications to TMS. All participants were deemed to be right-handed using the Edinburgh Handedness inventory (Oldfield, 1971). This study gained ethical approval through the School of Psychology ethics committee at the University of Nottingham and was conducted in accordance with the ethical standards specified in the 1964 Declaration of Helsinki.

3.2.2 Serial Reaction Time Task

Participants performed a modified version of the SRTT (Nissen & Bullemer, 1987), as depicted in Figure 3.1. During the task, the participants were presented with 4 boxes arranged horizontally across a screen directly in front of them. A cross appeared in one of those boxes in a manner that was designed to seem random to the participant, but were either a pseudo-sequence, or according to a sequence, depending on which condition they were completing. This is detailed further in the next

paragraph. Participants were instructed to respond to the location of the cross by pressing the corresponding key using their right hand as follows: number 1 to be pressed with the index finger, 2 with the middle finger, 3 with the ring finger and 4 with the little finger. The participant was required to make an accurate response as quickly as possible with the corresponding key press depending on its location. Speed of response was emphasised strongly to participants. If an incorrect key press was made the next trial was delayed until the correct one had been pressed.

There were two counterbalanced conditions which were completed in separate testing sessions by all participants; one where the sequence was a pseudo-sequence, and the other the sequence was repeated, meaning learning of the sequence was possible. The participants were naïve to the different conditions and were informed during the briefing that both sessions would consist of the visual cues appearing randomly. The sequences were a list of 14 locations of the cross, denoted by the digit linked to that location. The sequence used was the following: 1,4,2,3,2,1,3,4,1,2,4,3,1,2. On average participants took 5.61 seconds to complete a single run through the sequence. This dictated the inter pulse interval (IPI) as a single TMS pulse was delivered for each repeat. The 14 item sequence was used to maximise IPI without compromising learning, in order to avoid potential accumulative effects of TMS pulses, which have previously been reported with ISIs of less than 5s (Pellicciari et al., 2016). In this sequence pairwise transition probabilities between elements were equally probable. As shown in Figure 3.1, each testing session consisted of 5 experimental blocks in total, each of which consisted of a sequence containing 14 trials, which was repeated 30 times. This meant there were 420 trials per block, with the exceptions of block 4, which had the addition of 5 pseudorandom sequences, also consisting of 14 trials, meaning there was an additional 70 trials in block 4. The purpose of this was to assess learning, as we would expect to see an increase in RT in comparison to the other sequence blocks as a result of general practice and sequence learning. For both conditions, block 1 consisted of pseudorandom

sequences. This was used as a practice, to train the finger mapping and could also act as a baseline measure for the subsequent analyses. The pseudo sequence used in the pseudosequence condition and in the additional pseudosequence block 4b, were both balanced sequences, meaning that there was an equal likelihood that any of the locations could be presented in the subsequent trial, apart from the same location, meaning that there were no repetitions, nor were they the same.

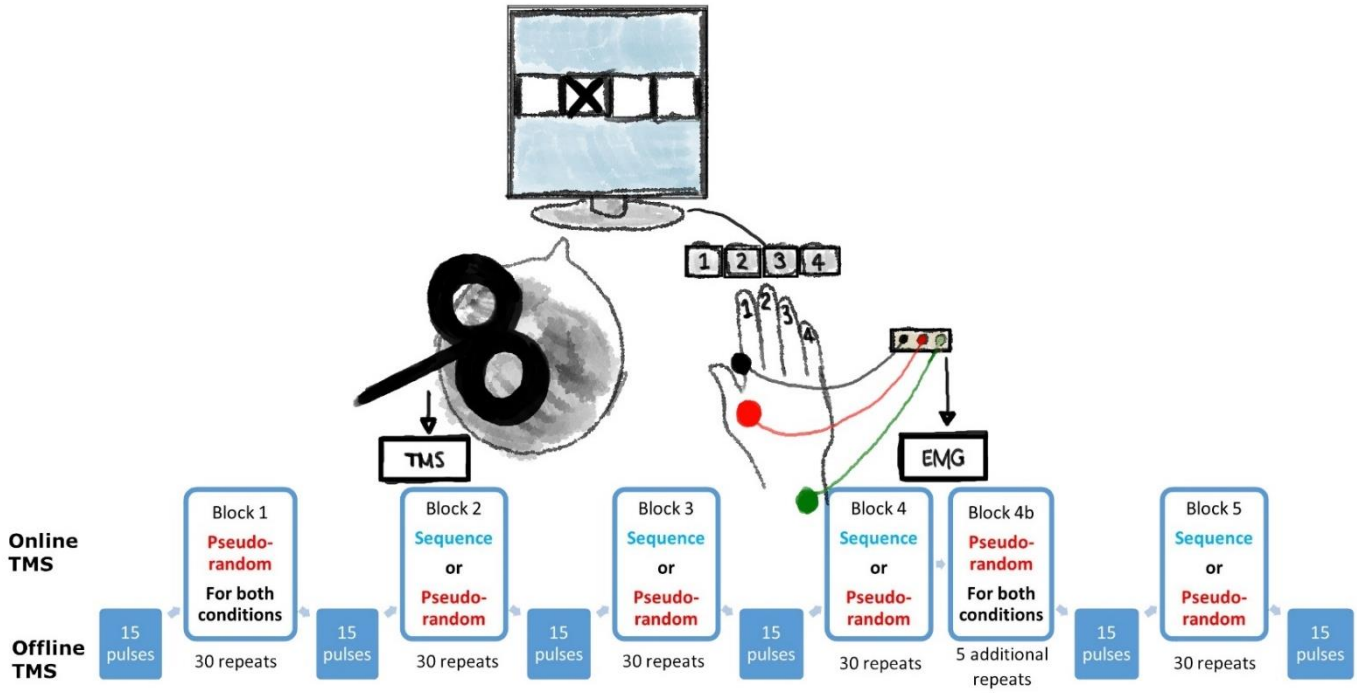

Figure 3.1 - A pictorial explanation of the experimental setup and a summary of the SRTT protocol. MEPs evoked from TMS stimulation of M1 were measuring from the right FDI muscle using EMG, before, during and after SRTT. Stimuli was presented on a computer screen directly in front of them and responses were made using a keypad. Digits are labelled to correspond with keys. In this example the correct response would be to use digit 2 (middle finger) to press the correspondingly numbered key. The protocol features 2 conditions, sequence and pseudo-random, which were completed on separate days, and 5 online experimental blocks which are presented alternately with blocks of offline TMS, where measures can be taken with the hand at rest. During the online experimental blocks, the TMS is applied every 13th correct response made in the SRTT.

3.2.3 Transcranial Magnetic Stimulation

TMS was delivered using a monophasic Magstim 200 stimulator (Magstim, Whiteland, Dyfed, UK) with a standard figure-of-eight, branding iron coil (diameter of one winding 70mm). This coil was held tangentially to the scalp, at a 45° angle from the midline, resulting in a posterior to anterior flow of current. The optimal stimulation site was defined as the site where the largest, most consistent MEP amplitudes were observed from the FDI. Neural navigation software (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used throughout the experiment with a MNI template which was scaled to the head of each individual. Registration accuracy was ensured by keeping any calibration errors below 3mm. This assisted with consistent coil placement over this location in the left primary motor cortex ensuring the same area was always being stimulated, with minimal movements from target location. We can therefore assume that any observed changes are due to the experimental procedure. MEPs were recorded using Ag-AgCl surface electrodes attached to the FDI muscle of the right hand in a belly tendon montage (see Figure 3.1). The signals were amplified, bandpass filtered (10Hz-1kHz, sampling rate 5kHz), and digitised using Brainamp ExG (Brain Products GmbH, Gilching, Germany). Resting motor threshold (RMT) was defined as the lowest stimulation intensity needed to yield a MEP with a peak-to-peak amplitude of >50μV in the FDI muscle whilst the subject is relaxed, in 5 out of 10 trials. All TMS pulses were triggered using an in-house Matlab program (Mathworks, MA, USA).

Offline TMS

Test pulse intensity during the offline blocks were set to 120% of RMT. 15 pulses were collected at this intensity prior to block 1 and then after every block subsequently. An interval of 5s separated each of these pulses, to avoid potential accumulative effects of pulses.

Online TMS

During online blocks, test pulse intensity was 110% RMT. Stimulation pulses during the task were triggered 100ms prior to the estimated response time of the 13th correct button response in the sequence. This would have matched with a response made by the index finger. The estimated response time was calculated as median RT of the previous four RTs. A total of 30 pulses were delivered per block. The mean total RT was 0.4 ± 0.05, meaning that the time it took to complete 14 key presses was approximately 5.61 seconds; establishing an interval between the pulses similar in length to the offline TMS.

3.2.4 Experimental Procedure

Subjects were sat at a table in an adjustable chair and instructed to sit as still as possible. During the task they were also instructed to minimise hand movement when completing the task, instead to just use the fingers to make a response, and otherwise keep them resting on the keypad. Each experimental session began with the identification of the FDI muscle 'hot-spot', followed by establishing the RMT. The procedure is summarised in Figure 3.1. This was a within subjects' paradigm, so all participants completed both conditions where subjects were presented with either sequences of locations for the visual cue, or pseudo sequences. Stimulation sessions were scheduled at the same time of day, and at least a day apart. Subjects were also naïve to what condition they were taking part in, and about the expectation of learning in one of them.

3.2.5 Data Analysis and Statistical Tests

EMG data was manually examined for muscle pre-contraction 500ms prior to the application of TMS pulses. If present, those MEP data values were removed. Peak-to-peak MEP amplitudes were measured using inhouse Matlab programmes (Mathworks, MA, USA). The statistical analysis programme jamovi (jamovi.org) was used to perform statistical analyses on generated data. All tests were conducted with a significance level of $p<.05$. Mauchley's test of sphericity was performed, and corrections were made using Greenhouse-Geisser when needed. Throughout the analysis process *p* values were uncorrected, meaning that interpretation of them in the subsequent results section should be interpreted with extreme caution. Median data was examined in addition with normalised data. In this instance normalised group data was formatted into percentage change values compared to the first experimental block.

Bayesian methods were applied as a parallel analysis strategy in addition to frequentist methods using the *p* value. The *p* value, an example of the null-hypothesis significance testing paradigm, is based on what can be expected if the H_0 were true rather than what can be expected if the H_1 were true (Marsman & Wagenmakers, 2017). Bayes factors (BFs) is a ratio that contrasts the likelihood of the data fitting under the null hypothesis (H_0), compared with fitting under the alternative hypothesis (H_1) (Jarosz & Wiley, 2014). Conventional significance testing only examines whether the H_1 is over a threshold to be labelled as significant compared to the H_0. Without these additional BFs examinations it is not possible to state evidence for the null hypothesis (Biel & Friedrich, 2018). This parallel approach was chosen as the BFs method is particularly suited to both the type of stimulation data generated here, and the circumstances under which this thesis is being written. The significant amount of missing data means that analysis is being conducted on small data sets, and BFs are not sensitive to the collection of more participants

if you want clearer evidence, it merely assigns degrees of plausibility to the existing data (Dienes, 2011). The classification scheme being used in this thesis for all BF analyses is depicted in Table 3.1.

Bayes Factor	Evidence Category
>100	Extreme evidence for H_1
30-100	Very strong evidence for H_1
10-30	Strong evidence for H_1
3-10	Moderate evidence for H_1
1-3	Anecdotal evidence for H_1
1	No evidence
0.33-1	Anecdotal evidence for H_0
0.1-0.33	Moderate evidence for H_0
0.03-0.1	Strong evidence for H_0
0.01-0.03	Very strong evidence for H_0
<0.01	Extreme evidence for H_0

Table 3.1 - A classification scheme for the interpretation of Bayes factors BF_{10} (Schönbrodt & Wagenmakers, 2018)

Behavioural

In each trial of the task, RT was recorded from the appearance of the visual cue, a cross in one of the four available positions, until the participant makes the correct corresponding key press. RTs longer than 1 second were excluded from further analysis. This should eliminate any instances where the subject took a break or was distracted, to be wrongly recorded. The median RT in each block was calculated, with block 4 trials 1-30, referred to as block 4a, and 30-35, referred to as block 4b, being treated as separate blocks. The median average of all included individual's data was then calculated for each block.

Offline

Trials in which there was evidence of precontraction 500ms prior to the TMS pulse were removed before median MEP amplitudes for each block were calculated.

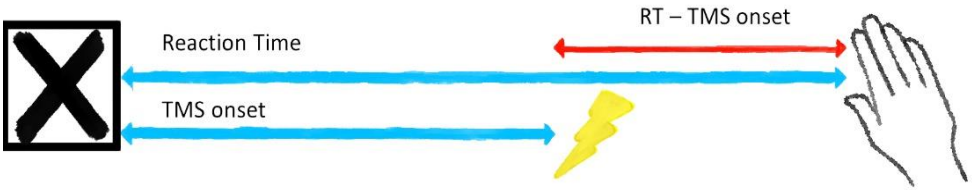

Figure 3.2 – Illustration to demonstrate that the RT is measured from the appearance of the visual cue, until the correct response is completed. TMS onset refers to the time between the visual cue appearing and the TMS pulse being discharged. And RT-TMS onset then equals the time from the TMS pulse to the response being completed

Online

Included MEP trials were ascertained by checking the RT of the 13th response subtracted by the onset of TMS for that trial was between .05s and .15s and was not a negative value. This ensures the TMS pulse was not triggered during, or after the movement, as we intend to measure excitability just prior to movement onset. Figure 3.2 explains this pictorially. This calculation is necessary to discard trials that would otherwise measure cortical excitability at the time that would not have been from the index finger, as they would have moved to the next trial in the sequence. 32% of the total data was discarded as a result (34% from the sequence condition, and 31% for the pseudo-sequence condition).

3.3 Results

3.3.1 Reaction times

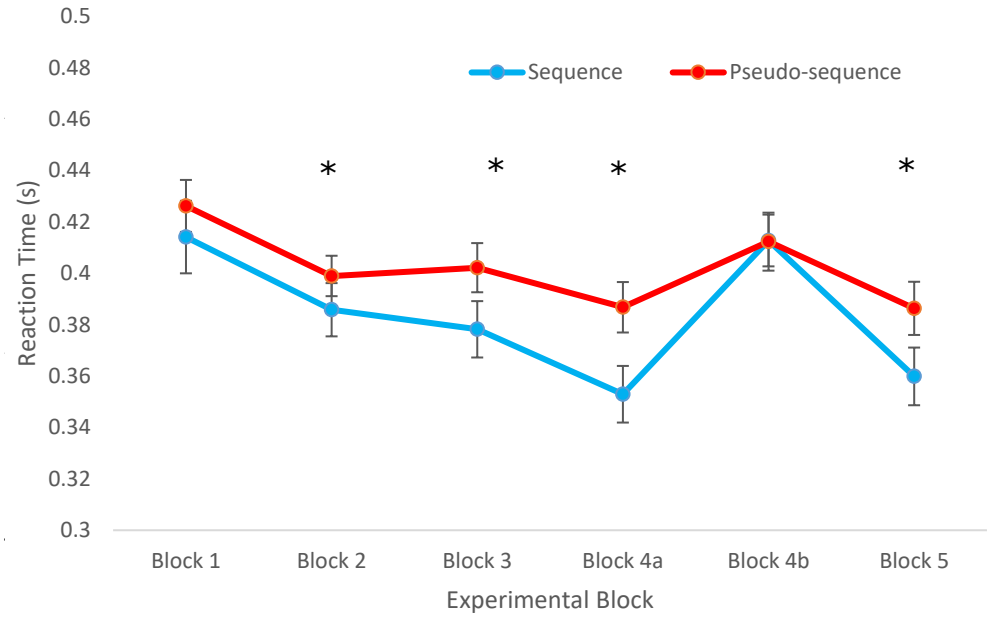

*Figure 3.3 - The median group reaction time data collected across all blocks during the SRTT for both the sequence and the pseudo-sequence conditions. Error bars depict standard error of the mean. * indicates a significant difference between the sequence*

Block	***statistic***	***df***	***p***	***Cohen's d***
1	0.0452	22	0.518	.0094
2	-1.9641	22	0.031*	.4096
3	-2.5609	22	0.009*	.534
4a	-2.8828	22	0.004*	.6011
4b	0.7591	22	0.772	.1583
5	-3.0453	22	0.003*	.635

*Table 3.2 - Results of one-tailed paired samples t-tests calculated for the behavioural data comparing the Sequence and the Pseudo-sequence conditions of the same block. An * indicates a significant value*

A one-tailed paired samples t-test was conducted on each of the blocks to compare between conditions and identify the blocks where the conditions RTs significantly differ. The results are shown in Table 3.2, with a reference of which blocks these are also shown in Figure 3.3.

Condition	***Block***	***statistic***	***df***	***p***	***Cohen's d***
Sequence	4(a) and 4(b)	-6.54	22	<.001*	1.364
	4(b) and 5	8.08	22	<.001*	1.684
Pseudo-Sequence	4(a) and 4(b)	-3.77	22	.001*	.786
	4(b) and 5	5.58	22	<.001*	1.163

*Table 3.3 - Results of two tailed paired samples t-tests comparing the median data from the additional blocks in 4(b) to the block appearing directly before and after it. * denotes a significant effect*

A two-tailed paired samples t-test was conducted to compare the RTs between specific blocks, the results are shown in Table 3.3. The blocks were chosen as an indication that learning had occurred, as the introduction of additional pseudo-random trials within the sequence condition should affect the RT. The analysis revealed a significant difference between the RTs of the chosen blocks in both conditions.

Figure 3.4 shows how, as the experiment continues, there is more evidence for the H_1. Blocks 4(a) and 5 are when we expected to see the most difference as participants will have had more practice, and that is where the BF is highest.

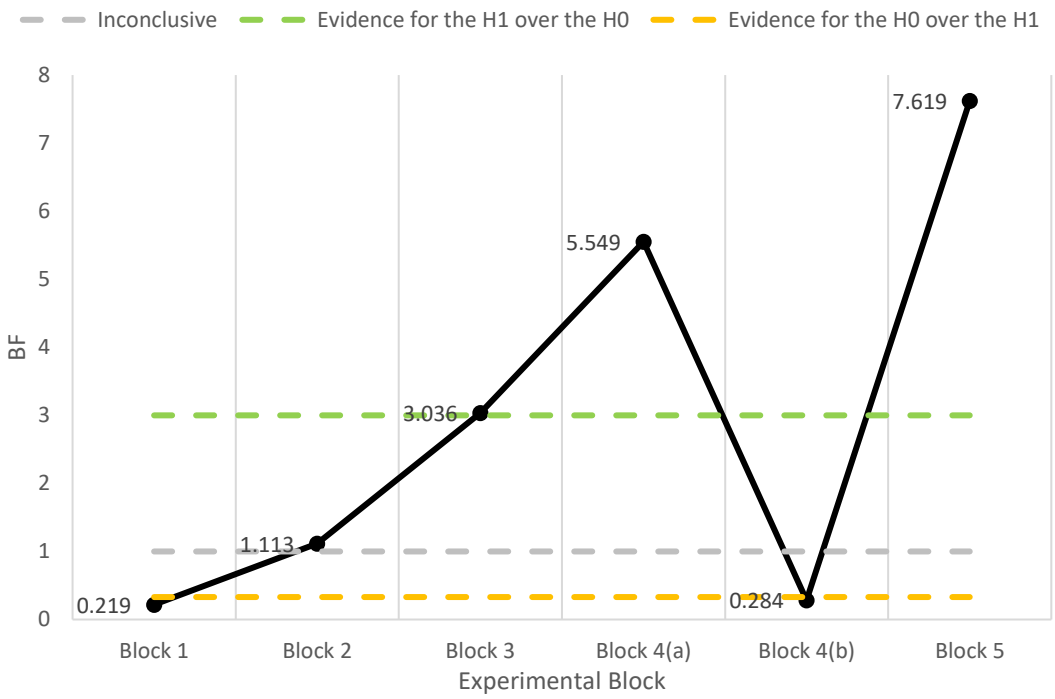

Figure 3.4 - Representation of the results of a Bayesian paired t-test. The horizontal lines indicate the thresholds for evidence for each hypothesis. Values lower than 1 indicate evidence for H_0, and values higher than 1 indicate evidence for H_1. The black line indicates the BF_{10} values for each experimental block

3.3.2 TMS Data

3.3.2.1 Offline MEP Data

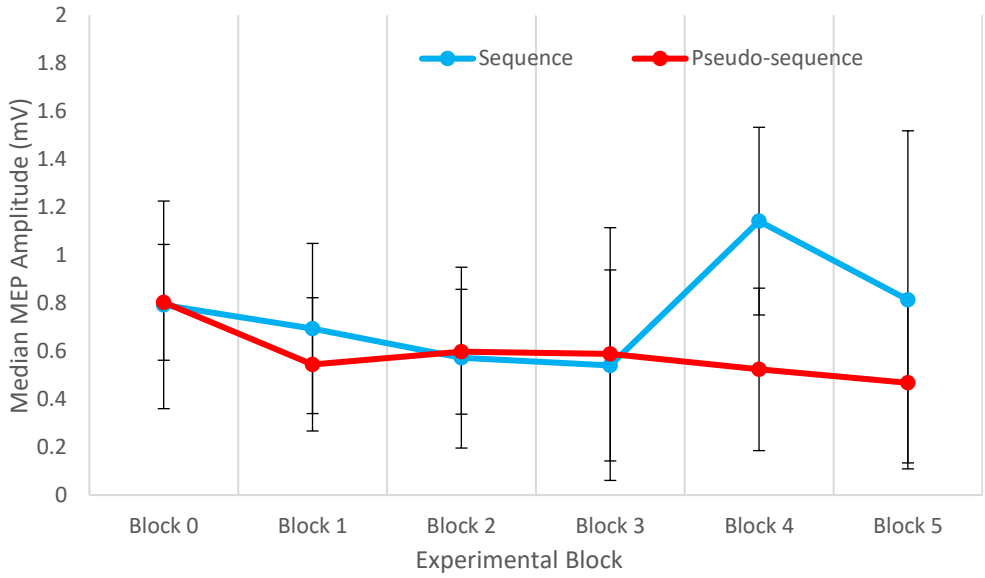

Figure 3.5 - Group median TMS pulse from offline TMS blocks, meaning the subject was at rest. Error bars depict the standard error of the mean

TMS data was analysed to examine the impact of condition on global excitability measures whilst the participant was at rest, between the blocks of the SRTT. This is shown visually in Figure 3.5. A repeated measures ANOVA was conducted on the median data to investigate the Time and Condition factors during the TMS measures taken whilst the participant was at rest in between blocks. No significant effects were found for either factor (Time (F [1.38, 30.45] = .621, p = .487, η^2p = .027), Condition (F [1, 22] = 1.299, p = .267, η^2p = .056), or interaction between these factors Condition*Time (F [1.71, 37.55] = 1.768, p = .188, η^2p = .074).

A Bayesian paired samples t-test was conducted on the median data to more closely investigate if this data was evidence for either the H_0 or H_1. The most promising value on inspection of Figure 3.6 is the Block 4 data, as the error bars have the least amount of overlap compared to the other blocks. The results are listed in Table 3.4, demonstrating how there was no substantial evidence for either hypothesis, with more evidence for the null hypothesis when examined as the median data and presented as percentage change data.

