Exploring Function and Effective Connectivity of the Motor Cortex and its Role in Tourette Syndrome

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Abstract

Tourette syndrome (TS) is a neurodevelopmental disorder characterised by vocal and motor tics. It is associated with cortical–striatal–thalamic–cortical (CSTC) circuit dysfunction and hyper-excitability of cortical motor regions. TS follows a developmental time course, in which tics often become increasingly more controlled during adolescence. Importantly, however, a substantial minority of patients continue to have debilitating tics into adulthood. This indicates that there may be important differences between adult TS patients and children and adolescents with the disorder.

The first aim of my thesis was to explore the excitability of the primary motor cortex (PMC) at rest, during motor preparation, motor execution and the inhibition of action. In **Chapters 3** and **4** I demonstrate that, in contrast to studies of adult patients, resting motor threshold (RMT) and the variability of motor-evoked potential (MEP) responses are increased in young people with TS, while the gain of motor excitability in reduced. Furthermore, these differences normalise with age over adolescence. I conclude that these effects are likely due to a developmental delay in the maturation of key brain networks in TS, consistent with recent brain imaging studies of structural and functional brain connectivity. Importantly, these findings suggest that the alterations in brain network structure and function associated with TS may be quite different in children and adult patients with the condition.

In **Chapter 4**, I demonstrate that whilst there is evidence of reduced gain during motor execution in young people with TS relative to controls (**Chapter 3**), the reduction is likely driven by baseline differences and when corrected to baseline patients with TS show an increased ramping of motor excitability during motor execution. In fact, patients' tic severity was inversely related to the modulation of

motor excitability whereby those with the most severe tics were least able to increase excitability. Patients showed largely the same patterns of change in excitability during motor preparation and response inhibition. However, the extent to which patients could modulate excitability during motor preparation was related to phonic tic severity whereby those with the least severe tics had higher excitability change from baseline. In addition, those that were able to suppress motor excitability to a greater extent whilst inhibiting action had the least severe tics, likely engaging inhibitory mechanisms to a greater extent with the consequence of slower response times during the task. I conclude that the ability to modulate motor excitability is both related to pathology and adaptive compensatory mechanisms that may help in tic suppression.

The second aim of this thesis was to explore effective connectivity, excitatory and inhibitory physiological mechanisms and the neurochemistry of PMC in young healthy adults. Subsequent experiments in **Chapters 5** and **6** used various transcranial magnetic stimulation (TMS) techniques and proton magnetic resonance spectroscopy (^{1}H-MRS) to investigate these issues. **Chapter 5** explored interhemispheric facilitation and inhibition (IHI and IHF) in two directions between bilateral PMC. The results provided evidence for an asymmetry of interhemispheric interactions using dual site TMS (ds-TMS) whereby the left-to-right direction is more inhibitory than right-to-left. Furthermore, females appeared to show greater interhemispheric modulation than males and whilst there was robust evidence for IHI (in the left-to-right direction) IHF appeared to not be robust.

Finally, **Chapter 6** explored how TMS-induced measures of excitation and inhibition related to ^{1}H-MRS measures of neurochemicals γ-aminobutyric acid (GABA), glutamate (Glu) and glutamine (Gln). GABA is the primary inhibitory

neurotransmitter in the human brain and is critical for the regulation of neuronal excitability and the orchestration of neuronal networks and is critically important in neurodevelopmental disorders such as TS. GABA was not found to be related to measures of synaptic neurotransmission as assessed by TMS and neither was Gln. In contrast, Glu was found to be related to a hub of TMS measures, in particular, Glu was positively related to both intracortical facilitation (ICF) and long intracortical inhibition (LICI). **Chapters 5** and **6** further uncovered relationships between ds-TMS, pp-TMS and ^{1}H-MRS showing that these various measures likely have overlapping mechanisms.