Block	BF_{10}	Category
0	0.275	Moderate evidence for H_0
1	0.307	Moderate evidence for H_0
2	0.277	Moderate evidence for H_0
3	0.245	Moderate evidence for H_0
4	1.452	Anecdotal evidence for H_1
5	0.768	Anecdotal evidence for H_0

Table 3.4 – The results of Bayesian paired samples t-tests to examine if there is any evidence that there are differences in offline MEP size between conditions

3.3.2.2 Online MEP Data

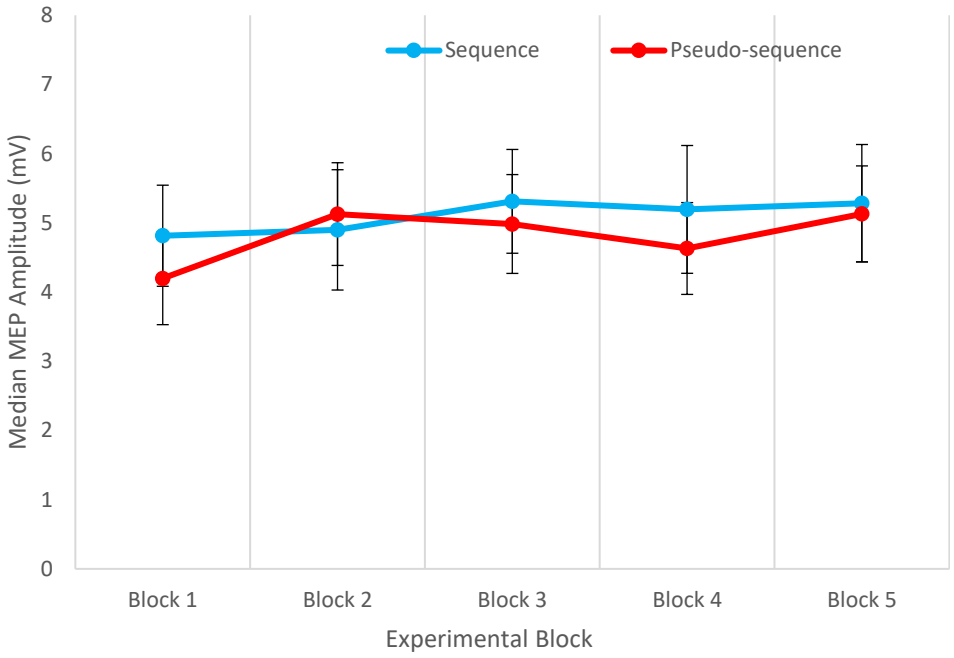

Figure 3.7 - Median group data for single pulse online TMS measures, meaning the subject was engaged with the SRTT task at the time of the pulses firing. Error bars depict standard error of the mean

The online TMS data was analysed for evidence of the impact of condition and time on global excitability measures during the task as motor learning occurred. A repeated measures ANOVA was conducted on the median data to investigate the Time and Condition factors during the TMS measures taken whilst the participant was at rest in between blocks. A significant effect was found for Condition during the Online blocks, but no significant effects were found for either Time, or Condition*Time (Condition (F [1, 22] = 4.511, p = .045, η^2p = .17), Time (F [1.82, 40.05] = .458, p = .618, η^2p = .02), Condition*Time (F [4, 88] = .123, p = .974, η^2p = .006).

A Bayesian repeated measures ANOVA was also conducted on the median data, and this revealed that there was extreme evidence for the H_1 for Condition (BF_{10} = 2927.86), very strong evidence for the H_0 for Time (BF_{10} = .0166), and very strong evidence for the H_1 for Condition * Time (BF_{10} = 51.146).

A two tailed paired samples t-test was conducted to compare the MEP size of the first online block with the final block. No significant change was expected for the pseudo-sequence as no learning is expected to occur, but it was hypothesised that there might be a difference in the online conditions. As depicted in Table 3.5, no significant effect was found for the median data. This was similarly supported by Bayesian paired sampled t-test between Block 1 and 5, with both sequence condition and pseudosequence conditions returning moderate evidence for the H_0 (Sequence BF_{10} = .219; Pseudosequence BF_{10} = .226).

Condition	***statistic***	***df***	***p***	***Cohen's d***
Sequence	0.075	22	0.941	.0156
Pseudo-sequence	-0.2765	22	0.785	.0576

Table 3.5 - Results of paired samples t-tests calculated for the median MEP data from the online blocks. Block 1 and Block 5 values are compared, but no significant differences are observed

3.4 Discussion

Plastic changes that take place during motor learning have been studied previously. In this study, our aim was to further investigate those connections between patterns of plasticity while performing a motor learning task, with a view to develop this protocol into one that would be applicable for use in research with individuals with TS. Our RT data findings in particular reflected that of previous research.

Behavioural Data

We achieved our aim of successfully replicating expected behavioural results through the use of a modified SRTT. Motor learning was demonstrated as occurring in this task by the significant difference in RT between conditions where subjects were presented with a reoccurring sequence, or pseudo sequences. Figure 3.3 shows a clear decrease in RT for both conditions as the participants become familiar with the finger mapping. Blocks 2, 3 and 4a show the reaction times starting to increasingly differ, with the pseudosequence condition significantly slower than the sequence conditions RTs. In block 4b, the additional trials of pseudosequence, there is a return to a time comparable to the baseline speed, where there is no significant difference between the conditions. This was as expected, with a significant increase of RT. Following that, block 5 shows a return to faster RTs, and a significant difference between conditions, with those in the sequence condition performing faster than those in the pseudosequence condition. This was the pattern of results we were hypothesising to take place over the course of the experiment. These findings align with previous research such as Ambrus et al., (2016), and suggest that learning of the sequence has occurred and is having a significant effect on RT, rather than the change in RT merely resulting from practice effects.

Some findings shown in Table 3.3 were unexpected. We hypothesised that in the sequence condition there would be a significant difference in RT between blocks 4a and 4b, and 4b and 5. As the pseudosequence trials were added in block 4b, it was thought that would cause a large contrast from the sequence condition, which the results did indicate. However, there was also a significant difference evident from the pseudosequence condition showing a slowing in RT at 4b despite these trials being a continuation of the same pseudosequence condition. We speculate that this could be due to fatigue, as the participant was not told the block contained additional trials, making it feel longer. They may have been anticipating when the block would have ended, resulting in the RTs slowing. Block 5 resumes after a block of offline TMS measures, meaning they have a rest from responding to the task. This perhaps explains the decrease in RT again. Block 4b is also a smaller number of trials from which to calculate an average, which may have affected the outcome.

TMS Data

Studying plasticity in the context of motor learning is not easy, since the synaptic changes are not likely to be the only factor influencing the learning rate, and therefore meaning it is not a straightforward relationship to interpret between MEP amplitude and motor output changes following learning (Bestmann & Krakauer, 2015). Amplitude does not correlate to something being learned, but if there are observed changes in the MEP amplitude, it may indicate that learning and plasticity is occurring.

No significant effects were found in the offline experimental blocks, whilst the participant's hand was at rest, when examining the data with frequentist statistics. There was also nothing more than moderate

evidence for the null hypothesis following analysis with Bayesian statistics, meaning we cannot reject the null hypothesis that motor learning over time does not have an effect on global excitability between blocks in the hand area in this motor learning protocol. This was similarly found in the control conditions of Ambrus et al., (2016), that MEP amplitudes were not changed during offline TMS measures.

A significant effect of Condition on the size of the MEP responses was demonstrated during the execution of the SRTT, but not for the factor Time. However, the Bayesian analysis suggests that there is extreme evidence for the H_1, and the size of the MEPs during the task is a result of which condition the participant is completing. Our findings also suggested that Time and Condition*Time also had very strong evidence supporting the H_1, that MEP amplitudes were also affected by the time course of the experiment and there was an interaction between both of those factors. This is a departure from Ambrus et al., (2016) and their findings, as they found no significant change of MEPs in their equivalent conditions. Examining this data using Bayesian statistics reveals a more nuanced result that requires further investigation with more subjects. It was hypothesised that amplitudes would increase in size, which does seem to be the case in Figure 3.7. More investigations may be necessary to examine if this is a result of learning during the SRTT or if this MEP variability is skewing the data to obscure a more representative average. It may be possible to further examine if the process of learning in the sequence condition supports consistency of MEPs over time.

Limitations

Very rarely are study designs perfect and able to account for all potential methodological issues. As with any TS study, coil placement is key to ensure consistent stimulation of the targeted brain area. Brainsight neuronavigation software was used throughout the experiment to

mitigate this. There would, however, be a higher degree of accuracy if the individual's anatomical MRI scans were available to reduce any error associated with the use of the template brain available within the software. Maintaining the coil position is also much harder for the TMS operator when a degree of movement is introduced into the experiment. Whilst subjects were instructed to keep as still as possible during the SRTT, this was not always guaranteed, and could therefore influence the MEP amplitudes. A further limitation is related to the subjects' attention to the task. On occasion it was noticed that subjects were not adhering to the instructions, such as moving their hands excessively or using the incorrect finger responses. Reaction times were checked for possible signs of inattention to try and mediate this.

The Bayesian statistical results also suggest that the sample size is not sufficient in this case to effectively capture a more representative sample. It highlights the need to incorporate this into brain stimulation research that is often undertaken using small sample sizes (Biel & Friedrich, 2018). This needs to be carefully balanced with the wish to incorporate more individual brain scans into the procedure to improve TMS targeting accuracy.

The subsequent design utilising this protocol, detailed in Chapter 4, combined the sessions to a single day, meaning subjects only needed to attend one experimental session. This may help with inattention and ensure full data sets are collected. It may also remove the variability of someone presenting at a data collecting session in a different state, for example, not having the same amount of sleep, recently having ingested alcohol and recreational drugs or other factors that may alter their attention and cortical excitability between sessions, making comparisons between sessions less valid. This will also reduce possible practice effects that may continue from the previous session.

Conclusions

This study was largely successful in replicating the previous findings from the behavioural data, relating to the RT changes during the SRTT. The pattern of these RT changes suggest that motor learning occurred, and is therefore present in our data sets. However, the TMS related data is less clear. It is not possible to reject the null hypothesis in the instance of the offline data, as no significant change in MEP amplitude was observed. However, in the online data there is some evidence that the presence of a sequence increased the MEP amplitude.

The protocol was designed to pilot the SRTT procedure and to make any necessary changes to the design before it was used to examine motor learning and plasticity difference in individuals diagnosed with TS. This is explored further in the next chapter.

Chapter 4 : Serial Reaction Time Task and Tourette Syndrome

Keywords: *Tourette's Syndrome (TS), cortical-striatal-thalamic-cortical circuits (CSTC), Gamma aminobutyric acid (GABA), Paired associative stimulation (PAS), Motor evoked potential (MEP), Electromyography (EMG), Motor threshold (MT), Long term potentiation (LTP), Long term depression (LTD), Short interval intracortical inhibition (SICI), Interstimulus interval (ISI), Premonitory Urge to Tic Scale (PUTS), Yale Global Tic Severity Scale (YGTTS), Wechsler's Abbreviated Scale of Intelligence (WASI), Yale-Brown Obsessive Compulsive Scale (YBOCS), Children's Yale-Brown Obsessive Compulsive Scale (CYBOCS), Social Communication Questionnaire (SCQ), primary motor cortex (M1), reaction time (RT), motor-evoked potentials (MEPs), Conditioning pulse (CP)*

It has previously been shown that those diagnosed with TS have an altered performance in some motor tasks, with accompanying imaging data showing a different neuronal activation pattern compared to typical developing individuals (Serrien et al., 2002).

The aim of the study described in this chapter was to build on the work of the previous SRTT work in Chapter 3, to demonstrate differences in motor learning and in plasticity between TS and controls. Due to restrictions on in-person testing during the Covid-19 pandemic, insufficient data was collected to make any supported conclusions for this experiment, however, initial Bayesian analyses were conducted to assess if this experimental design is suitable for this patient group.

4.1 Introduction

The progression of habit formation resembles that of the development of the tic symptoms associated with Tourette syndrome (TS); both are

inflexible and repetitive behaviours that are acquired over a period of time (Jackson et al., 2015). As a result it is thought tics develop from aberrant habit learning (Conceição et al., 2017). A study by Marsh et al., (2004) included an alternative learning task, similarly designed to test habit learning, with an accuracy and a reaction time component. Their conclusions further support that the dysfunction in the striatal learning systems subsequently mean habit learning is impaired with children and adults with TS in comparison to normal controls. Interestingly, an association between the severity of tics an individual experiences and their impairment in the learning task was made, which further suggests that the habit learning system in this group is dysfunctional and is relevant to tic formation. Whilst plasticity has been shown to be key in learning processes, such as the motor movements discussed, there is some contradictory evidence for exactly how plasticity is altered in patient groups compared to healthy populations. One procedure by Martín-Rodríguez et al., (2015) utilised TMS and a PAS protocol to evaluate motor cortical plasticity in adult patients diagnosed with severe TS. Electrical stimulation was applied to the median nerve on the wrist and paired with TMS stimuli over the APB muscle hotspot. Their findings consisted of an increase of LTP-like motor response, meaning, the authors concluded, that plasticity was abnormally increased in patients and this abnormal plasticity was associated with tic severity. This is in contrast, for example, to Brandt et al., (2014), who reported the majority of adult patients displayed LTD-like plasticity in protocols where the majority of the healthy sample were responding with LTP-like plasticity. However, both studies concluded there was an association between tic severity and cortical plasticity. One possible explanation for this contradictory result is that the recruited subjects differed slightly; Brandt et al., (2014) excluded patients with co-occurring diagnoses, therefore only uncomplicated TS subjects, with more mild tics were used, unlike the other study. This has problems in that this 'pure' TS may be much more of an exception, rather than the normal patient experience (Freeman et al., 2000). This clearly demonstrates that more investigations into the plasticity differences between healthy and patient groups is necessary.

There are many ways to measure altered plasticity in TS. Alternative methods to TMS include imaging techniques to assess structural differences or MR spectroscopy to investigate chemical concentrations in the brain. The most commonly associated areas with altered structure and function for those diagnosed with TS are those contained within the CSTC brain circuits, which is heavily implicated in motor learning (Leckman & Riddle, 2000). The neurotransmitter GABA has implications in the control of those cortical areas associated with TS, regulating and modulating the processes involved in development or changing those neuronal networks (Di Cristo, 2007). If these GABAergic mechanisms are dysfunctional, this directly impacts the CSTC as the structure and coordination of the network will be undermined. This has been clearly demonstrated in the instance of TS (Rapanelli et al., 2017) with evidence of altered concentration of GABA in motor areas of the brain (Draper et al., 2014; Puts et al., 2015). Reduced amounts and distribution of GABAergic interneurons have been observed in the striatum of severe TS in post mortem studies (Kataoka et al., 2010). TMS is a useful physiological technique with which to measure cortical excitability differences and physiological inhibition, in particular the paired pulse measure, SICI, mentioned in Chapter 2.2.2. This type of inhibition, which is dependent on specific GABA receptors, is decreased in individuals with TS (Jackson et al., 2015; Orth & Münchau, 2013). There are suggestions that there is a relationship between SICI and the severity of the individual's motor tics, but more evidence is required, and may be confounded by the presence of co-occurring conditions, ADHD in particular (Gilbert et al., 2004).

This updated protocol builds on the design piloted in Chapter 3 and enables a full data set to be collected in one session, including sequence and pseudosequence conditions within the same experiment. Such a protocol increases the likelihood of successful recruitment of sufficient participants whilst remaining a within subjects design, gathering full data

sets and being able to undertake studies with more statistical power. Additionally, there is the introduction of paired pulse measures, meaning we are able to examine differences in SICI between groups and throughout the course of motor learning. Previous studies examining the use of SRTT in TS populations have previously found no evidence for altered performance in individuals compared to controls, or to even display enhancement of sequence learning (Shephard, Groom, & Jackson, 2019; Takacs, Münchau, Nemeth, Roessner, & Beste, 2021). Whilst studying TS with the SRTT has been done before, by adding the single and paired pulse TMS measures it is hoped that this paradigm will be more sensitive to motor learning differences that may instead be observed within the cortical excitability levels rather than only in the behavioural response.

In this study we hypothesised that we would observe a similar behavioural pattern to that described in Chapter 3, to clearly demonstrate that motor learning is occurring throughout the experiment. In comparison to the control groups, we hypothesise that the individuals diagnosed with TS will have a quicker decrease in RTs, demonstrating that they were more quickly learning the sequence. However, when the pseudosequence block is introduced, we would expect there to be more significant increase in their RT. This would be in line with the theory that tic acquisition mimics an increased aptitude for habit learning, however this could suggest the presence of hyper-learning, and results in a greater difficulty in avoiding this learnt behaviour, resulting in those longer reaction times when the sequence is removed. It is also hypothesised that there will be an observable difference in the patterns of cortical excitability over the course of the protocol, evidenced using MEP amplitudes in the offline and online portions of the task compared to those without a TS diagnosis, especially when examining the paired pulse data, which we expect to see as having a lower inhibitory effect in those with TS in line with previous conclusions.

4.2 Methods

4.2.1 Participants

Prior to testing being suspended, 7 individuals with a confirmed diagnosis of TS had participated (4 male and 3 female, mean age: 29.6 ± 12.7 years, range: 15.7 – 51.4 years). All were tested using the questionnaire methods below. The aim was to collect 30 data sets with participants diagnosed with TS, and 30 controls, matched for age and gender and handedness. Exclusion criteria included an IQ score of 70 or below, any contraindications to TMS or a sight disability that could not be corrected with corrective measures such as glasses, as this would have prevented them from accurately completing the on screen SRTT.

						YGTSS					Strengths and Difficulties					
Participant	Age	M/F	Handedness	Resting Motor Threshold	WASI	Impairment	Motor	Phonic	Global	PUTS	Difficulties	Prosocial	AQ	C-YBOCS/Y-BOCS	Medication	Hand Tics reported on Yale?
TS061	15.7	M	Right	62	99	10	21	15	46	42	16	8	17	1	-	Yes
TS204	33.6	M	Right	56	130	0	17	11	28	26	16	9	18	0	Clonidine	Yes
TS182	18.6	F	Middle	43	85	10	12	7	29	62	18	10	16	0	Clonidine	Yes
TS234	51.4	M	Right	43	90	40	18	18	76	58	21	10	20	22	Fluoxetine, Lorazepam	Yes
TS235	19.1	F	Right	48	117	30	16	14	60	35	21	10	27	0	-	No
TS229	33.4	M	Right	42	114	20	14	6	40	57	17	8	23	21	Paroxetine	Yes
TS240	35.11	F	Right	41	-	15	22	0	37	19	19	7	22	15	Thyroxine, Citalopram	Yes

4.2.2. Questionnaire Methods

The following questionnaires and measures were administered by an experienced researcher.

Edinburgh Handedness Inventory

An adapted version of the Edinburgh Handedness Inventory was administered to access the degree of handedness (Oldfield, 1971). As the application of TMS is over the left M1, and therefore stimulating the right hand, it is preferable that the participants were either categorised as having no dominant hand or having a right-hand preference.

Assessing tic severity

The Yale Global Tic Severity Scale (YGTSS) (JAMES F. Leckman et al., 1989) was used to assess the participants current symptoms of TS. This takes the form of a semi-structured clinician-rated measure, which details the nature of the motor and vocal tics that the participant has been experiencing over the past two weeks. It records the number, frequency, intensity and complexity of both motor and phonic tics, as well as also rating the level of interference on an ordinal scale, and the level of impairment the participants experience in their daily life as a result of their tics. It takes into account the distress an individual may be feeling as a result of their symptoms in a variety of day to day contexts and is clear in the instructions that the individual should consider their academic, workplace and social interactions, for example. The YGTSS measure is widely used as it has been demonstrated to have good psychometric properties, in particular, internal consistency, stability and validity (Storch et al., 2005) (Storch et al., 2011).

Premonitory urge to tic scale (PUTS)

This scale is commonly used during tic disorder research to measure the associated premonitory sensory phenomena an individual may experience before the onset of a tic. PUTS allows researchers to measure this sensation quantitively, using a self-report measure to assess different properties of the urges and how they relate to an individual's tics. This measure was also found to have good internal consistency, reliability and validity when used in children over the age of 10 years old (Woods et al., 2005).

Y-BOCS or CY-BOCS (Yale-Brown Obsessive Compulsive Scale and Children's Yale-Brown Obsessive Compulsive Scale)

The Y-BOCS is a semi-structured measure of obsessive-compulsive symptom severity as experienced by the patients over the past week. The CY-BOCS is a modified version with altered wording to ensure it is more developmentally appropriate for children or adolescents (Scahill et al., 1997).

Additional Measures

In addition to the measures described above, further questionnaire data was collected by utilising carefully selected measures. These included the Strengths and Difficulties Questionnaire that assesses the psychological adjustment of the individual by questioning them on their positive and negative attributes. These generate indications of emotional symptoms, conduct problems, hyperactivity-inattention, peer problems and prosocial behaviour, which can then be culminated to create an overall difficulties score (Goodman, 2001). Participants within the patient groups were screened for symptoms of attention-deficit hyperactivity disorder (AHDH)

using the Conners-3 self-report measures (Conners, 2008), symptoms of autism using the 'current' portion of the Social Communication Questionnaire (Rutter et al., 2003) and the Autism-Spectrum Quotient (AQ) questionnaire (Baron-Cohen et al., 2001). IQ estimates of participants were collected using the vocabulary and matrix reasoning portions of the tests from the Wechsler's Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999).

4.2.3 Serial Reaction Time Task

Participants performed a modified version of the SRTT (Nissen & Bullemer, 1987). This design was a development of the version used in Chapter 3. The key difference was that subjects only needed to attend one session for data collection.

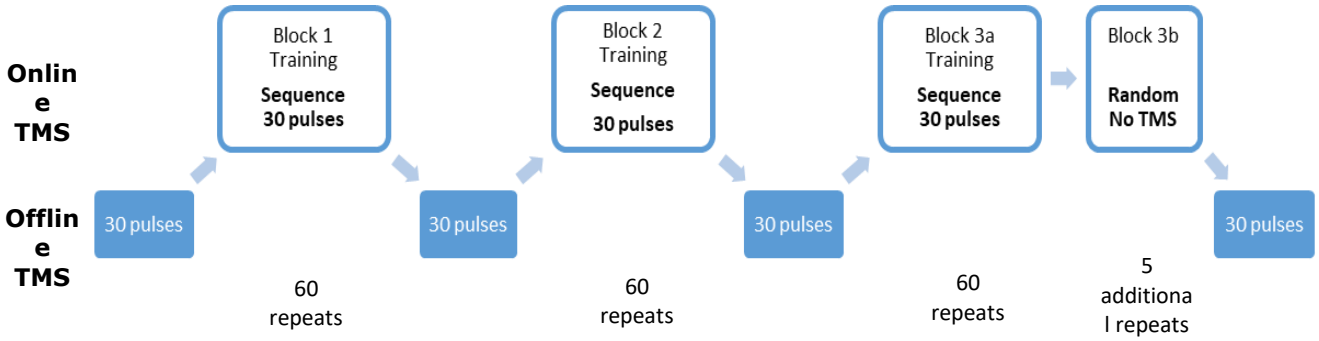

Figure 4.1 – Summary of the SRTT experimental protocol combined with the TMS measures

During the task, the participants were presented with 4 boxes arranged horizontally across a screen directly in front of them. A cross appeared in one of those boxes in the following sequence: **1, 4, 1, 2, 4, 2, 1, 3, 2, 3, 4**. Participants were instructed to respond to the location of the cross by pressing the corresponding keys using their right hand as follows: number 1 to be pressed with the index finger, 2 with the middle finger, 3 with the

ring finger and 4 with the little finger. The participant was required to make an accurate response as quickly as possible with the corresponding key press depending on its location. Speed of response was emphasised strongly to participants. If an incorrect key press was made the next trial was delayed until the correct one had been pressed. TMS was triggered just before the 7th key press in the sequence, which corresponds with the index finger.

As shown in Figure 4.1, each testing session consisted of 3 experimental blocks in total, each of which consisted of a sequence containing 11 trials, which was repeated 60 times, followed by the addition of random sequence additions, which were repeated 5 times at the end of the third and final block. The purpose of this was to highlight the presence of learning, as it is expected that participants would demonstrate a sudden increase in RT at the end of the 3rd block when compared to the other blocks, as the introduction of a random sequence slowed the participants.

4.2.4 Transcranial Magnetic Stimulation

Similarly, to the experiment detailed in Chapter 3, this design incorporates a mixture of online and offline TMS measures. TMS was delivered using a monophasic Magstim 200 stimulator (Magstim, Whiteland, Dyfed, UK) with a standard figure-of-eight, branding iron coil (diameter of one winding 70mm). This coil was held tangentially to the scalp, at a 45° angle from the midline, resulting in a posterior to anterior flow of current. The optimal stimulation site was defined as the site where the largest, most consistent MEP amplitudes were observed from the FDI. Neural navigation software (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used throughout the experiment with a MNI template which was scaled to each individual's head. Registration accuracy was ensured by keeping any calibration errors below 3mm. This

assisted with consistent coil placement over this location in the left primary motor cortex (M1). MEPs were recorded using Ag-AgCl surface electrodes attached to the FDI muscle of the right hand in a belly tendon montage (see Figure 3.1). The signals were amplified, bandpass filtered (10Hz-1kHz, sampling rate 5kHz), and digitised using Brainamp ExG (Brain Products GmbH, Gilching, Germany). Resting motor threshold (RMT) was defined as the lowest stimulation intensity needed to yield a MEP with a peak-to-peak amplitude of >50μV in the FDI muscle whilst the subject is relaxed, in 5 out of 10 trials. All TMS pulses were triggered using an in-house Matlab program (Mathworks, MA, USA).

Offline

There are 4 offline blocks, each consisting of 30 pulses. 15 of these were single pulse measures, and another 15 were paired pulse 3ms SICI measures. Conditioning pulse intensity was 65% RMT and test pulse intensity was set at 120% of RMT. The application of these pulses did not have a set order, and there was a 5s interval between each test pulse to avoid potential accumulative effects of pulses.

Online

There are 3 online blocks, plus the addition of the random sequence at the end of the 3rd one. During these online blocks, a single pulse of TMS was triggered every other iteration of the sequence. This was selected so that there was a minimum of 4 seconds between each pulse, giving the TMS stimulator time to recharge before having to fire again, and to prevent any priming effects of pulses that may have been too close together. The pulse was always fired when the subject was going from '4' to '1' in the sequence. This digit transition occurs twice during the sequence, so the experiment was designed to randomise whether the TMS pulse occurs during the early or late transition. This can reduce any

changes in RT in expectation of the loud clicking noise associated with TMS or the sensation. The timing of the pulse firing was calculated by tracking the median RT of each correct key press to estimate when the participant may be about to respond to an index finger key press, and therefore the TMS pulse coincided with the same point in movement preparation.

4.2.5 Experimental Procedure

Subjects were sat at a table in an adjustable chair and instructed to sit as still as possible. During the task they were also instructed to minimise hand movement when completing the task, instead to just use the fingers to make a response, and otherwise keep them resting on the keypad. Each experimental session began with the identification of the FDI muscle 'hot-spot', followed by establishing the RMT. Subjects were naïve about the expectation of learning a sequence.

4.2.6 Initial Analysis

Planned analyses included comparisons between the TS population and age-matched controls for observable differences in task performance, MEP data throughout the SRTT and also questionnaire scores. Every participant had notes made by the researcher during data collection regarding the individual completing the task. The full effects of the observations were more evident during data pre-processing and initial assessment of the RT. In those cases, the notes were useful to refer to and are an interesting commentary alongside the number of trials within the task that were necessary to be excluded from analysis. These are detailed below:

TS061

Experienced hand tics during the task that seems to have been interfering with them completing the task, they also struggled to keep still during offline measures. 61 RT trials were excluded due to being too slow and therefore outside of the necessary range.

TS204

Had severe tics that were interfering with both the task and keeping stationary when required. 91 RT trials excluded.

TS182

Hands were observed shaking throughout the task. 227 RT trials were subsequently excluded.

TS234

This individual could not prevent hand movements during offline measures. 84 RT trials excluded

TS235

No usable data collected due to a large amount of movement throughout, especially of the head. This meant EMG recordings were difficult to examine, and TMS coil placement was highly inaccurate.

TS229

Picked up the sequence immediately, made remarks about it and was aware when it wasn't present. At the end of testing the participant informed us they were a professional musician. 2 RT trials excluded.

TS240

This individual also experienced a lot of head tics which impacted the accuracy of the TMS coil placement. 26 RT trials excluded.

These notes clearly demonstrate that significantly more participants are needed as there is the potential for lots of individuals to experience this tic interference in the task and the offline measures, something that may be difficult to screen for prior to them taking part. The combination of circumstances here, where recruitment is difficult in these population groups and usable data sets may be difficult to acquire, is a good instance where Bayesian statistics can be a useful tool to assess if there are any findings worth pursuing.

4.3 Initial Results

With only 6 useable data sets, all with so many excluded trials, and no control matched data it is difficult to reach any conclusions about the data collected so far. An initial examination of the RT data necessitated the inclusion of the Bayesian analyses as these are a useful tool to use during the data collection process. Whilst this data set is incomplete, what was collected suggests a similar pattern of RT whilst performing the SRTT task as those taking part in the sequence condition in Chapter 3. Figure 4.2 shows this promising start with the expected shape of the RT changing during the SRTT. As the subject becomes more familiar with the sequence their RTs are decreasing as they become faster. The introduction of pseudorandom sequences in Block 3b prompts an increase in the RTs.

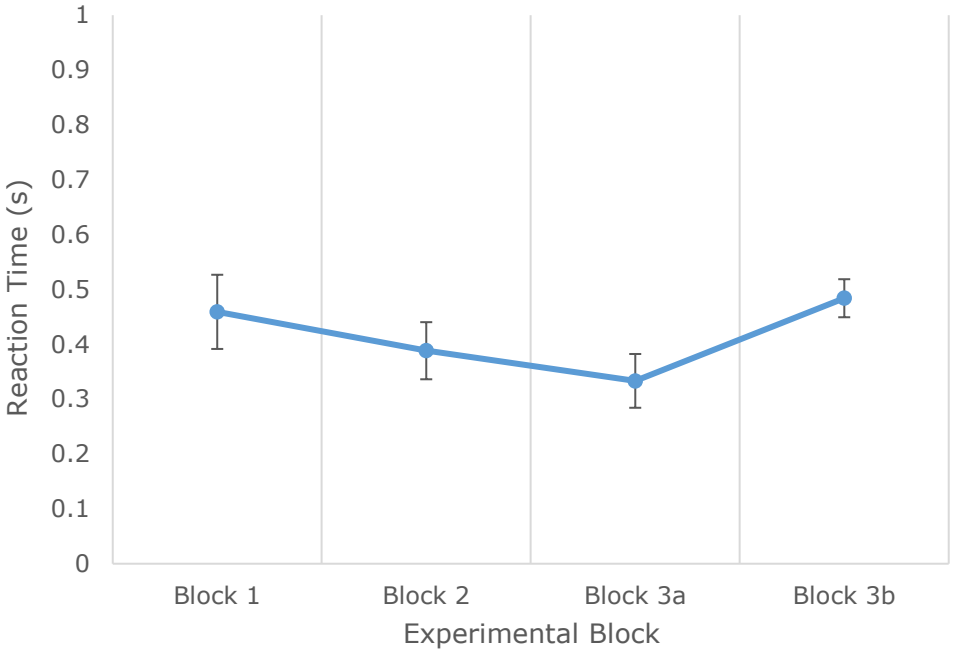

Figure 4.2 - Group median RT for each experimental block for 6 participants. Error bars show standard error of the mean. Block 1, 2 and 3a have sequences, and 3b introduces some pseudorandom sequences

Paired Variables		BF_{10}	Category
1	3b	0.471	Moderate evidence for H_0
2	3b	19.204	Strong evidence for H_1
3a	3b	124.839	Extreme evidence for H_1
1	3a	6.994	Moderate evidence for H_1
2	3a	5.585	Moderate evidence for H_1

Table 4.1 - Group results for Bayesian paired samples t-tests. The categories are determined by Table 3.1.

This was further supported by the analyses reported in Table 4.3. When comparing Block 1, the RT baseline and Block 3b, when the pseudosequence is imposed, there is moderate evidence that the null hypothesis is true, that there is no significant difference between the RTs. The other comparisons all returned at least moderate, or stronger, evidence for the alternate hypothesis that the RTs were significantly different between those blocks. This fits with the hypothesis that RTs will match the pattern depicted on Figure 4.2 and therefore, we are hopeful that this indicates that motor learning is occurring during this altered SRTT paradigm. By demonstrating that the SRTT paradigm is measuring motor learning skills as intended, this shows that theoretically, more in depth comparisons between patient and control groups about motor learning and associated TMS results, are possible.

4.4 Discussion

As mentioned in the Covid-19 impact statement, testing had to be suspended prematurely during this research, therefore almost all the data that had been planned to be collected is missing for this experimental protocol from both the patient and control groups. However, this exercise was still useful to test the protocol design and feasibility within this population group. After this small sample was collected, some limitations became apparent.

Limitations in the research were linked to movement whilst the session was underway. This was mentioned in the previous chapter, but remains relevant when testing individuals with TS as for most participants tested, their tics interfered with their reaction time. Notes were taken after each session by the researcher to record if anyone had any impairment completing the task. Most of these issues were because due to tic

movements or an inability to remain still, creating a considerable amount of noise on the EMG. This consequently made it harder to see MEPs during the pre-processing of the data, changing the size of the MEPs as the muscles were activated, and therefore giving an inaccurate measure of excitability. Hand tics were interfering with reaction times in many instances, and one individual seemed to have shaking hands, so there was a concern they may have felt anxious during testing. Head movements were also an issue, as this made it difficult to collect accurate TMS data. Coil placement is much more likely to be inconsistent compared to controls, and therefore the subsequent MEPs may not be representative of cortical excitability. It may also change the individual's reaction times as their eye line and focus is moved away from the screen. Due to the varied experiences of those who were recruited, there were also many instances which would need to be reviewed to decide whether they would be suitable for analysis within the group data. There was one example of an individual presenting with a hemifacial spasm, reported as unrelated to their TS diagnosis, but this still presented a similar difficulty of maintaining TMS coil positioning for the researcher. Another revealed themselves to be a professional guitarist, a skill that requires movements similar to those required in the SRTT, and pattern recognition. The researcher noted that they completed the task with extreme ease, picking up the sequence very quickly and commenting on its presence. Whilst the exact nature of the experiment was not revealed to them until the end, there may be some discussion required to decide whether or not this individual was 'blind' to the conditions, and if their advanced dexterity relating to their profession may also be significantly different to the group average and thereby skewing it. Another adult individual had the opposite problem and struggled to complete the task as they had tics in their hand that drastically interfered with their reaction times. These specific circumstances would all be difficult to screen for prior to testing, as well as many other circumstances not yet imagined. It should be noted this may require many more participants to be recruited than originally planned to account for instances that may need to be excluded from analysis if a subject has a high number of data points that require

removal. It is also important that there are enough individuals who can be included within the analysis to ensure that a proper cross section of TS experience is able to be tested, whilst still remaining feasible to test and there is not too much tic interference. This highlights the need for accuracy data as well as the RTs to examine if subjects are sacrificing their accuracy for speed in any instances (Shephard et al., 2019), especially if they're feeling self-conscious about tics, or if the RTs remain fairly constant throughout the blocks. For guidance on what that number of participants might be, a power analysis revealed that on the basis of the repeated measures, within factors comparison, for an effect size observed in the present study to be (F = .25), the number of useable participants data that would need to be examined would be approximately 176 would be needed to obtain statistical power at the recommended .80 level (Cohen, 1988). Taking into account that there will be a huge amount of excluded data if other participants experiences reflect what has been captured so far here, a very large number of participants would need to be tested to ensure complete data sets that may reveal differences between the experimental blocks. Despite these limitations, the initial results have allowed us to consider some lose comparisons with the data collected from the experimental design in Chapter 3. So far this supports the hypothesis that there will be a similar behavioural response pattern to the SRTT protocol between patient groups and control groups, however, no direct comparison can yet be made about the particular nuances of RT changes between groups when there is an introduction of pseudosequence trials.

Plastic changes that take place during motor learning have been studied previously. In this study, our aim was to further investigate those connections between patterns of plasticity with performing a motor learning task, with a view to develop this protocol into one that would be applicable for use in individuals with TS. Our RT data findings in particular reflected that of previous research and is as expected, indicating there are learning effects. The lack of significant difference between the RTs

from Block 1 and Block 3b indicate these are comparable. The Bayes factor result further consolidates that there is a large potential for significant evidence that learning has occurred once a larger data set has been gathered. Unfortunately, the key research questions were focused on making comparisons with the control groups, something which is unable to be reflected on in this instance.

Chapter 5 : Investigating plasticity changes following the application of Theta burst stimulation over the motor cortex

Keywords: *Plasticity, Transcranial magnetic stimulation (TMS), Repetitive transcranial magnetic stimulation (rTMS), Theta burst stimulation (TBS), Intermittent theta burst stimulation (iTBS), Continuous theta burst stimulation (cTBS), Short intracortical inhibition (SICI), Intracortical facilitation (ICF), Resting motor threshold (RMT), Motor evoked potential (MEP)*

TMS is a powerful investigative tool for observing cortical excitability and plasticity, as explored in previous chapters. However, it can also be used as a method of manipulating brain excitability and plasticity. This study utilises these possibilities to develop a thorough examination of the plasticity of the motor area following the application of rTMS measures. The aim is that this will better our understanding of these mechanisms in a small sample of control individuals before making comparisons with TS populations.

5.1 Introduction

In previous chapters of this thesis, TMS was used to deliver single, or paired pulses as a method of measuring cortical plasticity in that brief moment in time in the M1. By changing the pattern of this application and using rTMS, cortical excitability can be altered for longer than the period of stimulation (Chung et al., 2016). This is presumed to be via Hebbian plasticity mechanisms, described in Chapter 1.1. Using theta burst stimulation (TBS), a specific pattern of rTMS described in Chapter 2.2.3 and pictured in Figure 2.4 and 2.5, previous studies have employed both iTBS and cTBS to induce LTP and LTD-like plasticity in the M1. This specific

rTMS stimulation was selected, as many rTMS protocols require a large number of pulses over a long period of time, which can be arduous for the participant. TBS consists of much shorter stimulation durations, and therefore can be more comfortable and practical for those taking part. These experiments, reported in this chapter and Chapter 6, initially aim to replicate these findings, before developing to investigate how the excitability observations differ between control groups and those diagnosed with TS.

Our study aims to replicate the findings of Murakami, Müller-Dahlhaus, Lu, & Ziemann, (2012), in a healthy population prior to examining TS patient samples. In their first experiment, the researchers began by studying the effects of non-primed TBS, both intermittent and continuous, followed by the priming effects of both of these types of TBS on subsequent iTBS and cTBS, meaning there were 6 conditions in total. The TBS used was similar to that of the commonly applied protocol; a burst of three pulses at 50HZ with a 20ms inter-pulse interval, which was repeated at 240ms intervals. This repetition rate is therefore 4.2 Hz, which is slightly different from the original description of the protocol, which has a repetition rate of 5Hz as the interval is set at 200ms intervals (Huang et al., 2005). They discuss in their methodology that this repetition rate is still between 4-7Hz, the theta range, and that the results from the non-primed iTBS and cTBS on MEP amplitude still replicated those of previous findings. The iTBS consisted of 10 bursts of a train, which comprised of 30 pulses, repeated every 10s for a total of 600 pulses. In the cTBS, the train stimulation was applied without any interruptions for a total of 600 pulses. The intensity of stimulation was 80% of active motor threshold (AMT) and was applied over the left M1 hand area. Baseline IO-MEP amplitudes and IO-SICI were recorded in the first two conditions which were the non-priming conditions. This was then followed by iTBS or cTBS and the same measures were taken again immediately after stimulation, and 15 minutes post stimulation. In the remaining 4 conditions, baseline recordings were taken, followed by the priming TBS, then immediately

post TBS, and at 15 minutes post. Their second experiment was designed with the aim of investigating the extent of the influence priming TBS has on the subsequently induced TBS plasticity. The method was the same as the first experiment, except that the priming TBS stimulus intensity was 70% of AMT, not 80%. This was chosen as the authors stated that previous research by Mcallister, Rothwell, & Ridding, (2009) found that at this intensity, neither iTBS or cTBS produced any MEP amplitude change. Their findings for the non-primed TBS protocols reflected previous work and produced facilitatory and inhibitory effects as expected for the MEP amplitude, although no significant change was observed when examining SICI. In the priming protocols they observed that pairing of the same protocols meant the non-primed effects were supressed, and that the pairing of different protocols enhanced the effects on MEP amplitude. They also observed that non-primed TBS had no significant effect on SICI, however, iTBS primed with iTBS resulted in a decrease in SICI, and that cTBS primed with the same protocol increased SICI. These findings from this priming protocol provides evidence that plasticity is regulated by homeostatic metaplasticity, as it demonstrates that the previous activity is influencing the subsequent plasticity. The second experiment adds further support to this finding. By using a lower threshold of 70% and showing there is still an effect, it is providing extra evidence that this is due to metaplasticity as it is not in itself inducing any changes in synaptic efficacy. Prior to this study Doeltgen & Ridding, (2011), had tested a similar protocol, also examining SICF. They found no significant results for modulations of either SICI or SICF.

It is expected that there will be similar results to Murakami et al., (2012) for the healthy cohort. In our 1mV measures, selected as an alternative to their IO-MEP measures, we are expecting to see the same direction of results as the IO-MEP results reported, with iTBS resulting in an increase of MEP size, and cTBS decreasing in amplitude. Our IO-SICI parameters are similar to the original, however we selected a narrow range of three conditioning stimulus intensities, 70, 80 and 90% of RMT, compared to

Murakami et al.'s four. This was to prevent the protocol from taking too long, so as to ensure subjects would return to complete all 6 conditions. Similarly to Murakami et al., (2012) this study also has 6 conditions, two of which are non-primed and the remaining four consist of a TBS priming protocol prior to probe TBS. Our study does add more post stimulation measures, to more closely monitor changes to the MEP amplitude in comparison to the baseline following stimulation, particularly as previous studies suggest that the effect can last longer than the 15 minutes, which was the limit tested by Murakami et al (2012). Other research has demonstrated that for cTBS effects in particular, the most reliable time point to measure the extent of inhibition was 50 minutes post the application of stimulation (Jannati et al., 2019). However, the number of conditions and length of time of testing, coupled with the comfort of the subjects has to be taken into consideration. Hence why the effects are not being tested up to and beyond the 60 minutes that Chung et al., (2016) found to still show an effect from TBS protocols. Their investigation consisted of a meta-analytic review of the efficacy of the iTBS and cTBS paradigms, in altering corticospinal excitability, SICI and ICF in the primary motor cortex. Following their exploration of available data, overall they found that iTBS increased excitability, with no effect on SICI, and cTBS decreased excitability and SICI. Chung et al., (2016) also examined in more detail the importance of the time point when the excitability measures were taken. The greatest effect sizes reported for the iTBS condition were observed between 20-30 minutes after the application of TBS, and for cTBS at the very earliest time points of less than 5 minutes. Prolonged effects lasting longer than 30 minutes were only significant following the application of cTBS. These studies formed the basis of our hypotheses for this protocol undertaken in healthy controls.

Our aim for this protocol is to replicate previous findings regarding the application of iTBS or cTBS to individuals, and the resulting cortical excitability change over a longer period of time following the protocol. It is hypothesised that the data will resemble that of previous work, with

iTBS prompting a facilitatory response, evidenced by an increase in MEP amplitude and cTBS prompting an inhibitory response, evidenced by a decrease in MEP amplitudes, with additional evidence of SICI modulation following cTBS.

5.1.1 Safety

TBS has been deemed safe and effective as a technique, despite the use of high frequency pulses, with the risks related to it deemed similar to those associated with other rTMS protocols (Oberman et al., 2011). As with many other TBS studies, 600 pulses were used for each TBS session applied, which is the current safety limit, although 900 pulses has been safely performed. The stimulation intensity has been set for 70% of RMT in this case. The more common intensity of 80% active motor threshold (AMT) was decided against to avoid voluntary contractions prior to TBS which may interfere with the TBS-induced plasticity (Tse et al., 2018).

5.2 Method

5.2.1 Participants

Subjects recruited consisted of control individuals, although there had been plans to pilot these procedures with a small number of subjects diagnosed with Tourette syndrome, to investigate group differences in plasticity and metaplasticity in the M1. Complete data for 12 participants (6 female, 6 male) was collected in total; average age 24.9 ± 3.08 years. Participants were screened to ensure they were healthy, free from medication and any counter indications to TMS. All participants were deemed to be right-handed using the Edinburgh Handedness inventory (Oldfield, 1971). This study gained ethical approval through the School of

Psychology ethics committee at the University of Nottingham and was conducted in accordance with the ethical standards specified in the 1964 Declaration of Helsinki. No subject reported any adverse effects during or after the experiments.

5.2.2 Design

For clarity, the following terms are outlined in the box, and will be referred to throughout the subsequent chapters in reference to the experimental design.

Prime	– the first application of TBS
Probe	– the second application of TBS
Adjusted	– refers to the measures which are collected following possible changes in threshold to ensure S1mV remains constant, meaning SICI results are comparable
Unadjusted	– refers to measures being collected using the original threshold values, which enables an insight into global excitability changes
Unconditioned	– refers to the single pulse measures
Conditioning pulse	– refers to the first pulse in paired pulse measures
Test pulse	– refers to the second pulse in paired pulse measures
Pre-measures	– referring to those taken before TBS has been applied
Post-measures	– referring to the measures taken after TBS has been applied

This protocol was a within subjects' design; and all subjects returned for these two non-primed conditioned and the further sessions detailed in Chapter 6. At least 48 hours was necessary between experimental

conditions for all subjects, and the testing sessions were completed at the same time of day, always in the afternoon. This ensured plasticity of the human motor cortex was not influenced by different scheduling in the day, and by raised cortisol levels, which are at their highest in the morning (Sale et al., 2008), therefore limiting the individual variability (Corp et al., 2020).

5.2.3 TMS measurements

TMS was applied using two Magstim 200 stimulators (Magstim Co., Whitland, Dyfed, UK), connected through a BiStim Module (Magstim Co.) with a standard figure of eight, branding iron coil (diameter of one winding 50mm). The coil was held tangentially to the scalp, at a 45° angle from the midline, resulting in a posterior to anterior flow of current. The left M1 hand area was targeted for the TMS, and the motor hotspot was determined as the area where TMS was eliciting the largest MEPs from the FDI muscle on the right hand. MEPs were recorded using Ag-AgCl surface electrodes attached to the FDI muscle of the right hand in a belly tendon montage. The signals were amplified, bandpass filtered (10Hz-2kHz, sampling rate 5kHz), and digitised using Brainamp ExG (Brain Products GmbH, Gilching, Germany). Neural navigation software (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used throughout the experiment with a template brain to assist with consistent coil placement over this location in the left primary motor cortex. Resting motor threshold (RMT) was defined as the lowest stimulation intensity needed to yield a MEP with a peak-to-peak amplitude of >50μV in the FDI muscle whilst the subject is relaxed, in 5 out of 10 trials. This was calculated for both TMS machines and both types of coil used. The 1mV threshold was also established, this was the intensity where the average MEP size was 1Mv. All TMS pulses during the experiment were triggered using an in-house Matlab program (Mathworks, MA, USA).

Motor cortical excitability is being observed with both single pulse and paired pulse measures, similar to (Murakami et al., 2012). The paired pulse measures were for testing SICI at a 2ms interstimulus interval (ISI), selected as it is used to study the $GABA_A$ergic synaptic neuro-transmission and limits the contamination of short-intercortical facilitation (Ziemann et al., 1996). In this case, the intensity of the conditioning pulse was set at 3 different levels between 70% and 90% of the RMT, whilst test pulse intensity was adjusted in blocks where SICI is measured throughout the experiment to maintain an average of 1mV in peak-to-peak amplitude for the unconditioned MEPs. This is an important aspect of the method as variation in unconditioned pulse MEP amplitude as a percentage of RMT will result in a change in the observed SICI regardless of the TBS protocol (Ilić et al., 2002). As it is hypothesised that cortical excitability changes will occur and therefore the RMT may be altered, this is a way of limiting those variables when observing SICI specific modulation. Findings from previous work also suggests that as SICI has been shown to decrease with increasing test stimulus intensity, this suggests that the neurons involved in the generation of MEP amplitudes of ~1mV, are the ones most susceptible to SICI (Müller-Dahlhaus et al., 2008; Peurala et al., 2008), meaning the protocol is optimised for these specific observations whilst remaining at a comfortable level for subjects. The necessary changes to maintain the correct intensity are documented in Table 5.1 shows how these intensity changes, if they were necessary, were often small.