The final chapters extend our knowledge about the PMC and the methodologies used to assess its state. **Chapter 5** extends our understanding of the communication between right and left PMC and highlights a normal asymmetry in communication. This is important for understanding neurodevelopmental disorders such as TS of which asymmetry in effective connectivity and brain volume have been implicated. **Chapter 6** importantly shows that ^{1}H-MRS measured GABA is likely irrelevant for assessing synaptic neurotransmission and thus its interpretations should be limited to non-synaptic levels of GABA. This is particularly important for TS research in which both changes in $GABA_A$ receptor activity is present in the PMC and abnormalities in GABA concentration have been shown.

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Division of Work and Publications

The work detailed in **Chapter 6** was carried out equally between the author of this thesis and Katherine Dyke. The author collected all the ^{1}H-MRS data and Katherine Dyke collected all the pp-TMS measures.

Some of the results presented in this thesis have been published in the following papers:

Draper, A., Stephenson, M. C., Jackson, G. M., **Pépés, S. E.**, Morgan, P. S., Morris, P. G., & Jackson, S. R. (2014). Increased GABA contributes to enhanced control over motor excitability in Tourette syndrome. *Current Biology*, *24*(19), 2343–2347. http://doi.org/10.1016/j.cub.2014.08.038

Dyke, K., **Pépés, S. E.**, Chen, C., Kim, S., Sigurdsson, H. P., Draper, A., … Jackson, S. R. (2017). Comparing GABA-dependent physiological measures of inhibition with proton magnetic resonance spectroscopy measurement of GABA using ultra-high-field MRI. *NeuroImage*, *152*, 360–370. http://doi.org/10.1016/j.neuroimage.2017.03.011

Jackson, G. M., Draper, A., Dyke, K., **Pépés, S. E.**, & Jackson, S. R. (2015). Inhibition, Disinhibition, and the Control of Action in Tourette Syndrome. *Trends in Cognitive Sciences*. http://doi.org/10.1016/j.tics.2015.08.006

Pépés, S. E., Draper, A., Jackson, G. M., & Jackson, S. R. (2016). Effects of age on motor excitability measures from children and adolescents with Tourette syndrome. *Developmental Cognitive Neuroscience*, *19*, 78–86. http://doi.org/10.1016/j.dcn.2016.02.005

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^{1}H-MRS	Proton magnetic resonance spectroscopy
ACC	Anterior cingulate cortex
AD	Axial diffusivity
ADHD	Attention deficit hyperactivity disorder
ADM	Abductor digitor minimi
AMT	Active motor threshold
ASD	Autism spectrum disorder
BF_{10}	Bayes factor 10
BOLD	Blood-oxygen-level dependent
bpRT	Button press response time
CC	Corpus callosum
CoV	Coefficient of variation
CP	Conditioning stimulus
CRLB	Cramer-Rao Lower Bound
CS	Control subjects
CSE	Cortico-spinal excitability
CSP	Cortical silent period
CSTC	Cortical-striatal-thalamo-cortical
DBS	Deep brain stimulation
DLPFC	Dorsolateral prefrontal cortex
ds-TMS	Dual site TMS
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DTI	Diffusion tensor imaging
EMG	Electromyography
emgRT	Electromyography defined response time
FA	Fractional anisotropy
FDI	First dorsal interosseous
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
GABA	γ-aminobutyric acid
Gln	Glutamine
Glu	Glutamate
Glx	Glutamate + Glutamine composite
GWAS	Genome-wide association study
ICF	Intracortical facilitation
IHF	Interhemispheric facilitation