	Baseline		*Adjusted 1*		*Adjusted 2*		*Adjusted 3*	
Condition	%Change	SEM	%Change	SEM	%Change	SEM	%Change	SEM
1	0.00%	0.00%	-0.72%	0.56%	-0.72%	0.97%	-0.72%	0.68%
2	0.00%	0.00%	0.00%	0.69%	0.00%	0.87%	0.00%	0.75%

Table 5.1 - 1mV stimulus intensity changes, shown as a percentage change from the threshold taken prior to the condition starting. Values are from medians taken across participants within each condition

There can be a large variability of MEP amplitude within an individual's data between trials in the same testing session, and in subsequent ones, making it difficult to compare and can affect the reproducibility of findings. Methodological factors can play a role in this, such as movement of the coil, which may then be targeting an alternative brain area. There are other causes for this variability. For example, Goldsworthy, Hordacre, & Ridding, (2016) list changes in attention and arousal, activation of the target muscle prior to the TMS stimulus (Darling et al., 2006) and the timings of the TMS during ongoing cortical oscillatory rhythms (Bergmann et al., 2012). To best limit the effects of large inter-trial variability of MEPs on overall experimental data, Goldsworthy et al.'s, (2016) study suggests that between 20-30 pulses are needed for the most accurate and stable measure of corticospinal excitability for within and between session reliability, and that anything more than 30 provided no additional benefit. This was further supported by recommendations made by Corp et al., (2020).

5.2.4 Theta Burst Stimulation

TBS over the left M1 hand area was applied using a Magstim Rapid^2 (Magstim, Co., Whitland, Dyfed, UK) with a standard figure-of-eight

branding iron coil (diameter of one winding 70mm). Both continuous and intermittent TBS was used in these experiments, with the pattern of stimulation and parameters shown in Figures 2.4 and 2.5.

5.2.5 Procedure

Participants are seated on a comfortable chair and instructed to rest their arm on the table in front of them throughout the entire experiment. For Experiment 1, as depicted in Figure 5.1, first the resting motor thresholds and the S1mV threshold are determined, followed by the baseline measures. This consists of 100 pulses of a mixture of 2ms SICI at 70, 80 and 90% RMT, and test pulses at the 1mV threshold intensity. The assigned TBS protocol was then applied. Following this, 30 single pulses were applied at 1mV intensity as a measure of global excitability changes. This also indicated if the 1mV threshold intensity had changed, allowing for this to be altered for the adjusted measures of subsequent repeated mixed 100 pulses of single and SICI pulse measures taken during baseline. These Post 1 measures are repeated twice more, referred to as Post 2 and Post 3, meaning they were following the application of TBS. Figure 5.1 visually describes this protocol, and Table 5.2 depicts the timings of when these measures were taken. These show that between participants and conditions the measures were performed at consistent times.

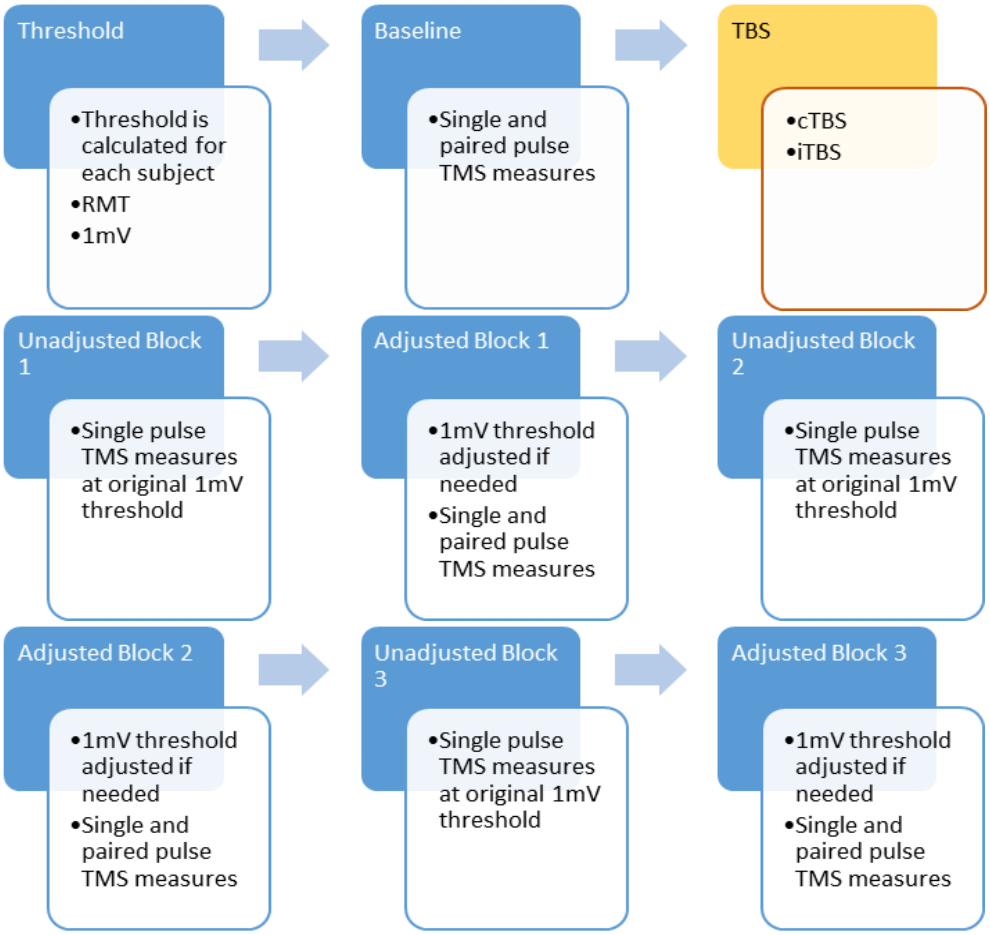

Figure 5.1 – Visual representation of the experimental protocol for investigating a single application of TBS. The yellow box highlights where TBS is applied

	Length of Interval between B1 and B2	Time of Unadjusted Post1 (approx min)	Time of Post1	Time of Unadjusted Post2	Time of Post2	Time of Unadjusted Post3	Time of Post3	End of data collection time
Mean (of medians)	20.60	5.58	10.67	26.08	32.25	38.67	45.67	49.33
SEM	0.98	0.45	0.33	1.14	0.36	2.32	3.17	3.41

Table 5.2 – Timings between measures across the experiment detailed in this chapter, and Chapter 6. This suggests that comparisons between sessions and experiments will be acceptable as there seems to be consistent blocks of time across subjects

5.2.6 Analysis and Statistical Tests

For the MEP data, following manual visual inspection of the EMG data, any traces showing muscle pre-contraction 500ms prior to the TMS pulse were removed from the data. Peak-to-peak MEP amplitudes were measured using inhouse Matlab programmes (Mathworks, MA, USA). A Grubbs test was applied (using the critical value of .05) to identify and remove any significant outliers, reported in Table 5.3.

Condition	**Unconditioned**	**Paired Pulse**
1	0	4
2	4	6

Table 5.3 – Number of excluded values in each condition, across all participants, from either the unconditioned pulses or the paired pulse measures

For any data points that were missing, group medians were used in their place for analysis. Median data was examined in addition with normalised group data, formatted into percentage change values compared to the baseline measures. This is often used as a way of seeing how MEP amplitude is changing over time and is a way of normalising the data across participants. Paired pulse trials were analysed by calculating the median MEP amplitude for each conditioning pulse intensity. These values were then divided by the median MEP amplitude for the unconditioned pulses in those blocks. Individual data was visually assessed as the exact intensity needed for peak SICI effect varies depending on the individual. Group effects looked most promising at ~80% intensity, so the decision was made to use a median of the 70% and 80% intensity individual values as this should be more representative of the group SICI effect, and to avoid averaging across too wide a spread of data and obscuring effects.

The statistical analysis programme jamovi (jamovi.org) was used to perform statistical analyses on generated data. All tests were conducted with a significance level of $p<.05$. Throughout the analysis process *p* values were uncorrected, meaning that interpretation of them in the subsequent results section should be interpreted with extreme caution. Mauchley's test of sphericity was performed and corrections were made using Greenhouse-Geisser when needed. Bayesian methods were applied as a parallel analysis strategy in addition to frequentist methods using the *p* value as a way to indicate the extent of evidence for either the H_0 or H_1. The BF_{10} and the corresponding categories are determined in Table 3.1.

5.3 Results

To ensure that there were no significant differences of MEP size resulting from an unconditioned pulse during the SICI measures, a repeated measures ANOVA and Bayesian repeated measures ANOVA was performed on this data, summarised in Table 5.4.

Factor	**d.f**	**F**	***p***	**η^2p**	**BF_{10}**	**Category**
Condition	1	.004	.95	.00	.212	Moderate evidence for H_0
Time	3	1.73	.18	.136	.276	Moderate evidence for H_0
*Condition*Time*	3	.498	.69	.043	-	-
Condition + Time	-	-	-	-	.058	Strong evidence for H_0
*Condition + Time + Condition*Time*	-	-	-	-	.009	Extreme evidence for H_0

Table 5.4 Summary of the results of a repeated measures ANOVA and a Bayesian repeated measures ANOVA on the median data of the unconditioned pulses in the baseline measures and those unconditioned pulses applied during the SICI measures following the application of TBS

This evidence is necessary to ensure that this portion of the protocol, where the researcher was required to adjust the thresholds, was completed correctly. Thus, any threshold changes were accurate and therefore limiting the variables that would alter the magnitude of SICI. The results suggest this has been managed evidenced by the BF_{10} results ranging from moderate to extreme evidence for the null hypothesis.

5.3.1 Single pulse measures

A repeated measures ANOVA conducted on the median data revealed that there was no significant effect of either Condition ($F(1,11) = 1.29$, $p = .28$, $\eta^2p = .105$) or Time ($F(3,33) = 1.8$, $p = .167$, $\eta^2p = .140$), but that the combination of the two factors did result in a significant difference in size of the MEPs ($F(3,33) = 3.91$, $p = .017$, $\eta^2p = .262$). Two tailed paired samples t-test were utilised to investigate the median data further. As shown in Figure 5.2 there is a significant difference between Cond 1 (M = 1288, SD = 646) and Cond 2 (M = 606, SD = 252) MEP sizes at the third time point post stimulation (t = 3.55, p = .005, d = 1.024). This is further supported by a test between the baseline measures taken in Cond 1 (M = 842, SD = 286) and at the 3rd time point (M = 1288, SD = 646) which also returned a significant result (t = -2.5, p = .03, d = .721). This is not the case for Cond 2 between the baseline measure (M = 844, SD = 580) and the third measure (M = 606, SD = 252) (t = 1.44, p = .179, d = .414). However, Figure 5.2 shows an unexpected increase in MEP size in Cond 2 in the first measure post TBS (M = 1144, SD = 626), followed by the predicted decrease in MEP amplitude in the third measure (M = 606, SD = 252), which was found to be significant (t = 2.79, p = .018, d = .805).

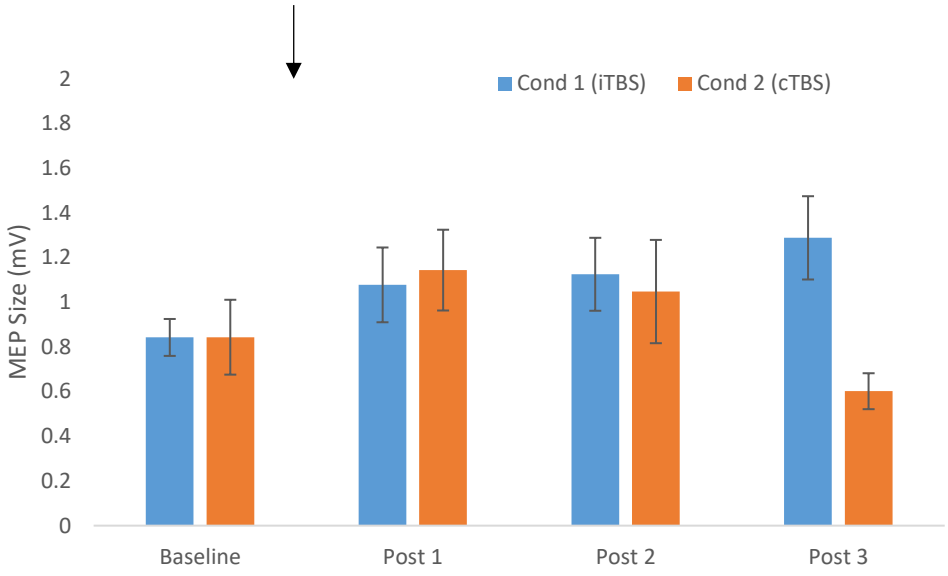

Figure 5.2 – Group median MEP size throughout the blocks of the protocol. The arrow indicates when the TBS was applied. Error bars are the standard error.

Factor	BF_{10}	Category
Condition	.698	Anecdotal evidence for H_0
Time	.264	Moderate evidence for H_0
Condition + Time	.192	Moderate evidence for H_0
Condition + Time + Time * Condition	.381	Anecdotal evidence for H_0

Table 5.5 – Summary of results for the Bayesian repeated measures ANOVA for the median data

The data was additionally examined using a Bayesian repeated measures ANOVA. The results, reported in Table 5.5, show that mostly there is small amounts of evidence for the null hypothesis and no evidence for the alternate hypothesis.

5.3.2 SICI measures

A repeated measures ANOVA of the median data revealed that there was no significant effect of any of the factors tested (Condition: F (1,11)= 1.827, p= .204, η^2p = .142, Time: F(1.73, 19.08)= 1.069, p= .354, η^2p = .089, Condition*Time: F(3,33)= .462, p= .711, η^2p = .040). Data is depicted in Figure 5.3.

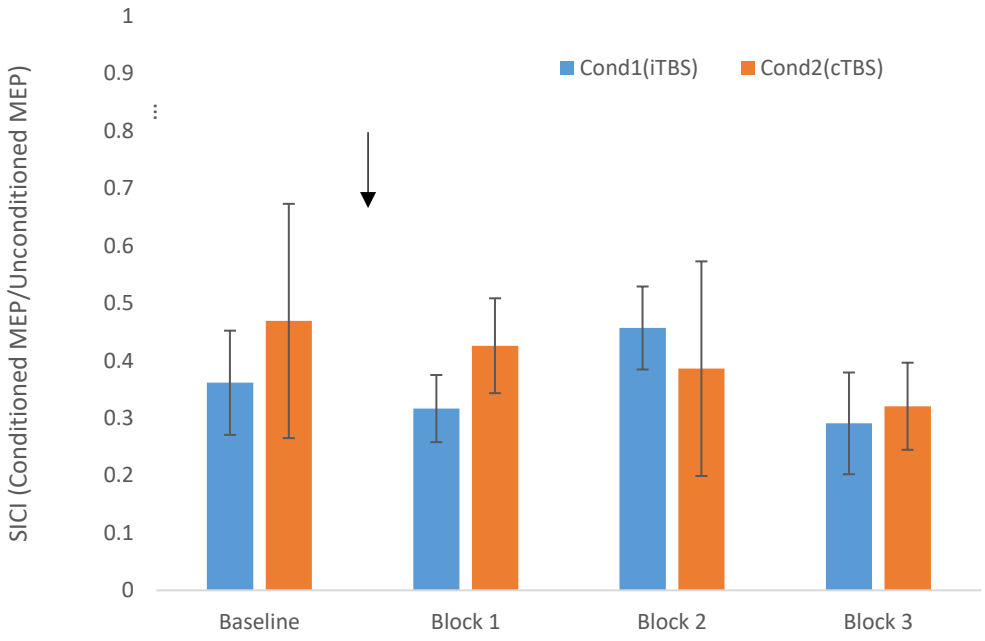

Figure 5.3 – Median group data showing changes in SICI response following either iTBS or cTBS over the experimental blocks. Error bars are standard error of the mean. The placement of the arrow indicates where the TBS is applied

Factor	**d.f**	**F**	**p**	**Cohen's d**
Condition	1	1.827	.204	.142
Time	1.73	1.069	.354	.089
*Condition*Time*	3	.462	.711	.040

Table 5.6 - Summary of repeated measures ANOVA results for the paired pulse measures to examine 2ms SICI, examined as medians

Factor	**BF_{10}**	**Category**
Condition	.071	Strong evidence for H_0
Time	.177	Moderate evidence for H_0
Condition + Time	.13	Moderate evidence for H_0
Condition + Time + Time * Condition	.02	Very strong evidence for H_0

Table 5.7 – Summary of results following the percentage change data being examined with a Bayesian repeated measures ANOVA

Bayesian analysis was similarly conducted on the median and percentage change data for SICI effects. This returned evidence for the null hypothesis, summarised in Table 5.7.

5.4 Discussion

Theta burst protocols have been demonstrated to alter cortical excitability for a period following application. In this study, our aim was to replicate the application of cTBS and iTBS with additional time measures to monitoring observable changes in MEP size and evidence for changes in SICI.

Following the examination of the single pulse TMS data, a significant difference was found in MEP size between conditions in the measures taken at the third time point following TBS application, and also when comparing the baseline measures to the final measure within the iTBS condition. The combined factors of Condition*Time were also found to be significant when analysing the median data. This supports our hypothesis and the previous literature that following an iTBS protocol, MEP amplitudes are significantly larger. Following application of cTBS, no significant difference was found. Surprisingly there was an increase in MEP size initially, which is when the largest inhibitory effect has previously been observed (Chung et al., 2016). Whilst the percentage change data made this easier to visualise in Figure 5.3, and highlighted the huge degree of variability, analysis of the data yielded no significant results. Similarly in Figure 5.2, very few significant differences are observable, however, visually there is a decrease in MEP size at the third measure compared to baseline. This is a good example as to why multiple measures over an amount of time is useful as MEP sizes decreased later in the experiment. This is interesting as this is often a time point not included in previous protocols. In this instance it depicts the largest difference from baseline for both conditions in accordance with our hypotheses. Rarely have measures been taken 30 minutes post application, yet this study, along with others who conclude 50 minutes post application is the most reliable time to measure cTBS effects (Jannati et al., 2019) are adding further weight to the suggestion that these effects are more observable

at a later point. Our multiple measures across a longer time span are a strength of this experimental design.

During analysis of paired pulse TMS data, mostly there were no significant changes to SICI observed, except from after the application of iTBS and when comparing the MEP size from baseline to the final measure. This is similar to the comparisons made with single pulse data, except MEPs were significantly smaller. An alteration in SICI response is not consistently found in other research, so the majority of the results returning insignificant is unsurprising, but the observed reduction of MEPs following iTBS does suggest it is possible. The varied results at each time measure in Figure 5.5 suggest some changes, even if insignificant ones, especially in contrast to a more stable looking SICI response during the cTBS condition. Further investigation may return more insight.

A lot of the Bayesian analyses mostly returned results that were in the middle, neither providing concrete evidence for the null or alternate hypothesis. This indicates, in addition to the small number of significant findings, that rather than an unsuccessful TBS protocol, there is simply inconclusive evidence. Even in such a thorough within subjects design, our sample size may be limiting the possible effects, and therefore more subjects are necessary to make those conclusions, especially in a protocol that has been confirmed to result in considerable inter-individual variability, as evidenced by the large error bars on the relevant figures (López-Alonso et al., 2014). It is hoped that the addition of further data may contribute to more in depth insights into these cortical excitability changes for both single and paired pulse TMS measures. Following the interruption to data collection due to the Covid-19 situation, this was not possible for this thesis but is something that researchers may return to in the future.

With regards to expected findings in the TS population, it is hypothesised there would be evidence of altered cortical excitability, altered response to TBS protocols and a demonstration of reduced SICI. Previous literature detailing the changes in M1 excitability within diagnosed TS, which is thought to arise from abnormal plasticity, speculates how this plays a role in the pathophysiology of the syndrome. An early study by Ziemann et al., (1997) found reduced SICI when examining this brain area in TS individuals over the M1, a finding that has been replicated numerous times since (Berardelli et al., 2008; Jackson et al., 2015). Specifically in the case of TBS it has been shown that expected responses to applied iTBS and cTBS protocols are reduced in individuals with TS (Antonio Suppa et al., 2011; Wu & Gilbert, 2012). We expect future findings from our study design to therefore to provide further evidence of plasticity differences within this patient population.

Conclusion

There is evidence to support our hypothesis that TBS can influence cortical excitability, however, most of the analyses results remained inconclusive in this case, with no strong evidence for either hypothesis. When further data collection can resume, it is hoped that additional data can be pooled with this existing data set and enable more thorough and enlightening conclusions to be made.

Chapter 6 : Investigating metaplastic changes in the motor cortex using a primed Theta Burst Stimulation protocol

Keywords and terms: *Metaplasticity, Bienenstock Cooper Munro theory of synaptic plasticity (BCM), transcranial magnetic stimulation (TMS), Theta burst stimulation (TBS), Intermittent theta burst stimulation (iTBS), Continuous theta burst stimulation (cTBS), Prime, Probe, Resting motor threshold (RMT)*

In Chapter 5, TBS techniques were introduced and explored, combined with single and paired pulse TMS to examine plasticity by measuring how these may alter the cortical excitability in the M1. In this chapter, using the same participants and consistent parameters, the experiment was extended to examine priming TBS protocols and therefore allowing direct comparison as to how effective these techniques may be across all conditions.

6.1 Introduction

In the previous chapter, the application of either cTBS or iTBS was expected to change the excitability of the M1 hand area for the recruited group of participants. There are many examples of previous work testing responses to one application of TBS, but now this technique is being developed with two applications of TBS, a prime and a probe. This study aims to build on that by introducing some additions to the experimental method that can help us glean further insight about metaplasticity.

In addition to investigating plasticity, TBS can also be used to investigate the phenomena of metaplasticity, briefly described in Chapter 1.1 as the

'plasticity of synaptic plasticity' and a higher order form of synaptic plasticity (Abraham & Bear, 1996). This is where the response of a synapse to a plasticity inducing event is influenced by the activity that happened prior to that response (Doeltgen & Ridding, 2011). This demonstrates that the thresholds for synaptic plasticity to occur are not static, instead they vary according to an enduring trace from previous synaptic activity (Abraham & Bear, 1996). This enables homeostatic regulation of synaptic plasticity, keeping activity within a safe range. It may manipulate subsequent learning ability and may also be implicated in neurological conditions (Hulme et al., 2013). This was proposed in the Bienenstock, Cooper, Munro theory of synaptic plasticity, where they incorporated these ideas with experimental evidence within the visual cortex (Cooper & Bear, 2012). Their model for bidirectional synaptic plasticity predicts that previous instances of low post-synaptic activity will lower the synaptic modification threshold for LTP, and increase the threshold for LTD. This also works in reverse. A history of high synaptic activity will shift the threshold for LTD so that it is more likely to be induced and the threshold for the induction of LTP will be increased. Subsequently these influential theories of a sliding threshold have been applied and since established within other areas of the brain and have led to establishing the phenomenon of metaplasticity and other cortical mechanisms (Karabanov et al., 2015).

To examine metaplasticity a priming TBS protocol can be utilised, such as the one by Murakami et al., (2012), who investigated non-primed TBS protocols, described in the previous chapter, and also primed TBS protocols. Experiments consist of an initial application of TBS referred to as the prime, and an additional application of TBS, referred to as the probe. From their results they concluded that plasticity in both the excitatory and inhibitory circuits within the M1 are regulated by homeostatic metaplasticity.

Based on previous literature, it is hypothesised the following observations for each condition:

For Condition 3, which consists of two applications of cTBS, It is predicted that in the unconditioned measures there will be a decrease in MEP amplitude measured in Post 1, but that the amount of reduction in MEP size will then become smaller over the subsequent measures; the effects of the primed TBS are suppressed. An alteration is expected in the responsiveness of SICI, like Murakami et al., (2012), in conditions where the priming protocol and the probe protocol are the same.