IHI	Interhemispheric inhibition
IO Curve	Recruitment curve
ISI	Inter-stimulus interval
ITI	Inter-trial interval
LICI	Long intracortical inhibition
MEP	Motor evoked potential
MP-RAGE	Magnetization-Prepared Rapid Gradient-Echo
MRI	Magnetic resonance imaging
MT	Motor threshold
MSO%	Maximum stimulator output
Naa	N-acetyl aspartate
NMDA	N-methyl-d-aspartic acid
NA	Noradrenaline
NRXN1	Neurexin 1
OCD	Obsessive compulsive disorder
OFC	Orbitofrontal cortex
ODD	Oppositional defiant disorder
PFC	Prefrontal cortex
PMC	Primary motor cortex
pp-TMS	Paired pulse transcranial magnetic stimulation
RMT	Resting motor threshold
rTMS	Repetitive transcranial magnetic stimulation
SD	Standard deviation
SICI	Short intracortical inhibition
SMA	Supplementary motor area
SNR	Signal to noise ratio
sp-TMS	Single pulse transcranial magnetic stimulation
SVM	Support vector machine
TMS	Transcranial magnetic stimulation
TP	Test stimulus
TS	Tourette syndrome
VOI	Volume of interest
YGTSS	Yale Global Tic Severity Scale

Chapter 1: General Background and Thesis Overview

1.1 Thesis Overview

The aims within this thesis are two-fold: to explore the functional state of the primary motor cortex (PMC), characterised by motor excitability and its neurochemistry, and its role in both the pathology and cognitive control of action in Tourette syndrome.

In **Chapter 3** and **4**, I examined core measures of motor excitability in young people with TS using transcranial magnetic stimulation (TMS), namely: resting motor threshold (RMT), recruitment (IO) curves, motor-evoked potential (MEP) variability and motor excitability during two tasks that require cognitive control of action (volitional movement and response inhibition). I was particularly interested in how these measures related to neurodevelopment (how they related to age) and whether they were mediated by tic severity in order to explore the interplay of TS pathology and compensatory mechanisms that may be of benefit to tic suppression and tic remission. TMS measures in adults with TS have shown that in comparison to healthy controls, they have a reduced gain in motor excitability characterised by shallow recruitment curves (Orth, Münchau, & Rothwell, 2008) and reduced intracortical inhibition (Gilbert et al., 2004; K. F. Heise et al., 2010; Orth, Amann, Robertson, & Rothwell, 2005; Ziemann, Paulus, & Rothenberger, 1997). During the execution of a volitional movement both children and adults show a reduction in gain in excitability (Draper, Jude, Jackson, & Jackson, 2015; C. A. Heise et al., 2008; K. F. Heise et al., 2010) and normalisation of intracortical inhibition (only adults: [K. F. Heise et al., 2010]). However, no experiments to date have been done to explore how these measures change over development despite the evidence that suggests

that neural networks in TS are functionally immature in children and adults (Church et al., 2008; Worbe et al., 2012). Importantly, given that TS is a disorder of neurodevelopment one may hypothesise that there is a differential interaction of motor output, which may be important for tic genesis and/or suppression, with age. In **Chapter 3**, I demonstrate that, consistent with previous studies of adult TS patients, children and adolescents with TS exhibit reduced gain in motor excitability when indexed by TMS IO curves and ahead of volitional movements. However, and in direct contrast to studies of adult patients, I show that: RMT is significantly different (higher) in children and adolescents with TS compared to age-matched controls; that differences in RMT vary with age and are most pronounced in the youngest individuals and absent in young adults (18 years or older); that TMS-induced MEP responses are more variable in children and adolescents with TS relative to controls.

In **Chapter 4** I used a two-cued Go/ No-Go task that disassociated motor preparation from motor execution in order to explore whether this reduction of gain was specific to the execution of a volitional movement or whether the motor excitability during motor preparation was related to tic severity. I tested motor excitability of the left PMC but the task involved preparing both left and right movements, which allowed me to explore MEP suppression in a group of healthy control and patients with TS and determine whether patients with TS were as effective at 'stopping' as healthy controls. Whilst experiments have generally shown undisturbed or enhanced cognitive control of action in simple inhibitory control tasks the motor excitability related to this has not been explicitly tested in TS despite evidence for MEP suppression (Hoshiyama et al., 1996; Pascual-Leone, 2000) that is related to thalamo-cortical pathways modulating $GABA_A$ receptor activity (Stinear, Coxon, & Byblow, 2009). In this experiment, I found that patients with TS showed increased gain during the execution of volitional movements compared to age and

sex-matched controls. Patients with the biggest increases in gain had the least severe tics supporting the concept that those with more severe tics are least able to modulate their motor excitability (Draper et al., 2015).