In Condition 4, which consists of a priming application of cTBS, followed by a probe application of iTBS, it is predicted that in the unconditioned measures there will be an initial decrease in MEP amplitude measured in the Post 1 time point. However, following the application of iTBS an increase is expected in unconditioned MEP size, larger than observed following a single application of iTBS. A change in the SICI response is not expected to be observed.

For Condition 5, starting with a priming protocol of iTBS and followed by the probe application of cTBS, it is expected that an initial increase in unconditioned MEP size at the Post 1 measure. However, after the second application of TBS it is expected to reverse and to see a greater inhibitory effect and the MEP size to decrease more than after one single application of cTBS. No observable changes in the SICI response are expected.

Finally, in Condition 6, which consists of a prime and probe application of iTBS, it is expected that the unconditioned measures will increase in MEP amplitude measured in Post 1, but that the amount of facilitation in MEP size will then become smaller over the subsequent measures; the effects of the primed TBS are suppressed. Again, as the prime and probe TBS protocol are the same, therefore a change in the responsiveness of SICI is expected.

The aim of this study is to further the investigation described in Chapter 5, with additional priming TBS to test how the first application of TBS modulates cortical excitability following a second application of TBS, and how that cortical excitability changes over a period of time following that application. By using the same participants across the experiments in Chapter 5 and 6, it is hoped that a greater insight can be gained into an individual's response to this variety of stimulation patterns.

6.1.1 Safety

TBS has been deemed a safe and effective technique despite the use of high frequency pulses, with the risks related to it deemed similar to those associated with other rTMS protocols (Oberman et al., 2011). As with many other TBS studies, 600 pulses were used for each TBS session applied, which is the current safety limit, although 900 pulses in one application has been safely performed. Within the 4 experimental conditions described in this chapter TBS is performed only twice in each session. A minimum interval of 15 minutes between TBS sessions was set for all subjects to align with safety guidance (Rossi et al., 2009). The stimulation intensity has been set for 70% of RMT in this case. The more common intensity of 80% active motor threshold (AMT) was decided against to avoid voluntary contractions prior to TBS which may interfere with the TBS-induced plasticity (Tse et al., 2018).

6.2 Method

6.2.1 Participants

Complete data for 12 participants (6 female, 6 male) was collected in total; average age 24.9 ± 3.08 years. Participants were screened to ensure they were healthy, free from medication and any counter indications to TMS. All participants were deemed to be right-handed using the Edinburgh Handedness inventory (Oldfield, 1971). This study gained ethical approval through the School of Psychology ethics committee at the University of Nottingham and was conducted in accordance with the ethical standards specified in the 1964 Declaration of Helsinki. No subject reported any adverse effects during or after the experiments.

6.2.2 Design

The protocol was a development from the experiment detailed in Chapter 5.2. These additional sessions were also a within subjects' design. Similarly to the first iteration of this study, at least 48 hours was necessary between experimental conditions for all subjects, and the testing sessions were completed at the same time of day, always in the afternoon. For a summary of terms used within this experimental design, please refer to Chapter 5.2.2. Below, Table 6.1 depicts a brief outline of the two experiments, showing which TBS protocols were used in which condition, and in what order, and more details as to when the TMS measures were recorded.

Experiment	Condition	Priming			Probing			
		TBS			TBS			
1	1	-	-	Baseline 1	iTBS	Post 1	Post 2	Post 3
	2	-	-	Baseline 1	cTBS	Post 1	Post 2	Post 3
2	3	Baseline 1	cTBS	Baseline 2	cTBS	Post 1	Post 2	-
	4	Baseline 1	cTBS	Baseline 2	iTBS	Post 1	Post 2	-
	5	Baseline 1	iTBS	Baseline 2	cTBS	Post 1	Post 2	-
	6	Baseline 1	iTBS	Baseline 2	iTBS	Post 1	Post 2	-

Table 6.1 - An outline of the conditions within each experiment of this investigation into plasticity and metaplasticity

6.2.3 TMS measurements

TMS was applied using two Magstim 200 stimulators (Magstim Co., Whitland, Dyfed, UK), connected through a BiStim Module (Magstim Co.) with a standard figure of eight coil, branding iron coil (diameter of one winding 50mm). The coil was held tangentially to the scalp, at a 45° angle from the midline, resulting in a posterior to anterior flow of current. The left M1 hand area was targeted for the TMS, and the motor hotspot was determined as the area where TMS was eliciting the largest MEPs from the FDI muscle on the right hand. MEPs were recorded using Ag-AgCl surface electrodes attached to the FDI muscle of the right hand in a belly tendon montage. The signals were amplified, bandpass filtered (10Hz-2kHz, sampling rate 5kHz), and digitised using Brainamp ExG (Brain Products GmbH, Gilching, Germany). Neural navigation software (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used throughout the experiment with a template brain to assist with consistent coil placement over this location in the left primary motor cortex. Resting motor threshold (RMT) was defined as the lowest stimulation intensity

needed to yield a MEP with a peak-to-peak amplitude of >50μV in the FDI muscle whilst the subject is relaxed, in 5 out of 10 trials. This was calculated for both TMS machines and both types of coil used. The 1mV threshold was also established, this was the intensity where the average MEP size was 1Mv. All TMS pulses during the experiment were triggered using an in-house Matlab program (Mathworks, MA, USA). The necessary changes to maintain the correct stimulation intensity are documented in Table 6.1, and shows how these intensity changes, if they were necessary, were often minor adaptations.

	Baseline		*Adjusted 1*		*Adjusted 2*		*Adjusted 3*	
Condition	%Change	SEM	%Change	SEM	%Change	SEM	%Change	SEM
3	0.00%	0.00%	0.00%	0.70%	0.00%	0.88%	0.00%	0.45%
4	0.00%	0.00%	0.00%	0.64%	-0.83%	1.28%	0.00%	0.56%
5	0.00%	0.00%	-1.43%	0.74%	-1.45%	0.58%	-1.69%	0.47%
6	0.00%	0.00%	0.00%	0.48%	-1.82%	0.40%	-1.69%	0.45%

Table 6.2 - 1mV stimulus intensity changes, shown as a percentage change from the threshold taken prior to the condition starting. Values are from medians taken across participants within each condition

6.2.4 Theta Burst Stimulation

TBS over the left M1 hand area was applied using a Magstim Rapid^2 (Magstim, Co., Whitland, Dyfed, UK) with a standard figure-of-eight branding iron coil (diameter of one winding 70mm) Both continuous and intermittent TBS was used in these experiments.

6.2.5 Procedure

Participants are seated on a comfortable chair and instructed to rest their arm on the table in front of them throughout the entire experiment. For this second experiment, there is the addition of a further TBS session. As shown in Figure 6.1, the protocol matches the one described in Chapter 5 until the second baseline measures. These are the same as Post 1 measures in Experiment 1, except they are followed by another TBS session which is either the same pattern of TBS, or a different one. A set minimum interval of 15 minutes is between the two TBS applications for safety. Following the second application, two more post measures are taken, consisting of the 30 single pulses, then the 100 pulses. Test stimulus intensity was updated where necessary following the first 30 pulses of single pulse measures, and a note of the intensity changes was made. This is important as variation in unconditioned single pulse MEP amplitude may result in SICI change (Müller-Dahlhaus et al., 2008).

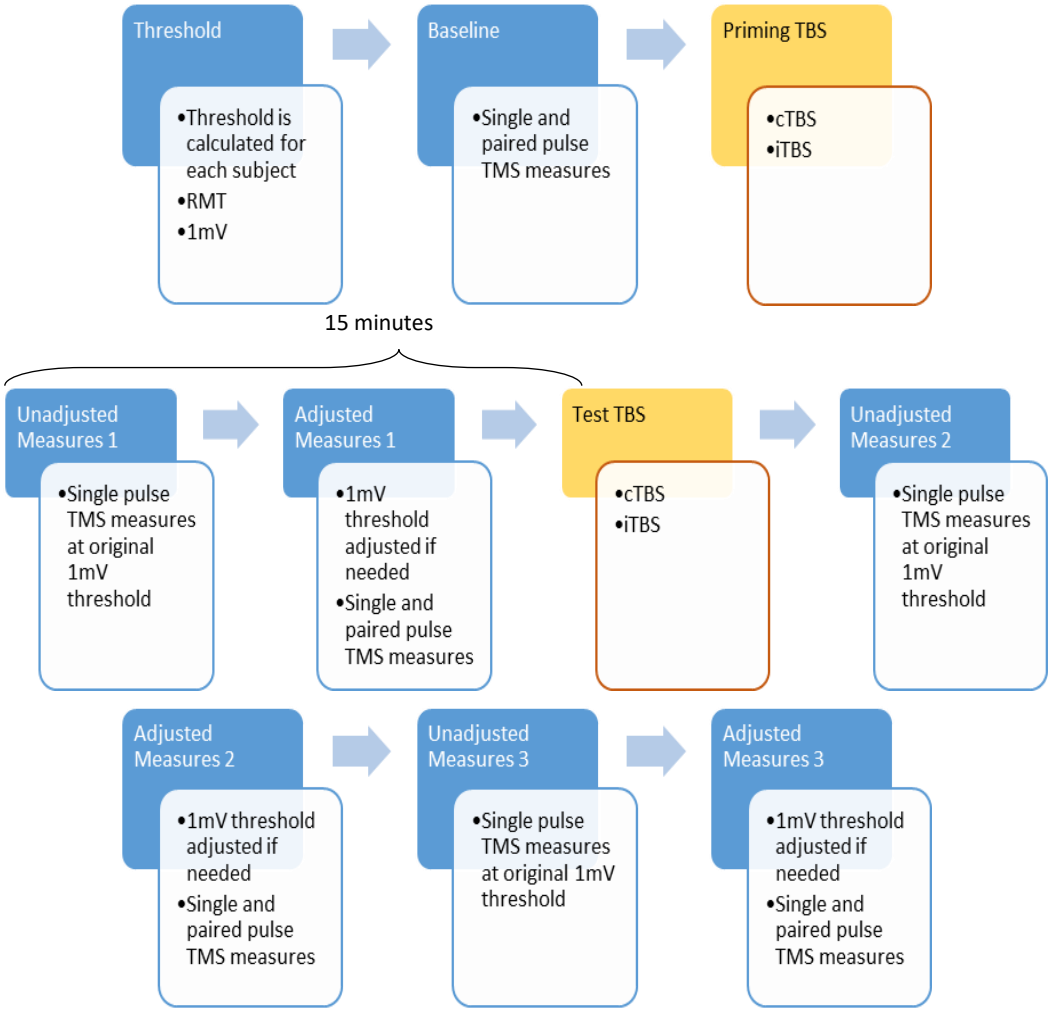

Figure 6.1 - A representation of the primed TBS protocols. The yellow boxes highlight when TBS is applied.

Timings were recorded for everyone, with a timer from when the first TBS session ended, and a note being made when each post measure began. There was no set time for these to begin as this varies between subject due to TMS coils needing cooling down, subjects asking for a brief break, or coil trackers used in Brainsight needing changing over. Instead the measures were taken as soon as possible, so all measures were recorded within 60 minutes of the last TBS session where possible. Table 5.2 depicts the timings of each measure in this experiment and the previous

one, showing that between participants and conditions the measures were performed at consistent times, enabling comparisons across them all.

6.2.6 Analysis and Statistics

For the MEP data, following manual visual inspection of the EMG data, any traces showing muscle pre-contraction 500ms prior to the TMS pulse were removed from the data. Peak-to-peak MEP amplitudes were measured using inhouse Matlab programmes (Mathworks, MA, USA). A Grubbs test was applied (using the critical value of .05) to identify and remove any significant outliers as reported in Table 6.3.

For any data points that were missing, group medians were used in their place for analysis. Median data was examined in addition with normalised group data, formatted into percentage change values compared to the baseline measures. Paired pulse trials were analysed by calculating the median MEP amplitude for each conditioning pulse intensity. These values were then divided by the median MEP amplitude for the unconditioned pulses in those blocks. Individual data was visually assessed as the exact intensity needed for peak SICI effect varies depending on the individual. Group effects looked most promising at ~80% intensity, so the decision was made to use a median of the 70% and 80% intensity individual values as this should be more representative of the group SICI effect, and to avoid averaging across too wide a spread of data and obscuring effects.

The statistical analysis programme jamovi (jamovi.org) was used to perform statistical analyses on generated data. All tests were conducted with a significance level of $p<.05$. Throughout the analysis process *p* values were uncorrected, meaning that interpretation of them in the subsequent results section should be interpreted with extreme caution.

Mauchley's test of sphericity was performed and corrections were made using Greenhouse-Geisser when needed. Bayesian methods were applied as a parallel analysis strategy in addition to frequentist methods using the *p* value as a way to indicate the extent of the evidence for either the H_0 or H_1. The BF_{10} and the corresponding categories are determined in Table 3.1.

Condition	**Unconditioned**	**Paired Pulse**
3	10	4
4	6	4
5	1	4
6	7	8

Table 6.3 – Number of excluded values in each condition, across all participants, from either the unconditioned pulses or the paired pulse measures

6.3 Results

6.3.1 Single pulse measures

For the single pulse measures, repeated measures ANOVA revealed that there was a significant effect of Condition ($F(3,33)= 6.7$, $p= .001$, $\eta^2p = .379$), however there was not of Time ($F(3,33)= 2.79$, $p= .056$, $\eta^2p = .202$, and the combination of the two was not significant (Condition*Time $F(3.82, 42.02)= 2.31$, $p= .077$, $\eta^2p = .173$), reported in Table 6.4. Figure 6.2 does vaguely show the expected pattern described in the introduction alongside the hypotheses. One tailed paired samples t-tests were used to investigate the median data further. There does seem to be a significant difference between the baseline measures in Condition 6 (M = 860, SD = 505) and the final measure taken at the third time point in Cond 6 (M = 1204, SD = 730) (t = -1.97, p = .037, d = .569); For Condition 5 a

significant difference in MEP amplitude was found between the second time point (M = 1114, SD = 493) and the third (M = 1812, SD = 852) (t = -3.255, p = .004, d = .9395).

Factor	**d.f**	**F**	**p**	**Cohen's d**
Condition	3	6.7	.001*	.134
Time	3	2.79	.056	.025
*Condition*Time*	3.82	2.31	.077	.059

*Table 6.4 – Summary of repeated measures ANOVA results for the single pulse measures. The * next to a number denotes that it is a significant value*

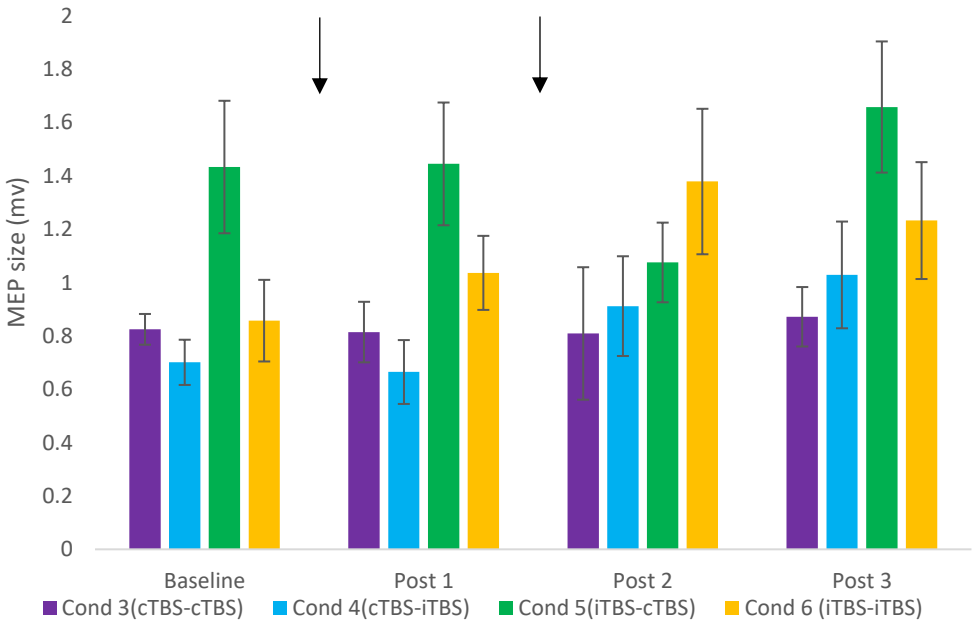

Figure 6.2 - Group median values of the single pulse measures across the four conditions of primed TBS followed by probe TBS. The error bars represent standard error of the mean, and the arrows indicate where the TBS was administered.

Factor	BF_{10}	Category
Condition	32215.278	Extreme evidence for H_1
Time	.273	Moderate evidence for H_0
Condition + Time	13270.537	Extreme evidence for H_1
Condition + Time + Time * Condition	10029.409	Extreme evidence for H_1

Table 6.5 – Summary of results for the Bayesian repeated measures ANOVA for the median data

The median was additionally examined using a Bayesian repeated measures ANOVA. The results, reported in Table 6.5, show that depending on whether the median or percentage change data is being analysed then considerably different conclusions can be made about the effects on MEP data.

6.3.2 SICI measures

For the paired pulse measures, repeated measures ANOVA revealed that there were no significant effects of Condition ($F(1.79,19.70)= 2.971$, $p= .079$, $\eta^2p = .213$) or of Time ($F(3,33)= .357$, $p= .784$, $\eta^2p = .031$, or the combination of the two (Condition*Time $F(3.9,42.94)= 1.551$, $p= .206$, $\eta^2p = .124$)). The median data for SICI measures was additionally examined using a Bayesian repeated measures ANOVA, with the results reported in Table 6.7.

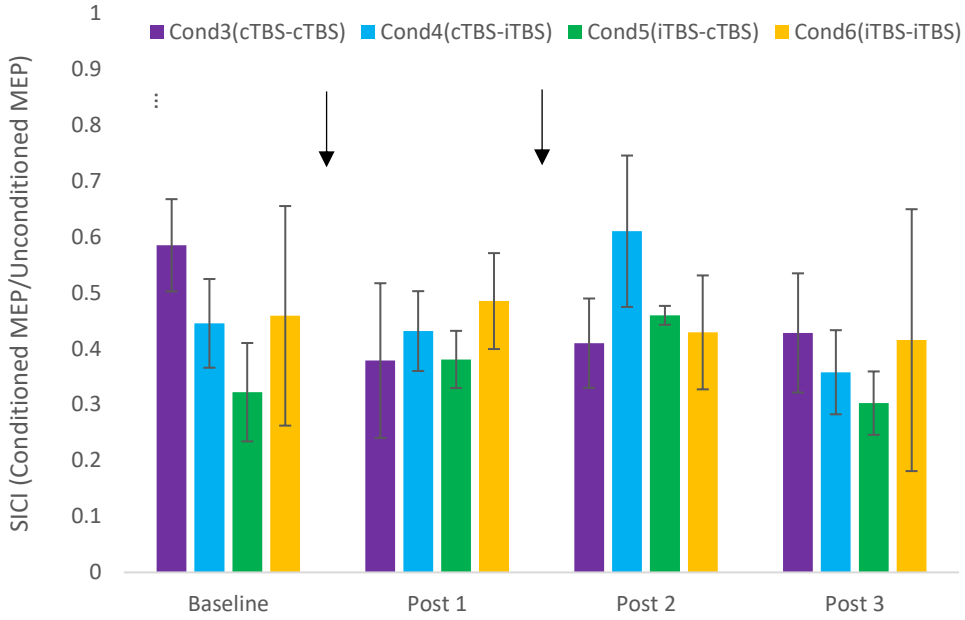

Figure 6.3 – Median change in MEP amplitude for 2ms SICI protocol following the first application of TBS after the baseline measures are taken, and again after the Post 1 measures. Error bars standard error of the mean. The placement of the arrows indicates where the priming TBS and the probe TBS are applied

Factor	**d.f**	**F**	**p**	**Cohen's d**
Condition	1.79	2.971	.079	.046
Time	3	.357	.784	.003
*Condition*Time*	3.9	1.551	.206	.047

Table 6.6 - Summary of repeated measures ANOVA results for the paired pulse measures to examine 2ms SICI, examined as medians

Factor	BF_{10}	Category
Condition	4.269	Moderate evidence for H_1
Time	.037	Strong evidence for H_0
Condition + Time	.159	Moderate evidence for H_0
Condition + Time + Time * Condition	.048	Strong evidence for H_0

Table 6.7 - Summary of results for the Bayesian repeated measures ANOVA for the median data for SICI measures.

6.4 Discussion

Primed theta burst protocols have been demonstrated to alter cortical excitability in such a way as to further support the presence of homeostatic metaplasticity. In this study, our aim was to demonstrate this with the different combinations of prime and probing TBS and to observe changes in MEP size as well as evidence for changes in SICI.

After an examination of the single pulse TMS data, it is clear there is significant evidence that the MEP amplitude is altered depending on the condition being undertaken. Condition 3 (cTBS-cTBS) demonstrated global excitability changes similar to our predictions and did not dramatically alter the MEP amplitudes of the single pulse measures. Condition 4 (cTBS-iTBS), depicted on Figure 6.2, does visually show the changes expected, with an initial decrease in MEP amplitude and then a subsequent increase, although these do not look significant. For Condition 5 (iTBS-cTBS) the MEP size is much more changeable throughout the different time measures. At measure Post 2, this looks promising to show the expected homeostatic shift to increase the amount of inhibition.

However, this is reversed in the Post 3 measures. Finally, Condition 6 (iTBS-iTBS) does seem to demonstrate initial facilitation of MEP amplitude, with a plateau after the application of the probe TBS. Despite this, the overall observation that condition is a significant factor in the alteration of MEP size is only observable when analysing the median data with frequentist and Bayesian analyses. This effect is not observed when the percentage change data is used. There could be a variety of reasons for this, including the variability of MEP amplitudes, or that there are not enough participants.

In almost all analyses no significance was observed for the paired pulse data, used to examine intracortical excitability. The only exception was that following Bayesian analysis of the median data, moderate evidence that condition had an effect on MEP amplitudes was found. The same reasons previously mentioned for the single pulse data, may additionally be obscuring significant results in the paired pulse data.

Responders and Non-Responders

In the literature regarding TMS there is a lot of discussion about individual variability in response to brain-stimulation protocols, and especially TBS. Factors that can alter this include age, sex, and genetic factors which can interact and alter the individual's response in complex multifactorial ways (Ridding & Ziemann, 2010). Indeed, some studies suggest these factors are not the most significant causes of such a large variability between individuals in their response to both iTBS and cTBS, instead citing differences in the latency of I-wave recruitment (Hamada et al., 2013), or even methodological reasons (Corp et al., 2020). These variables and their possible combinations within individuals make it clear that to attempt to understand the typical response to these protocols for a control population is a difficult task. Large sample sizes would be necessary, with the minimum number of individuals required to make comparisons

between groups suggested to be 30 people within each experimental group (Suppa et al., 2016). Others have calculated that the sample size necessary to detect the hypothesised iTBS effects is over 800 subjects (López-Alonso et al., 2014). Our study had to compromise on the number of participants due to the large number of conditions and subsequently the large time commitment asked of them to take part, as each was required to complete all 6 conditions. For guidance on what the necessary number of participants might be for this experimental design, a power analysis revealed that on the basis of the repeated measures, within factors comparison, for the effect size to be (F = .25) in the outcome of this study, an n of approximately 24 useable data sets would need to be obtained for statistical power to be at the recommended .80 level (Cohen, 1988) for each condition outlined in Chapter 5 and Chapter 6. This therefore limits the possibility of making population generalisations from this data alone.

In an effort to clearly show that plasticity changes are taking place, some studies examine their data in clusters, categorising the responders, those that demonstrate a response to a brain stimulation protocol in either a facilitatory or inhibitory way, and the rest as non-responders and then making their conclusions from the data accordingly. This method is attempting to negate the issue of obscuring possible significant effects by averaging across group data. This may be a good approach to examine the extent of the response from those classed as responders, but this would only be representative of a minority of the subjects. One study examining inter-individual variability to a number of different stimulation protocols found that only 43% of participants responded as expected to the application of iTBS (López-Alonso et al., 2014). This is clearly a key area of concern and warrants further study and consideration about how best to observe what a 'typical' response looks like, before being able to make true comparisons with neurological conditions that may have different patterns of plasticity. This is important not just for accurate

observations and the resulting conclusions but also for those wishing to use these stimulation paradigms therapeutically.

Recent work combined TMS and electroencephalography (EEG) to examine the reproducibility of these modulations, and further highlighted how the large degree of response variability between and within subjects is still a major concern (Ozdemir et al., 2021). Whilst single pulse TMS generated consistent responses between sessions, the effects of TBS modulation varied between and within subjects. If significant modulatory effects were observed in the first session, these were not able to be reproduced in the second session. Group level data showed significant differences in their experimental measures from the baseline measures following the application of iTBS and cTBS. However, most of these effects, including MEP amplitude, when compared with sham TBS showed no significant difference. On reporting these findings, the researchers made the following suggestions for future TBS research: to integrate a TBS sham control to rule out cumulative effects of single pulse TMS measures, incorporate EEG to make more direct measures of cortical responses and to establish reproducibility through the repetition of sessions.

There is a lot of potential for development in the field of non-invasive brain stimulation, but an increasingly complex understanding of the underlying mechanisms and molecular pathways is a growing necessity. Evidence is increasingly pointing to the view that these stimulation protocols do not directly act by inducing LTP or LTD like changes. This explanation seems too simplistic, and instead the modulation of the homeostatic cellular background, or metaplasticity, actually has a far more nuanced role in controlling the direction and strength of synaptic plasticity (Cirillo et al., 2017). A study by Gamboa, Antal, Moliadze, & Paulus, (2010) highlights this with their exploratory adaptations of the conventional TBS protocol. They reasoned that this type of rTMS is

favoured due to the efficiency. This is a key consideration for many researchers and an attractive quality when developing therapeutic treatments. The researchers stated that this is a powerful neuromodulation tool to generate these changes in a few minutes and hypothesised that these after-effects could be enhanced if a prolonged application of iTBS or cTBS was administered. A prolonged application in this instance was double that described as the conventional dose (Huang et al., 2005). They reported that they unexpectedly observed that the cortical excitability state was not only reduced but could be reversed to the opposite direction merely by changing that parameter (Gamboa et al., 2010). On reflection, this could be explained by metaplastic mechanisms, with similar results to what was expected to be observed in our priming TBS design. By better exploring the consequences of parameter changes it may be possible to determine the best way to achieve the desired after effect. In this instance, as the stimulation is applied for a length of time that reverses the initial effect, reflecting a U shape, it means it is possible to question if the application of a combination of a prime and probe stimulation with a break in between is necessary. In the experiment design discussed in this chapter it was possible to observe the changes in response by taking excitability measures between the applications. The pause between stimulation meant that the changes could be more closely observed. However, this pause in stimulation and its duration is another parameter that the induced excitability changes are sensitive to (Gamboa et al., 2011). It would be interesting to compare our priming protocol design with various iterations, changing the duration of TBS or of breaks in stimulation to examine if there are any differences. Small changes in parameters making such a large difference to the after-effects highlights just how necessary a more thorough understanding of the mechanisms behind this phenomena is, especially as there are many considerations to be explored for optimising this method for clinical protocols.

With regards to expected findings in the TS population, whilst this particular experimental design may not be sensitive enough to observe

any significant differences between population groups, there are still predictions for what might be observed in those with a TS diagnosis. In line with previously reported work, it would be expected to find further evidence of M1 specific plasticity abnormalities (Suppa et al., 2011). Whilst you might expect there to be a significant difference between the patient and control groups, within the patient group you would not expect a significant change in baseline single pulse measures following the application of iTBS or cTBS. Similarly, it is expected that there would be evidence of reduced SICI in all conditions for those with TS. To replicate these findings with this depth of investigation within subjects would be an interesting opportunity, especially with the primed TBS conditions. This may be an achievable way of using what is known about metaplastic changes to influence and modulate the plasticity of an individual, in the desired direction, most effectively.

Conclusion

The aim of this study was to replicate the findings of Murakami et al., (2012), who were similarly interested in examining global excitability, and intracortical excitability of the M1 following the application of primed TBS. There is evidence to support our hypothesis that with the addition of a priming TBS protocol, you can further influence cortical excitability and intracortical excitability. Whilst many of the results remained inconclusive, there was some promise with the predicted MEP and SICI changes depicted visually in the included figures. When further data collection can resume, it is hoped that this additional data may provide further and more conclusive evidence as to how the M1 adapts to this plasticity inducing TMS protocol.

Chapter 7 : Mapping Study with Theta Burst Stimulation

Keywords: *Transcranial magnetic stimulation (TMS), Plasticity, Motor maps, Functional magnetic resonance imaging (fMRI), Magnetic resonance spectroscopy (MRS), Theta burst stimulation (TBS), Conditioning pulse (CP), Test pulse (TP), First dorsal interossei (FDI), Abductor digiti minimi (ADM), Abductor pollicis brevis (APB), Resting motor threshold (RMT), Intermittent theta burst (iTBS), Interstimulus interval (ISI), Centre of gravity (COG)*

In this chapter, there is continued use of TMS as a tool to investigate plasticity changes in the M1, however, this time with a different technique termed cortical motor output maps. These are used to infer the changing excitability of the hand area in the motor cortex following the application of a TBS protocol.

7.1 Introduction

In previous chapters, TMS application has been very specifically targeting the FDI muscle in the right hand, using MEPs as an indirect measure of cortical excitability in that exact area. TMS generated cortical representations, also termed motor maps, are a useful tool as they are structured by the relationship between a point on the cortical surface and the muscles that are activated by the TMS pulse over it (Harrison & Murphy, 2014). Along with examining the gross somatotopy of the motor homunculus, this method is useful for assessing motor cortical function and plasticity in healthy individuals and those with disease, examining skill acquisition or the presence of lesions. It can be used for pre-surgical planning, and as a tool to assess recovery in those areas. Using coil tracking software such as Brainsight, the x, y and z coordinates of the coil are recorded at the site of each pulse, and following the combination of these data points, the resulting MEPs can be used to make inferences about the motor homunculus when mapped onto scans (Siebner &

Rothwell, 2003). Previously, this method was described as time-consuming, which is a particular problem when wishing to investigate excitability changes quickly, such as during motor learning, where changes are short term. However, following work by Van De Ruit, Perenboom, & Grey, (2015), a method for obtaining a reliable map in a shorter space of time has been developed, termed the pseudorandom walk method, during which MEPs are sampled randomly within a predetermined area on the scalp. This method consists of a single TMS pulse being delivered in a predefined grid, without the need for targeting additional predefined positions within that space. As a result of pulses not being fired in as close proximity the ISI can be reduced, making the protocol shorter. The development of this method is possible due to the advances in coil tracking technology and its ability to capture the 3D coordinates of the location of the coil when a pulse is fired, allowing this data to be combined with MEP amplitudes to generate the map. This has allowed TMS corticospinal excitability maps to become a more useful way to investigate plasticity in the corticospinal tract.

Enabling the measurements of adjacent muscles is a more nuanced way of examining plasticity as not only can you observe MEP amplitude changes, but also the location and outline of motor representations in the M1. This means cortical maps are a well-placed technique to be used to study the effects of modulation or motor learning in that area as these induce neuroplasticity. It is on this basis that this experimental protocol was developed. By examining the differences before and after the TBS protocol, we can better understand the ways in which this is altering plasticity, as it prompts the changing of the cortical representations of a number of muscles in the hand. The incorporation of this technique is a natural development of the work done in previous chapters, examining changes in plasticity following the application of a TBS protocol. In earlier chapters, articles have been cited that discuss the subsequent facilitation of MEPs in the hand area following an application of iTBS. However, there is also evidence of the expansion of the motor map area following the

application of this TBS protocol (Lee et al., 2013). In this instance, the researchers used cortical motor maps to compare 3 conditions, all of which induce facilitation within the M1. These are a behavioural motor training condition, an iTBS protocol and a combination of the two. Their aim was to examine and compare the influence these may have on the motor cortex. The researchers were able to collect a large sample, 82 participants in total, with 23 individuals assigned to the iTBS condition. They found that those in the iTBS condition had increased excitability and a greater expansion of motor map areas than the observed increase in the motor learning alone condition. With our developed version of this technique, involving sophisticated neuronavigation methods, it was hoped to replicate this finding. Other studies investigating the possibility of short-term cortical reorganisation often use functional magnetic resonance techniques, such as the protocol described by Kolasinski et al., (2019). In this instance, researchers utilised brain scanning techniques to investigate the changing concentrations of GABA in the M1 during the completion of a SRTT. This design partially informed a portion of the research discussed in this chapter, along with Kolasinski et al., (2016). In this instance, the researchers use scanning methods in a 7 T MRI, this time to map digit representations within the somatosensory cortex of individuals at multiple time points. Our study consisted of two parts, one which used MRI techniques like the ones described, and the other which utilised TMS measures. Only the TMS portion of the research is focused on in this thesis, however, it is worth noting the larger experimental design encompassing the TMS work, which will enables us to make more in depth conclusions, correlate the results between the methods, and hopefully enable further use of the TMS version to collect larger data sets in future studies.

The primary aim for this experiment was to investigate the plasticity inducing protocol iTBS and measure the extent of the change using a variety of TMS techniques. In doing so the aim was to replicate the previously reported facilitatory findings (Lee et al., 2013). It is

hypothesised that an increase in MEP amplitude would be observed following iTBS application to the M1 hand area, as opposed to the control site. It is also expected that the cortical motor mapping data to resemble previous work and for the area to expand in those undertaking the experimental condition.

7.2 Methods

7.2.1 Participants

Data was collected for 20 participants (16 female, 4 male) in total; average age 23 ± 3.7 years, range: 19.9 – 34.1 years. 10 subjects were assigned to a condition in which they received iTBS over M1, and the other 10 were assigned to a condition in which iTBS was given over the vertex, this acted as a control. Participants were screened to ensure they were healthy, free from medication and any counter indications to TMS. All participants were deemed to be right-handed using the Edinburgh Handedness inventory (Oldfield, 1971). This study gained ethical approval through the School of Psychology ethics committee at the University of Nottingham and was conducted in accordance with the ethical standards specified in the 1964 Declaration of Helsinki.

7.2.2 Design

Individuals were randomly assigned to one of two conditions, one was targeting the M1 hand area, and the other was the vertex, which acted as a control site. This study consisted of two testing sessions, the first session is detailed in Figure 7.1. In this design TMS was used only to apply TBS in between MRI scanning sessions. The fMRI hand map was acquired by instructing participants to tap each finger on their right hand, in a

sequence from their index finger to their little finger during the scans. The magnetic resonance spectroscopy (MRS) was used to quantify the concentration of GABA in the M1.

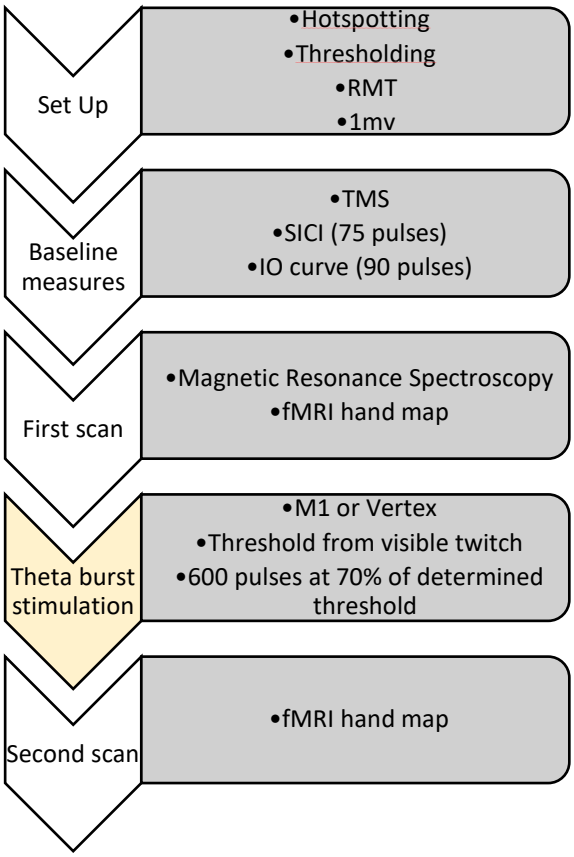

Figure 7.1 – A representation of the experimental procedure undertaken in Session 1, where the application of TBS is combined with MRI scans.

Here, only the TMS mapping undertaken in Session 2, shown in Figure 7.2, is discussed. It is worth noting the overall design for this experiment, as the anatomical scans collected in Session 1 were essential to generate accurate mapping data in the second session, as this enabled better targeting of the hand muscles for each individual. Additionally, by combining these methods, there is the opportunity for more thorough

analyses and conclusions at a later date. The measure of an fMRI hand map, taken before and after the application of TBS, refers to the task the individuals were required to complete during the scans. They were instructed to follow the cues on a screen with blinking dots, these corresponded to each of the fingers on the right hand. Participants were instructed to move each finger up and down in a regular rhythm along with the pattern indicated on screen. During this, fMRI scans were taken. This data could be combined with the TMS measures for a better understanding of the mapping of the hand area.

Testing sessions were scheduled a week apart, and at the same time to avoid any excitability, and therefore plasticity, differences that may arise from differing cortisol levels (Sale et al., 2008).

7.2.3 TMS measurements

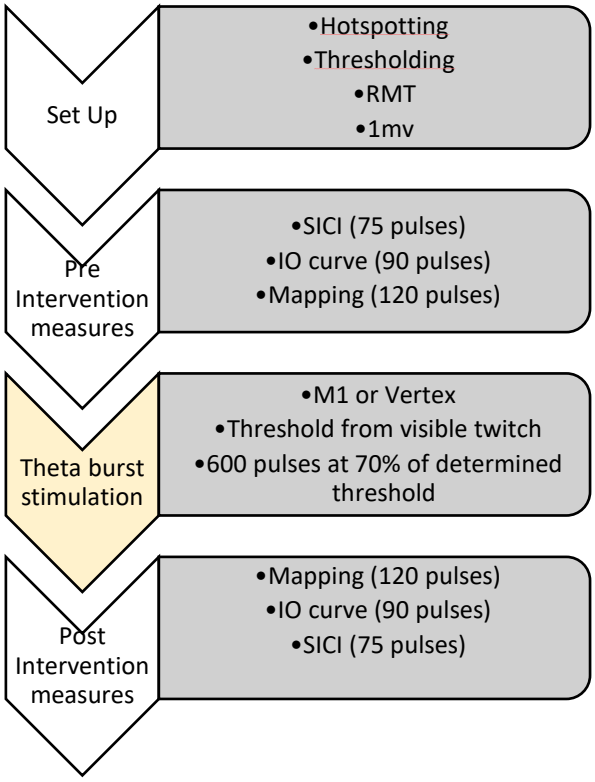

Figure 7.2 – A representation of the experimental protocol undertaken in Session 2, consisting of TMS measures prior and post TBS application

TMS was applied using two Magstim 200 stimulators (Magstim Co., Whitland, Dyfed, UK), connected through a BiStim Module (Magstim Co.) with a standard figure of eight, branding iron coil (diameter of one winding 50mm). The coil was held tangentially to the scalp, at a 45° angle from the midline, resulting in a posterior to anterior flow of current. The left M1 hand area was targeted for the TMS, and the motor hotspot was determined as the area where TMS was eliciting the largest MEPs from the FDI muscle on the right hand. MEPs were recorded using Ag-AgCl surface electrodes attached to the FDI muscle of the right hand in a belly tendon montage. Abductor digiti minimi (ADM) and abductor pollicis brevis (APB) muscles were also targeted. Their locations on the hand are illustrated in Figure 7.3.

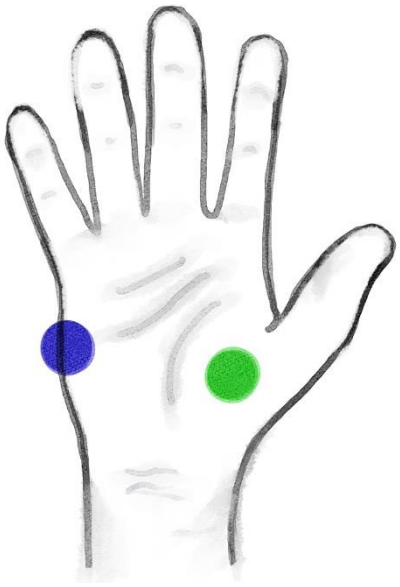

Figure 7.3 - illustration of the right hand, palm facing up. The green dot highlights the location of the APB muscle, and the blue dot highlights the location of the ADM. The FDI muscle that is also being measured is visible when the hand is in a palm down position, see the red dot on Figure 3.1

The signals were amplified, bandpass filtered (10Hz-2Hz, sampling rate 5Hz), and digitised using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by BrainVision Recorder (Brain Products GmbH, Gilching, Germany). The neuronavigation system, Brainsight (Rogue

Research Inc., Montreal Quebec, Canada) was used to ensure consistent placement of the coil, and to record the coordinates of the coil positioning. MRI scans taken in the first session of this protocol, loaded into Brainsight software, were used for accurate identification of the left primary motor cortex and the omega hand shaped area in the brain, as well as for consistent coil placement for the mapping in session 2, see Figure 2.2 for more details.

Resting motor threshold (RMT) was defined as the lowest stimulation intensity needed to yield a MEP with a peak-to-peak amplitude of >50μV in the FDI muscle whilst the subject is relaxed, in 5 out of 10 trials. This was calculated for both TMS machines and both types of coil used. The 1mV threshold was also established, this was the intensity where the average MEP size was 1Mv. All TMS pulses during the experiment were triggered using an in-house Matlab program (Mathworks, MA, USA).

The parameters described below were chosen as they have demonstrated good reliability in previous work performed by the research group (Dyke et al., 2018). The only alterations were that more pulses were added to measure SICI, but the weakest CS intensity was discarded so as to not extend the length of the protocol.

Single pulse

Single pulse measures were taken before and after the application of TBS to generate comparable IO curves. The selected intensities were 100, 110, 120, 130, 140, 150% of RMT, and each intensity had 15 pulses. The total number of pulses was 90 per measure.

Paired pulse

SICI levels were recorded pre and post TBS applications. The chosen intensities of the CP were 65, 70 and 75% of RMT and the TP intensity was the intensity needed for the average MEP size to be 1mV for that individual. There was an ISI of 3ms between pulses in this measure, there were 30 unconditioned pulses, and each CP intensity had 15 pulses, meaning there were 75 pulses in total for the paired pulse measures.

Mapping

Based on the identification of the hand area in each individual anatomical MRI image, a virtual 6x6cm grid was superimposed, using Brainsight, encompassing the target hand muscles. This grid provided a visual guidance for collecting the mapping data. During data collection, the coordinates of the 4 corners of the grid were mapped first, then the remaining pulses were applied using the pseudorandom walk method (Cavaleri, Schabrun, & Chipchase, 2018; Van De Ruit et al., 2015) within this predefined boundary. The stimulation intensity selected was 120% of RMT, chosen to ensure MEPs are elicited, without being so big as to recruit the whole hand and obscure more nuanced comparisons between hand muscles. An ISI of 5s was used to allow for coil movement into a new position. During each mapping session there were 120 pulses in total. The coordinates of the coil when pulses were fired were collected in 3D space.

7.2.4 Theta Burst Stimulation

TBS over the left M1 hand area was applied using a Magstim Rapid^2 (Magstim, Co., Whitland, Dyfed, UK) with a standard figure-of-eight branding iron coil (diameter of one winding 70mm) at a 45° angle. To identify the location for the TBS condition over the M1, the exact

placement for the TMS over the M1 was identified in the first session by locating the FDI muscle hotspot and marking the scalp with pen as neuronavigation was not possible. In session 2, the location was determined by using a combination of Brainsight and the participant's anatomical brain scans. The target was also the same as the hotspot for the FDI muscle as in the first session. For the vertex condition the location was determined using scalp measurements to determine the centre point of the individual's scalp. A mark was made on the scalp at the midpoint between the tragus of each ear, going over the head, and also at the midpoint of the measure taken from the nasion and the inion. Where these met, this was deemed the vertex point. This location is frequently selected as a control site as this area is assumed to not have an active role in the brain function assessed in this experiment, whilst allowing the inclusion of the same noise and scalp sensation associated with TMS that is not used in sham TMS, as in that instance the coil is held away from the head (Jung et al., 2016). Throughout this experiment iTBS was used, at 70% the RMT in the M1 condition, and 30% of visible twitch in the vertex condition. In the vertex condition the coil angle was 0° from the midline. 600 pulses were applied in each session.

7.2.5 Analysis and Statistical Tests

MEPs

All MEP data was visually inspected for noise levels, prior movements or obvious artifacts. These values were discarded. In house Matlab scripts were then used to correct noise and remove any data points in a more objective manner that may have been missed by eye. The noise calculation was based on measuring the minimum and maximum peak within the 500ms before the TMS artefact that is visible for all trials. Trials are excluded if there is a peak during this time window of over 50mV. Once these peaks are removed, the median MEP sizes were found and the

remaining data is Z scored. Values that score above 4 are also excluded as this indicates those values are over 4 standard deviations above the mean. This is a better approach for this experiment as there are multiple channels measuring multiple muscles. To determine SICI, the ratio of the median amplitude of the CS MEPs and the unconditioned MEPs was calculated.

TMS Mapping

The MEPs from each muscle EMG channel from the recording were synchronised to the TMS scalp coordinates, extracted from the neuronavigation software. This 3D data is projected onto a 2D plane and rotated to align with the axis. The cortical maps are then calculated for each muscle, consisting of pixels containing approximated MEPs based on triangular interpolation. The map is standardised, smoothness levels for all maps were set to level '6', and thresholded to eliminate potential false positive MEP activation in the map pixels and increase the likelihood that the muscle map areas are reflective of the true area.

The inclusion criteria for the data to be used in further analysis required the data sets of both pre and post TBS measures, peak MEP amplitude to be within the map, and that the number of excluded MEPs within each measure to be below 40. This is in accordance with previous work that suggests you can map an area in approximately 80 pulses (Van De Ruit et al., 2015). Any more MEPs necessitating exclusion will reduce the number of available data points to below this value. With these criteria there are 12 data sets that can be included in this analysis, 5 assigned to the M1 condition and 7 to the vertex condition.

From the collected cortical maps, the primary parameters that were collected were the centre of gravity (COG) which concerned the location,

the Euclidean distance between muscles, dice similarity coefficient, meaning the overlap of different muscles, and the area of each muscle which is a good measure of variability between maps taken before and after the application of TBS. The mapping parameter reported in this chapter is the area in mm^2. The other measures will be examined fully when subsequent useable data is collected, allowing for better comparisons that may otherwise be attributed to individual variability in such small sample sizes.

The statistical analysis programme jamovi (jamovi.org) was used to perform statistical analyses on generated data. All tests were conducted with a significance level of $p<.05$. Throughout the analysis process *p* values were uncorrected, meaning that interpretation of them in the subsequent results section should be interpreted with extreme caution. Mauchley's test of sphericity was performed and corrections were made using Greenhouse-Geisser when needed. Bayesian methods were applied as a parallel analysis strategy in addition to frequentist methods using the *p* value as a way to indicate the extent of evidence we have for either the H_0 or H_1 in these small sample sizes collected. The BF_{10} and the corresponding categories are reported in Table 3.1.

7.3 Results

7.3.1 Mapping results

A key measure in determining if the map outputs have quantifiably changed following application of iTBS is to look at how the area variability differs for each muscle being measured. Figures 7.4 - 7.7 depict two individual's mapping results, one from each condition, showing MEP amplitudes as a heatmap, before and after the application of the iTBS protocol. Figure 7.8 depicts the compiled group data, comparing both conditions and all the hand areas. Independent samples t-tests (one-tailed, Mann-Whitney U) revealed no significant difference between the two conditions for a change in area in the post measures (FDI: $t(12)=16$, $p=.153$, $d = .644$, ADM: $t(12)=18$, $p=.228$, $d = .413$, APB: $t(12)=23$, $p=.306$, $d = .233$). To investigate further, paired samples t-tests were used to compare the area before TBS application and after, for each hand area, however, these were also found to be non-significant differences (FDI: $t(6)=-1.213$, $p=.135$, $d = -.458$, ADM: $t(6)=-.166$, $p=.437$, $d = -.063$, APB: $t(6)=-1.286$, $p=.123$, $d = -.486$). Similarly, when examined using a Bayesian paired samples t-test, the results suggest that there is no convincing evidence for any of the 3 measured muscles.