Whilst I did not find evidence for the modulation of motor excitability during motor preparation, patients that showed the largest increases in motor excitability compared to baseline were those with the most reduced phonic tic severity scores. In addition, both patients and healthy controls showed evidence of MEP suppression during motor preparation for the non-selected effector and during No-Go trials. Interestingly, the patients that exerted less suppression of MEPs had more severe motor tics; suggesting that No-Go suppression may be related to a core reduction in $GABA_A$ receptor activity and disinhibited thalamocortical pathways. Finally, whilst RTs in healthy controls were inversely related to increased excitability during motor execution; in patients RTs were inversely related to motor excitability during No-Gos suggesting that increased RTs were a consequence to increased GABAergic action.

The second aim of this thesis was to explore other methodologies assessing a) the effective connectivity of the primary motor cortices and b) the neurochemical constituents of the PMC and how they relate to physiological measures of excitation and inhibition in young healthy adults. In **Chapter 5** I explored effective connectivity using ds-TMS. I was specifically interested in whether both facilitation and inhibition can be evoked by ds-TMS from one PMC to the other, transcallosally; whether there was symmetry between left-to-right and right-to-left stimulation and effects of sex. I tested a range of conditioning stimuli (50, 70, 100 and 110% RMT) and inter-stimulus intervals (ISI [6,8,10,12ms]) in the left-to-right and right-to-left direction in the same session. I sadly did not find robust evidence for interhemispheric facilitation (IHF), however, some evidence for effective IHI. IHI was only significant unidirectionallly: I

found that left-to-right interhemispheric influences were more inhibitory than right-to-left. When assessed using slopes fitted for each inter-stimulus interval (ISI) to the effect of each CP I found that females had a significantly different interaction in each direction, however, slopes appeared to be fairly similar for each ISI bidirectionally in males.

In **Chapter 6** I explored the use of proton magnetic resonance spectroscopy (^{1}H-MRS) to measure neurochemicals alongside TMS measures of synaptic function (using paired pulse TMS [pp-TMS] and single pulse TMS [sp-TMS, e.g. RMT and IO curves]). ^{1}H-MRS is a tool that has gained popularity in recent years for exploring abnormalities of neurochemicals within specific regions of the brain in neuropsychiatric and neurodevelopmental disorders. In TS research there is now a great drive to quantify neurochemicals within striatal, thalamic and cortical regions in order to greater our understanding of the neurobiology of TS. Whilst ^{1}H-MRS measures total concentration of neurochemicals (e.g. GABA, glutamate [Glu] and glutamine [Gln]) some researchers interpret GABA concentration, for instance, as related to phasic $GABA_A$ receptor action (Puts et al., 2017; Tinaz et al., 2014). The findings from this chapter demonstrate ^{1}H-MRS measurements of GABA are seemingly unrelated to physiological measures of inhibition (and excitation) as measured by TMS; importantly the GABA signal is not neatly associated to short intracortical inhibition (SICI) which measures $GABA_A$ receptor action. Glu on the other hand was found to be related to several TMS measures and was most significantly predicted by long interval intracortical inhibition (LICI) and intracortical facilitation (ICF) but was also significantly related to IO plateau (maximum MEP) and marginally related to MEP variability at 100% RMT. There were no statistically significant relationships between Gln and TMS measures.