Muscle	BF_{10}	Category
FDI	1.036	Anecdotal evidence for H_1
ADM	.399	Anecdotal evidence for H_0
AMP	1.117	Anecdotal evidence for H_1

Table 7.1 - table of Bayesian results for paired samples t-test for median data for participants within the M1 condition, comparing the muscle areas before the application of theta, and after the application of theta.

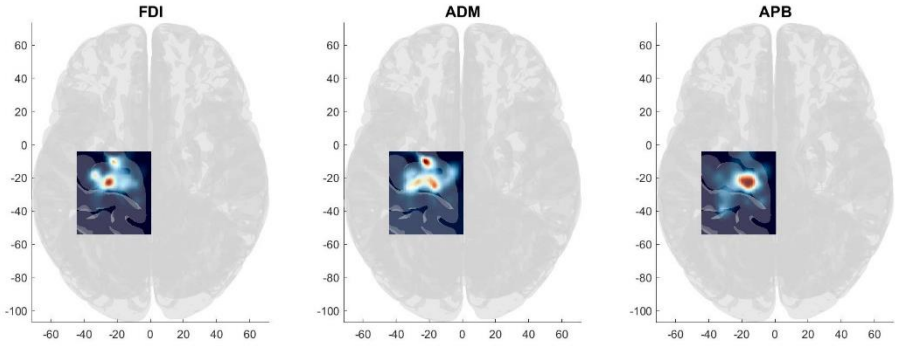

Figure 7.4 – Mapping results for Participant 1, in the M1 condition, prior to the application of iTBS

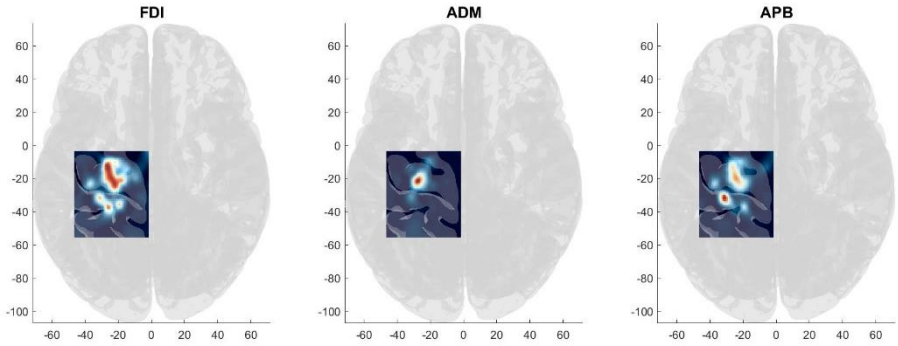

Figure 7.5 - Mapping results for Participant 1, in the M1 condition, after the application of iTBS

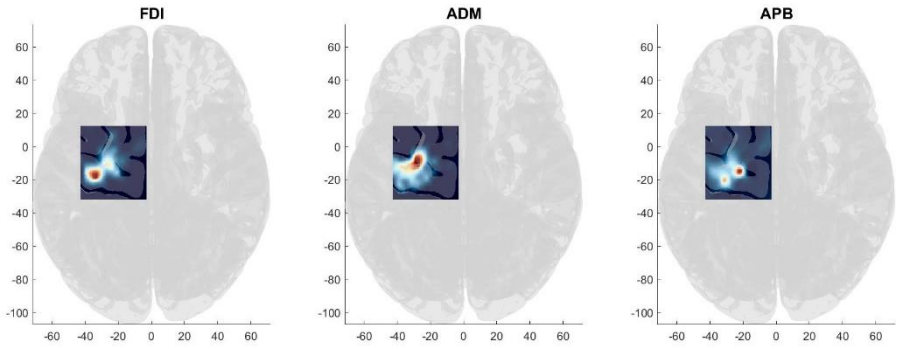

Figure 7.6 – Mapping results for Participant 21, in the Vertex condition, prior to the application of TBS

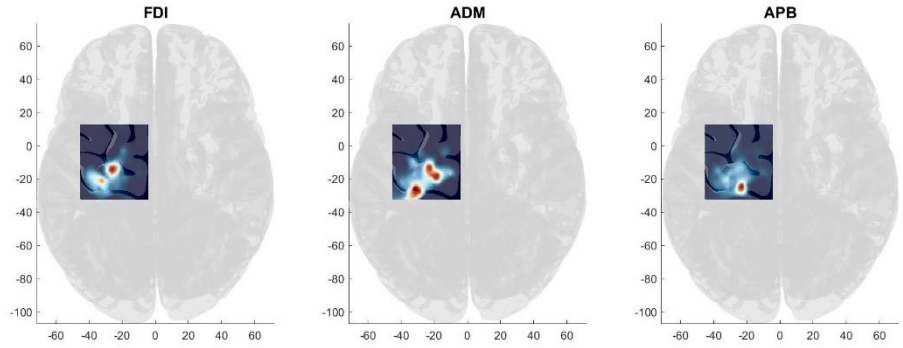

Figure 7.7 - Mapping results for Participant 21, in the Vertex condition, after the application of TBS

The control condition was checked with the same statistics (two tailed) and no significant results were found, as predicted (FDI: $t(6)=.98$, $p=.365$, $d = .370$, ADM: $t(6)=.813$, $p=.448$, $d = .307$, APB: $t(7)=1.82$, $p=.112$, $d = .643$).

Muscle	BF_{10}	Category
FDI	.514	Anecdotal evidence for H_0
ADM	.460	Anecdotal evidence for H_0
AMP	1.058	Anecdotal evidence for H_1

Table 7.2 - table of Bayesian results for paired samples t-test for participants within the vertex control condition, comparing the muscle areas before the application of theta, and after the application of theta.

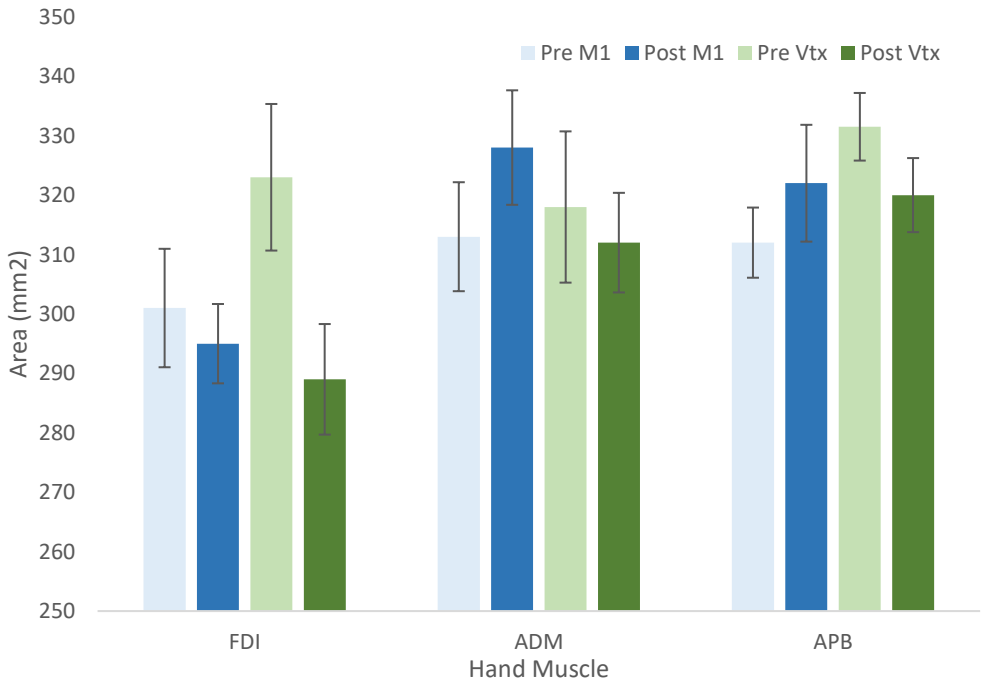

Figure 7.8 – Median area size in mm^2 for each condition and hand area measured. Error bars show standard error

7.3.2 TMS results

To examine the effects of iTBS on MEP size, the median MEP sizes at each stimulation intensity prior to iTBS application was subsequently compared to MEPs post application. As predicted the MEP size increased in the M1 condition, a one tailed paired samples t-test was conducted. For the control condition, there was not a hypothesis, and therefore a two tailed paired samples t-test was used. This is visually reported in Figure 7.9, and the statistics are documented in Table 7.3.

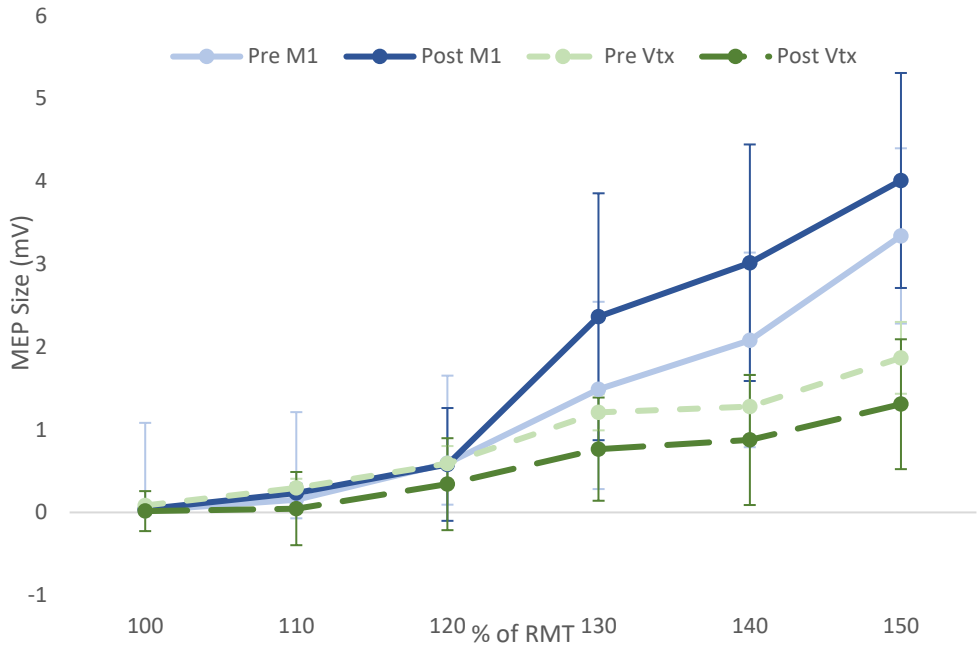

Figure 7.9 - Graph depicting the recruitment of MEPs at an increasing % of the RMT between the M1 condition and the Vertex condition. The lighter lines show the median MEP size at each stimulation before the application of TBS, and the darker lines are the same measures following the application of TBS. Error bars are representative of the standard error of the mean.

	Stimulus Intensity (% of RMT)	d.f	t	p	Cohen's d
M1	100	4	-.789	.237	.353
	110	4	.424	.653	.190
	120	4	-1.175	.153	.525
	130	4	-2.358	.039 *	1.055
	140	4	-2.432	.036 *	1.087
	150	4	-2.145	.049 *	.959
Vertex	100	6	-.889	.408	.336
	110	6	-.862	.422	.326
	120	6	-.633	.550	.239
	130	6	-.431	.682	.163
	140	6	-.529	.616	.200
	150	6	-.463	.660	.175

*Table 7.3 – Table of results of paired samples t-tests, comparing pre and post measures for both locations of TBS application. The * denotes a significant result*

	Stimulus Intensity (% of RMT)	BF_{10}	Category
M1	100	.753	Anecdotal evidence for H_0
	110	.307	Moderate evidence for H_0
	120	1.072	Anecdotal evidence for H_1
	130	3.06	Moderate evidence for H_1
	140	3.248	Moderate evidence for H_1
	150	2.561	Anecdotal evidence for H_1

Vertex	100	.483	Anecdotal evidence for H_0
	110	.475	Anecdotal evidence for H_0
	120	.416	Anecdotal evidence for H_0
	130	.382	Anecdotal evidence for H_0
	140	.397	Anecdotal evidence for H_0
	150	.386	Anecdotal evidence for H_0

Table 7.4 - Table of results following Bayesian paired samples t-tests comparing the medium MEP size pre and post TBS application

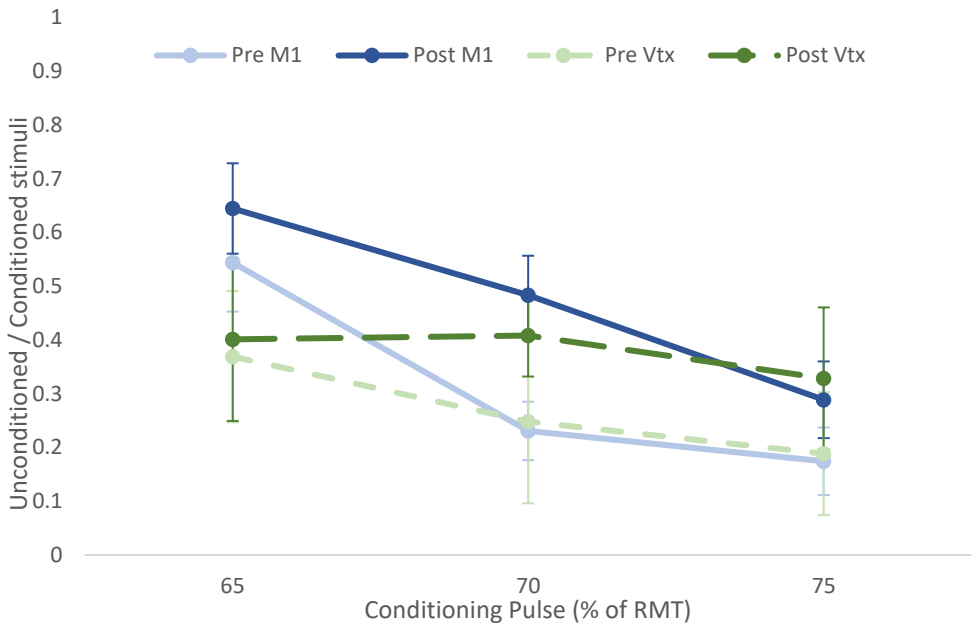

Figure 7.10 – Graph depicting the SICI results as ratio data (unconditioned/conditioned stimuli) for both M1 and vertex conditions. Values higher than one indicate facilitation, and smaller numbers indicate a larger amount of inhibition. The error bars are representative of the standard error of the mean.

	Stimulus Intensity (% of RMT)	*d.f*	*t*	*p*	*Cohen's d*
M1	65	4	-.793	.472	.355
	70	4	-2.782	.05 *	1.244
	75	4	-1.032	.360	.462
Vertex	65	6	-3.084	.022 *	1.166
	70	6	.0048	.996	.002
	75	6	-2.141	.076	.809

Table 7.5 – Results of two-tailed paired samples t-tests comparing pre and post measures after the application of TBS in different locations on the scalp

	Stimulus Intensity (% of RMT)	**BF_{10}**	**Category**
M1	65	.508	Anecdotal evidence for H_0
	70	2.227	Anecdotal evidence for H_1
	75	.589	Anecdotal evidence for H_0
Vertex	65	3.732	Moderate evidence for H_1
	70	.353	Anecdotal evidence for H_0
	75	1.461	Anecdotal evidence for H_1

Table 7.6 – Table of results following examination of SICI data using a Bayesian paired samples t-test comparing measures taken before and after the application of TBS for each location that was tested

Figure 7.11 depicts the group median MEPs in each measured hand area, pre and post stimulation for each condition. Independent samples t-tests (two-tailed, Mann-Whitney U) revealed no significant difference between the two conditions for a change in area in the post measures (FDI:

$t(12)=13$, $p=.165$, $d = .859$, ADM: $t(12)=24$, $p=1$, $d = .148$, APB: $t(12)=18$, $p=.281$, $d = .683$). To investigate further, paired samples t-tests (one-tailed) were used to compare the median MEP size before TBS application and after, for each hand area, however, these were also found to be non-significant differences (FDI: $t(6)=-.818$, $p=.222$, $d = .309$ ADM: $t(6)=-1.12$, $p=.154$, $d = .422$, APB: $t(6)=0.096$, $p=.536$, $d = .036$). No evidence was found for the H_1 after the application of a Bayesian paired samples t-test, reported in Table 7.7.

Muscle	BF_{10}	Category
FDI	.700	Anecdotal evidence for H_0
ADM	.938	Anecdotal evidence for H_0
AMP	.331	Anecdotal evidence for H_0

Table 7.7 - Table of Bayesian results for paired samples t-test for participants within the M1 control condition, comparing the median of the median MEP sizes before the application of theta, and after the application of theta.

The control condition was checked with the same statistics (two-tailed) and no significant results were found, as predicted (FDI: $t(6)=-1.022$, $p=.346$, $d = .386$, ADM: $t(6)=-1.837$, $p=.116$, $d = .695$, APB: $t(7)=0.264$, $p=.799$, $d = .094$). On applying Bayesian paired samples t-tests, a small amount of evidence for an altered MEP size in the ADM was found, reported in Table 7.8.

Muscle	BF_{10}	Category
FDI	.530	Anecdotal evidence for H_0
ADM	1.082	Anecdotal evidence for H_1
AMP	.346	Anecdotal evidence for H_0

Table 7.8 - Table of Bayesian results for paired samples t-test for participants within the vertex control condition, comparing the median of the median MEP sizes before the application of theta, and after the application of theta.

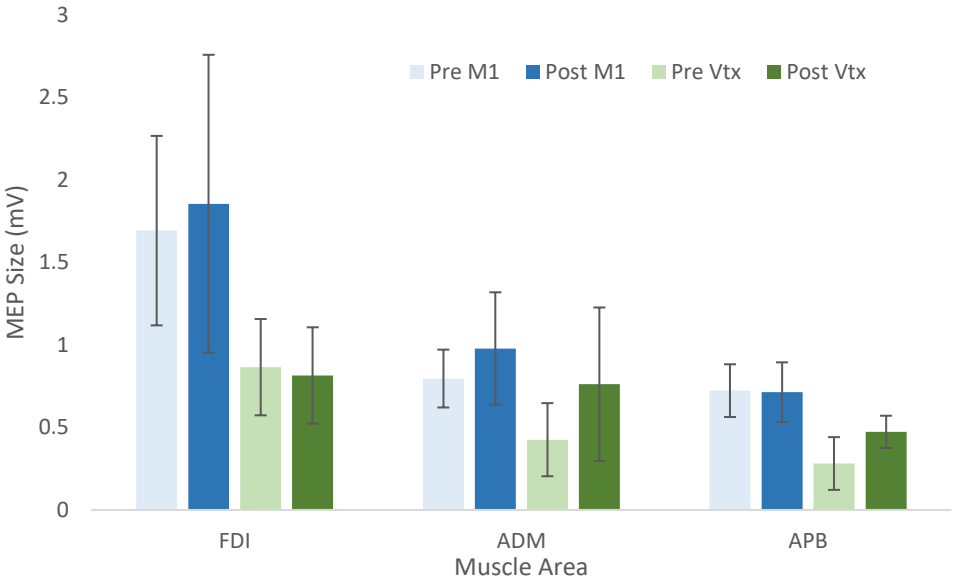

Figure 7.11 - Median MEP size for each condition and hand area measured. Error bars show standard error of the mean

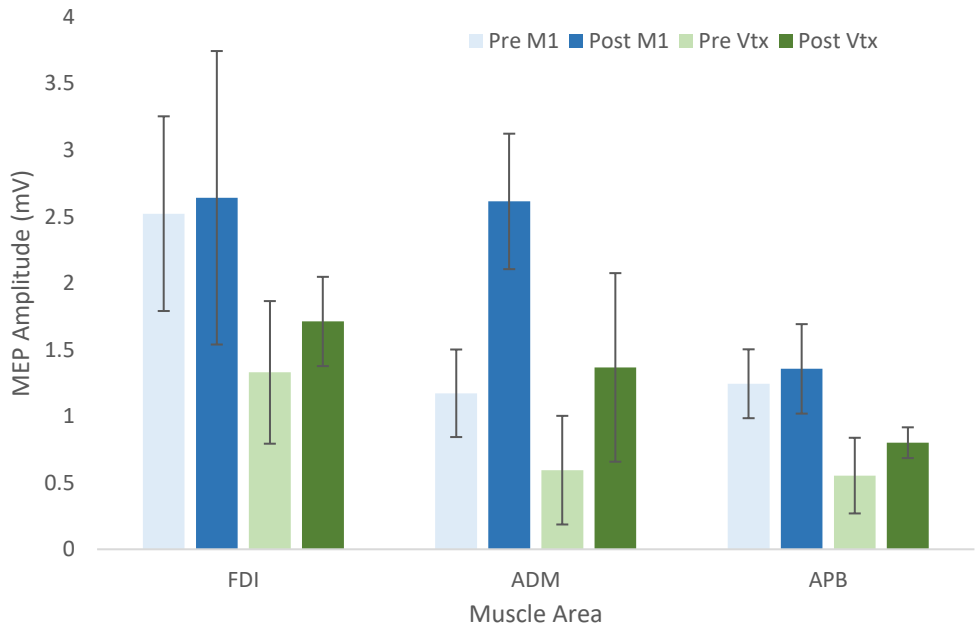

Figure 7.12 - Median of the maximum MEP amplitudes in each condition and for each hand area. Error bars are standard error

The median of the maximum MEP size was also examined for an indication of the expected facilitatory effects of iTBS. Independent samples t-tests (two-tailed, Mann-Whitney U) revealed no significant difference between the two conditions for a change in area in the post measures (FDI: $t(12)=13$, $p=.082$, $d = .526$, ADM: $t(12)=19$, $p=.267$, $d = .225$, APB: $t(13)=20$, $p=.198$, $d = .730$). To investigate further, paired samples t-tests (one-tailed) were used to compare the median MEP size before TBS application and after, for each hand area. These were also found to be non-significant differences (FDI: $t(6)=-0.638$, $p=.274$, $d = .241$, APB: $t(6)=-0.194$, $p=.426$, $d = .074$) apart from the one for the ADM muscle ($t(6)=-2.98$, $p=.012$, $d = 1.126$), which returned a significant result when comparing MEP maximum amplitude before and after the application of TBS.

Muscle	BF_{10}	Category
FDI	.592	Anecdotal evidence for H_0
ADM	6.596	Moderate evidence for H_1
AMP	.408	Anecdotal evidence for H_0

Table 7.9 - Table of Bayesian results for paired samples t-test for participants within the M1 control condition, comparing the median of the maximum MEP amplitude before the application of theta, and after the application of theta.

Following the application of Bayesian statistics, recorded in Table 7.9, there is further evidence of a significant effect in the MEP amplitudes elicited from the ADM muscle. The control condition was checked with the same statistics (two-tailed) and no significant results were found, as predicted (FDI: $t(6)=-0.58$, $p=.583$, $d = .219$, ADM: $t(6)=-1.695$, $p=.141$, $d = .64$, APB: $t(7)=0.462$, $p=.658$, $d = .163$).

Muscle	BF_{10}	Category
FDI	.406	Anecdotal evidence for H_0
ADM	.944	Anecdotal evidence for H_0
AMP	.368	Anecdotal evidence for H_0

Table 7.10 - Table of Bayesian results for paired samples t-test for participants within the vertex control condition, comparing the median of the maximum MEP amplitudes before the application of theta, and after the application of theta.

7.4 Discussion

The main aim of this study was to replicate the previously reported facilitatory findings of MEP amplitude changes and the expansion of cortical motor maps following the application of an iTBS protocol. In this study it was possible to demonstrate that there is an increase in MEP size following the application of this plasticity inducing protocol.

This cortical mapping technique is useful for identifying additional plasticity details rather than simply observing facilitatory responses in MEP size. It enabled us to establish more detail by examining the location, median MEP size and the maximum size of MEPs elicited in the pre and post measures. Using just frequentist statistics, no difference between the control stimulation site and the M1 was observed when examining the cortical motor map areas. This was disappointing, but not surprising for such a small sample size. A small amount of evidence for a change in the FDI muscle was reported following the use of Bayesian statistics. As expected, there were no changes reported in the control condition between cortical maps captured before and after iTBS application. However, the maximum MEP size data did return a significant result for an increase in MEP size following iTBS in the ADM muscle in the M1 condition. The vertex condition did not show any changes. This is more in line with our hypotheses.

One question that arises with these results is the precision of the iTBS application and whether the field of activation is only targeting the FDI or if it has spread to the surrounding muscles. The aim was to control this by using a stimulation intensity below threshold to minimise the spread of activation. This protocol lends support to the use of control stimulation sites. The spread of activation to other muscles is not the only consideration for TMS protocols involving multiple muscles. The

stimulation intensities used throughout the whole procedure are calculated from the individual's RMT from the FDI muscle. As this is not the optimum intensity for targeting the APB or ADM, this may cause an issue when calculating the muscle representation in a cortical map due to a loss of sensitivity. Intensity selection during SICI also remained the same, rather than changing with the possible fluctuations of the 1mV threshold in an individual. This was highlighted as a necessity in Chapters 5 and 6, as the failure to adjust this may obscure alterations in SICI following the application of the theta protocol. These adaptations need serious consideration alongside concerns of the length of the protocol and the comfort of the participant. However, by altering the 1mV threshold in the process it may be possible to gain further insight into whether GABA can predict the variability within the cortical motor maps. This rigidity of technicalities relating to the setup similarly extends to the coil placement. It was chosen for the optimum position with which it best evokes MEPs from the FDI. Throughout the procedure in the experimental condition, a coil angle of 45° from the sagittal midline is used, which is less readily able to activate the APB (Corp et al., 2020), also worth considering for future iterations of the methodology.

The additional TMS data collected before and after the cortical mapping established further evidence that an iTBS protocol will result in an increase of MEP amplitude, as numerous stimulus intensities in the IO curve measure returned significant results when comparing the pre and post stimulation measures. No statistically significant difference was observed in the vertex condition, as hypothesised. For the paired pulse data, there was evidence that at the stimulus intensity of 70% of RMT in the M1 condition, there was a significant difference between the amount of SICI when comparing pre and post measures. Figure 7.10 suggests that the amount of SICI reduced after the application of iTBS. Unexpectedly there were also significant differences between pre and post measures in the vertex condition, supported by both frequentist statistics and Bayesian analysis. It will be interesting to examine these calculations with a larger

sample group in the future to check if these SICI findings are robust, and not just a result of individual variability. It is also worth noting that TMS can induce changes in cortical excitability when delivered at fixed ISIs, such as those in the mapping paradigm (Pellicciari et al., 2016). As a result this may have altered many TMS measures, especially through the large number of pulses that are applied in the duration of the study. It may be one possible explanation for some of the unexpected results in the control condition. An attempt was made to mediate this by ensuring the participant experiences of the TMS sessions was always identical, and therefore the effects would still be comparable across the subject's data.

Other research has begun to query the use of the vertex as a control site in TBS. Whilst this is a control stimulation site that is used almost exclusively, evidence is emerging that TBS protocol when applied over the vertex may still lead to changes in a task measuring cognitive performance (Pizem et al., 2022). The changes observed were not found to be any more superior in the experimental stimulation site, leading to the researchers hypothesising that stimulation is reaching adjacent areas such as the supplementary motor area, which are also involved in the cognitive performance task used in their study. In an effort to counter these potential problems, the researchers suggest utilising the available neuronavigation software, brain scans and MNI atlas to create a trajectory that will best inform the correct angle so as to avoid inducing other areas as much as possible. Whilst the experimental design tested in this chapter did not include a cognitive performance task, it is a possible explanation for the large amount of variability and unexpected significant differences in measures observed in the control condition, which has obvious implications for future research.

A common discussion theme throughout this thesis has been the available sample size for analysis. This has been especially evident during the pre-processing of the data in this study, when it became apparent that a large

number of exclusions were necessary. Our methodology necessitated the use of an individual's anatomical brain scan to ensure accuracy in the TMS portion of data collection. Whilst this undoubtedly improved the accuracy of the TMS targeting, it was hoped that it would prevent so many data sets from being excluded from analysis. During the setup of the TMS portion of this study, when hotspotting and thresholding was taking place, if the hotspot did not match the location selected in Brainsight prior to testing, then this was visually judged to determine if the hotspot was still near the centre of the grid set up for the mapping protocol. Some data has proven to not be within this parameter, resulting in graphs where MEP peaks in amplitude are outside of the grid area being measured. This is possible if an individual has multiple hotspots, or if the hand area on the brain scan is less clear. Unfortunately, our individualistic methods were unable to prevent every instance of this, resulting in participant exclusions from analysis. In this case, these exclusions were based on data quality, and did not distinguish between those that were responders and non-responders. This is a point of discussion for many researchers using TBS protocols, mentioned briefly in Chapter 6.4, with some choosing to separate the two for individual analysis. Previous investigations found that only 43% of subjects responded to an iTBS protocol in the expected way (López-Alonso et al., 2014). This categorization is something that can only be done after the data collection portion of the experiment, or would necessitate additional data, such as genetic information, before you are able to predict an individual's categorisation (Cheeran et al., 2008). López-Alonso et al., (2014) used their study data to calculate the sample size necessary to detect a significant effect in an iTBS protocol and found that it would require 830 subjects. They subsequently suggest an enrichment of responders in the sample to lower the overall number of subjects required, but this constrains a researcher's ability to suggest these findings are representative of a wider population. Within the context of our research, comparing typically developing individuals and TS populations, it seems more applicable to build a better understanding of what a typical response is for each group rather than only examining those who respond as expected to a protocol.

In conclusion, this study has further demonstrated that TMS mapping is a safe, well tolerated and potentially valuable tool (Giuffre et al., 2021). The results were highly variable and specific conclusions are difficult to make with such a small sample. A key finding however, is that there was evidence of an observable expansion in the cortical motor mapping areas, and a facilitatory response in MEP measures. This is a promising start for this protocol, which will greatly benefit from a larger sample size for subsequent more nuanced analysis. Ideal map characteristics and outcomes are yet to be determined in the literature and studies within this methodology examining the developing brain are lacking. There is room to use this technique on adolescents with TS and control groups to examine possible alterations in motor development, but only after further studies of the developing brain.

Chapter 8 : General Discussion

Keywords: *Transcranial magnetic stimulation (TMS), Theta burst stimulation (TBS), Intermittent theta burst stimulation (iTBS), Continuous theta burst stimulation (cTBS), Interstimulus interval (ISI), First dorsal interossei (FDI), Abductor digiti minimi (ADM), Abductor pollicis brevis (APB), Resting motor threshold (RMT), Yale Global Tic Severity Scale (YGTTS), Serial reaction time task (SRTT)*

As is abundantly clear throughout the majority of this thesis, there is a significant amount of data not present due to Covid-19 restrictions suspending data collection. TMS literature is frequently underpowered due to experiments performed on small sample sizes, but in this instance they have been further restricted by unforeseen circumstances. Detailed below is the methodology for the final experimental protocol that was planned for this thesis, along with how it was expected to develop into a study comparing TS and control groups. At the end of this chapter some tentative conclusions are attempted for this portion of research. It is difficult to form conclusions in the way that was initially intended. Despite this, there is some promising evidence that the experimental designs tested could be highly insightful methods for further investigations of this topic, resulting in better understandings of the plasticity differences in TS.

8.1 Planned Study

The next step in this research narrative was to further the cortical mapping protocol described in Chapter 7. Recruitment of more subjects was required, as well as slight alterations in the protocol, to combine the TBS methods previously used to investigate both cTBS and iTBS effects. An additional measure post TBS application was also going to be added to allow for comparisons of maps between three time points, similar to that of the design described in Chapter 5 and 6. The point after stimulation where possible excitability changes are measured are a key factor in TBS

research (Corp et al., 2020). Following an analysis of a large number of TBS studies, investigators concluded that induced plasticity from each TBS protocol lasted for different durations. Extending the number of data points across a longer time period will enable a more detailed understanding of the course of that short term plasticity alteration. This was evident in the data collected in Chapter 5 and 6, and therefore it is intended to keep investigating across a longer timespan. In an attempt to capture more data, at the time of the closure of the research space we were testing the feasibility of capturing additional time points with the pseudorandom walk method previously used. Van De Ruit et al., (2015) who initially described this method, stated that it was possible to map a cortical area in approximately 80 pulses with an ISI of 1.5s. To be able to collect so much more data in the same amount of time would be of huge benefit. However, on initial testing of the equipment, it became clear that this would not be possible as the available TMS machine available was not able to recharge fast enough after each stimuli.

Another change to highlight in this protocol is that the vertex condition used in the previous chapter as a control, has been altered. Instead, the control condition will still be targeting the FDI hotspot, and instead a sham iTBS protocol applied at an intensity level low enough that no activation should penetrate the skull. Whilst the intensity of stimulation was considerably lower than RMT in the previous experimental design, this was none the less a key discussion point. Such a method should further avoid the possibility of stimulation spreading to other brain areas. The aim of this experiment is to develop the cortical mapping methodology tested in Chapter 7, as a means of investigating TBS protocols using a variety of TMS techniques. It is hypothesised that some of the procedural changes made, such as the alternative control condition, will allow for fewer data exclusions and more thorough conclusions as a result. We would expect that there would be an increase of MEP amplitudes observed following iTBS application, and a decrease in MEP amplitudes following cTBS application, compared to the control condition. It is also expected to

be reflected in the cortical motor mapping data, with an expansion of some or all of the target muscle areas following an application of iTBS, and a reversal of this observed effect in the cTBS condition.

8.1.1 Design

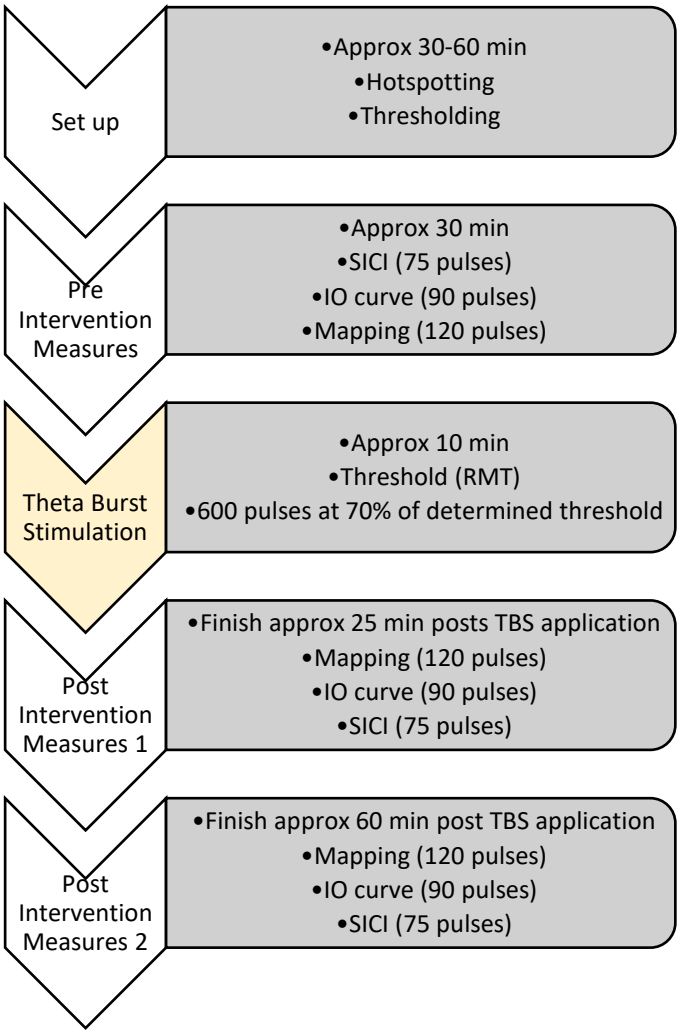

Figure 8.1 – A representation of the planned protocol for this second iteration of a mapping study. The yellow box highlights when TBS takes place

Individuals will be randomly assigned to one of three conditions. The first condition is the iTBS condition, the second is testing an individual's

response to cTBS, and the third condition is the control condition, where iTBS is applied to the M1 FDI hotspot but at 5% RMT. 5% RMT is chosen so as to mimic the noise produced by a TMS coil during an experimental condition, without the manipulation of cortical excitability levels. The aim is to collect 12 usable data sets for each condition, necessitating 36 subjects after any exclusions due to data quality. It would also be appropriate to consider an open-ended sequential Bayes factor design, adding participant data as the sample size grows to monitor the level of evidence during the recruitment process. This could save time and resources by preventing over recruitment and it also means the researcher is aware if more data is necessary earlier on in the process (Schönbrodt & Wagenmakers, 2018). For guidance on what the necessary number of participants might be for this experimental design, a power analysis revealed that on the basis of the repeated measures, within factors comparison, for the effect size to be (F = .25) in the outcome of this study, an n of approximately 156 would be needed to obtain statistical power at the recommended .80 level (Cohen, 1988).

TMS Measures

TMS will be applied using two Magstim 200 stimulators (Magstim Co., Whitland, Dyfed, UK), connected through a BiStim Module (Magstim Co.) with a standard figure of eight coil, branding iron coil (diameter of one winding 50mm). The coil will be held tangentially to the scalp, at a 45° angle from the midline, resulting in a posterior to anterior flow of current. The left M1 hand area will be targeted for the TMS, and the motor hotspot will be determined as the area where TMS is eliciting the largest MEPs from the FDI muscle on the right hand. MEPs will be recorded using Ag-AgCl surface electrodes attached to the FDI muscle of the right hand in a belly tendon montage. Abductor digiti minimi (ADM) and abductor pollicis brevis (APB) muscles will also be targeted. Their locations on the hand are illustrated in Figure 7.3. The EMG signals will be amplified, bandpass filtered (10 Hz – 2kHz, sampling rate 5kHz), and digitised using Brainamp

ExG (Brain Products GmbH, Gilching, Germany) controlled by BrainVision Recorder (Brain Products GmbH, Gilching, Germany).

Resting motor threshold (RMT) is defined as the lowest stimulation intensity needed to yield a MEP with a peak-to-peak amplitude of >50μV in the FDI muscle whilst the subject is relaxed, in 5 out of 10 trials. This will be calculated for both TMS machines and coils used. The 1mV threshold will be established, this is the intensity where the average MEP size was 1Mv. All TMS pulses during the experiment are triggered using an in-house Matlab program (Mathworks, MA, USA).

Single pulse

Single pulse measures will be taken before and at two time points after the application of TBS to generate comparable IO curves. The selected intensities are 100, 110, 120, 130, 140, 150% of RMT, and each intensity has 15 pulses. The total number of pulses will be 90 per measure similar to the protocol described in Chapter 7.

Paired pulse

SICI levels will be recorded pre and post TBS applications, with two post measures to examine how cortical excitability will change over a longer period of time following a TBS protocol. The chosen intensities of the CP are 65, 70 and 75% of RMT and the TP intensity is the intensity needed for the average MEP size to be 1mV for that individual. There will be an ISI of 3ms between pulses. There are 30 unconditioned pulses in this measure, and each CP intensity has 15 pulses, meaning there will be 75 pulses in total for the paired pulse measures, similar to the previous chapter design.

Cortical Mapping

Based on the identification of the hand area in each individual anatomical MRI image, which will be a necessity during the recruitment stage, a virtual 6x6cm grid will be superimposed, using Brainsight, encompassing the target hand muscles, the FDI, ADM and APB. This grid will provide a visual guidance for collecting the mapping data. Each mapping session will begin with the mapping of the 4 corners of the grid, before the remaining pulses are applied using the pseudorandom walk method (Cavaleri et al., 2018)(Van De Ruit et al., 2015). The stimulation intensity selected is 120% of RMT, and an ISI of 5s. During each mapping session there are 120 pulses in total. The coordinates of the coil when pulses are fired will be collected in 3D space.

Theta Burst Stimulation

TBS over the left M1 hand area will be applied using a Magstim Rapid^2 (Magstim, Co., Whitland, Dyfed, UK) with a standard figure-of-eight branding iron coil (diameter of one winding 70mm) at a 45° angle. To identify the location for the TBS condition over the M1 the FDI hotspot will be highlighted using Brainsight (Rogue Research Inc. Montreal Quebec, Canada). During the experimental conditions the selected intensity will be 70% of RMT, and during the control condition this will be reduced to 5% RMT. In this design, both iTBS and cTBS are used, both of which consists of pulses applied in bursts of three at 50Hz, with an inter-burst interval at 5Hz. The pattern of application determines whether it is a facilitatory or inhibitory protocol, with iTBS requiring 2s of TBS trains repeated every 10 seconds for 20 cycles for a 600 pulse protocol. In contrast for cTBS, the pulses are applied as uninterrupted TBS trains for 40s for a 600 pulse protocol (Chung et al., 2016).

8.2 Development to a TS Study

The next step for this protocol is to develop it into a viable study for those with TS. Sigurdsson, Jackson, Kim, Dyke, & Jackson, (2020) conducted a sensorimotor cortical mapping study using the same techniques described in Chapter 7, clearly demonstrating the feasibility of using these methods with patient groups. Whilst the focus of the research was of the somatosensory area and the sensory aspects of TS as opposed to the motor area, there is still relevance with this research. The researchers found limited evidence that supports the existence of spatial organisation differences in TS, but they did observe a possible shift in the location of the FDI muscle compared to control groups (Sigurdsson, 2018). A possible shift in muscle representation between groups would be an additional interesting aspect to examine further in our more motor area focused design with additional target hand muscles.

The primary aim for this planned development would be to investigate if there are significant differences in the muscle representations of the hand area, and the cortical excitability of the hand area in the M1 between control participants and patients with TS, and to examine how these change in each group following the application of a TBS protocol. It is possible to predict that the patient groups measures of plasticity would not be in the expected direction following iTBS or cTBS, therefore demonstrating abnormal plasticity compared to control groups, as evidenced by previous research (Suppa et al., 2011; Marsili et al., 2017). It would be interesting to correlate mapping and TMS measures with individual's YTGGS, particularly the observations of their motor tics as hands are frequently involved in tics and sensory urges. The mapping measures and the extensive TMS measures before and after the cortical mapping will also be an opportunity to contribute to our understanding of possible aberrant GABA synaptic activity in TS and the pattern of change over a short period of time. In the future this design could develop to include tasks involving motor learning, such as the SRTT, to examine the

plasticity changes associated with those actions, and compare the cortical excitability levels to typically developing individuals.

8.3 Conclusions

Following some invaluable discussion, it has become clear that throughout this thesis there is a lack of consideration about statistical power throughout the research. Whilst it is frequently alluded to with regards to not having fulfilled the recruitment of participants that was hoped for, this is only something that has been considered at the conclusion of this body of work. A priori power analyses would have been a more measured way to approach data collection. This would have enabled better estimates of the necessary participant numbers required and enabled more insightful conclusions. In an attempt to salvage more thorough scientific contributions from this data some post hoc calculations were performed for a few key experimental questions. For future work, including the planned study described in Chapter 8.1, a priori tests for statistical power will be better utilised. By following these preferred analytical methods, then measures that are frequently used in TMS research can be used. For example, TMS data is often described as percentage change, or other examples of normalised data. These methods were initially attempted with the data collected in this thesis, but were often found to exaggerate observations made in such small sample sizes.

In the meantime this thesis has contributed to the existing body of knowledge by focusing on the use of TMS as a non-invasive brain stimulation technique, and establishing protocols to investigate plasticity within the motor area of the brain. Limitations of the research conducted have been extensively detailed within the discussion points in each chapter. The conclusions that were hoped for following this portion of research work have been disappointingly limited due to the inadequate amount of data. The planned comparisons between the typically

developing population groups and those diagnosed with TS were unable to be made. However, there are still some useful findings from the existing data that can shape future research. The key research questions this thesis aimed to explore are detailed below with a short summary of findings:

Is there an observable change in the pattern of cortical excitability during a motor learning task?

The first experimental protocol in Chapter 3 successfully demonstrated with behavioural data that motor learning occurred during our version of the SRTT. Although less clear, there was some evidence to support our hypothesis that a significant difference in cortical excitability, measured in MEP amplitude, would be observed whilst the participants were undertaking the experimental condition. Using the protocol changes outlined in Chapter 4, this may be an easier and more reliable method to examine both online and offline changes to MEP amplitudes and gain an insight into cortical excitability changes.

Is this observable pattern altered in those with a diagnosis of TS?

This is explored in Chapter 4, but due to the extreme constraints on recruitment a conclusion is hard to reach, particularly with the data measuring cortical excitability. The experience highlighted that there is an additional difficulty in testing TS with TMS and may need either additional thorough screening or a larger pool of participants as it is difficult to predict exactly how their tic manifestations may interfere with the testing process, particularly if they become anxious about the situation. It was expected that evidence would be found of MEP changes throughout the SRTT that indicated altered motor learning, in addition to differences in the behavioural data compared to typically developing individuals. None

the less, the work undertaken in Chapter 4 was a valuable opportunity to pilot the procedure with individuals experiencing tics, enabling future researchers to access interesting insights when these methods can be applied. As a direct result of the lockdowns experienced recently, there has been an interesting increase of childhood tics and tic-like attacks during the Covid-19 pandemic (Heyman et al., 2021). It is already altering previously accepted information, particularly on the gender split of those who develop tic and TS disorders. This provides a wealth of opportunity to investigate TS onset, the role of stress and upheaval and whether or not those diagnoses will reverse when restrictions related to the pandemic ease and the amount of stress these individuals are experiencing reduces. There is a potential for studying a possible spectrum of plasticity differences between those who show no tic symptoms, those with sudden onset tic attacks, or those who have had a more typical development of TS from childhood.

Can we replicate the findings of previous research that has investigated the use of theta burst stimulation?

Chapters 5 and 6 were designed as within subject protocols to limit the variability between individuals, enabling a better understanding of how these plasticity inducing protocols can create excitability changes. We did conclude there was a significant effect on MEP amplitude following the application of iTBS in Chapter 5. However, there was no evidence for the inhibitory effects we expected to see for cTBS applications. In the primed protocols in Chapter 6, there was some evidence to suggest MEP amplitude was being manipulated by the condition that was applied. This was not a significant enough amount to conclude that we were able to fully replicate previous research with the data sets collected in this instance.

Are there improvements we can make to this theta burst stimulation protocol?

A strength of the theta burst protocols examined in Chapter 5 and 6 was the within subject design. It provided the opportunity to reduce some possible sources of variability by using the same group of participants. Unfortunately, this is not always a viable option for many, as it would be a considerable burden on time and may prompt participants to withdraw before all conditions can be tested. An easier alteration is to include more time points to repeat measures, which we did in our TBS protocols and our cortical mapping protocols. In doing so this allowed for a more detailed understanding of cortical plasticity fluctuations over a longer time period compared to a single measure after the application of TBS. Indeed, the addition of the cortical mapping measures combined with the TBS protocol may subsequently provide better conclusions relating to a variety of plasticity measures, as there will be a greater degree of sensitivity to changes as opposed to merely observing MEP amplitude changes. In developing this protocol, the aim is to enable a better understanding of the processes behind motor learning and cortical excitability changes.

Does the application of theta burst stimulation alter the cortical motor representations of the hand area?

The results of our study in Chapter 7 suggest there is some evidence that TBS is altering the cortical representations of the hand area, although at this stage it remains limited. A compromised sample size, due to data being excluded, meant a full conclusion is difficult to make at this stage. In addition, the final experiment detailed in this chapter did not proceed. This had the potential to provide many more insights about facilitatory and inhibitory excitability changes in the M1. Despite this, there were some significant excitability changes observed in the TMS measures and in the cortical motor maps, suggesting that this avenue of research still has potential.

Although more work is necessary, this thesis enabled the development of a number of methods designed to give valuable insight into the neuroplasticity mechanisms that may be altered in patient groups. Ultimately this may lead to further research with the aim to improving the lived experience of those diagnosed with TS.

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