I further explored the relationship of TMS measures to each other and ^{1}H-MRS measured neurochemicals to each other (given that they are tightly linked). I found several important findings, namely: RMT is inversely related to SICI (3ms), TP MEP amplitude is negatively related to ICF and LICI, SICI (1ms) and SICI (3ms) are positively correlated, the slope fitted to SICI (1ms) is negatively related to ICF, ICF using 10ms and 12ms are positively related, IO plateau and IO slope are negatively associated with LICI. Thus, demonstrating that various pp-TMS measures largely measure overlapping neural mechanisms. Finally I found that Glu and Naa were positively associated and GABA and Gln were negatively associated.

Prior to the experimental chapters I will first give an introduction to Tourette syndrome and give an overview of the current state of research regarding pathology, tic generation and tic suppression (**Chapter 1**). I will then give an introduction to the two main methods I have used throughout the thesis in **Chapter 2**, namely: TMS and ^{1}H-MRS and how they have been used in TS research.

1.2 Tourette syndrome

1.2.1 What is Tourette syndrome?

Tourette syndrome (TS) is a neurodevelopmental disorder that is characterized by the occurrence of tics with a worldwide prevalence in children and adolescents of approximately 1% (Robertson, 2008). Tics are involuntary, repetitive and stereotyped behaviour, which occur frequently throughout the day (Leckman, 2002). They tend to occur in bouts, and wax and wane over the course of the day. Whilst tics differ in nature across patients, they can be broadly categorized into motor or phonic and simple or complex tics. Simple tics are rapid movements that are seemingly purposeless in nature; examples include jerking of the head, inappropriate eye blinking, sniffing and grunting. Complex tics on the other hand tend to be inappropriately timed seemingly purposeful behaviours including touching, biting, echolalia (repeating words heard) and in around 10% of patients coprolalia (obscene utterances). The diagnosis criteria for TS according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), requires patients to have experienced tics (starting in childhood) for at least one year with two or more motor tics and at least one phonic tic.

The experience of a tic for a patient with TS can range dependent on the tic; typically, patients may not be aware of simple tics such as eye blinking whilst other tics may be preceded by a sensation known as premonitory sensory phenomenon (PSP). Sometimes tics can feel like a response to the PSP, a tic can be considered semi-volitional where patients feel they act the tic to relieve the discomfort from the PSP. Awareness of PSP occurs in around 92% of adults with TS (Kwak, Dat Vuong, & Jankovic, 2003) whereas only 37% of children are aware of a premonitory sensation preceding their tics (24% in 8-10 year olds increasing up to 57% of 15-19

year olds) (Banaschewski, Woerner, & Rothenberger, 2003). One theory proposes that given the proportion of patients that experience PSP rises with age, PSP may result from increased attention given to the somatic signals that immediately precede a tic (Ganos et al., 2012). These signals can be considered similar to the urge that occurs prior to other biological phenomenon such as a sneeze or micturition (S. R. Jackson, Parkinson, Kim, Schüermann, & Eickhoff, 2011). Like PSP, those with TS are usually able to suppress their tics for short periods of time with increasing ability throughout childhood (48% of children ages 8-10 can suppress tics whilst 79% of those aged 15-19% can [Banaschewski & Rothenberger, 2003]). However, some find this particularly uncomfortable and stressful; anecdotally some believe that an increased bout of tics can occur after tic suppression as a rebound to their efforts. However, no evidence of such a rebound effect exists (e.g. [Meidinger, 2005]).

TS follows a specific developmental time course in which tic onset usually begins between the ages of 3-8 (Leckman, 2002) and the peak of the disorder tends to occur around the ages of 10-12 (Leckman et al., 1998). Tic severity considerably decreases by the age of 19-20 (Leckman et al., 1998) where only 19% of those with TS continue to have the moderate-severe tics in adulthood (M. H. Bloch et al., 2006). Importantly, patients with TS do not tend to grow out of tics entirely. However, in adulthood tics tend not to be debilitating and the numbers of those still taking medication are hugely reduced (Pappert, Goetz, Louis, Blasucci, & Leurgans, 2003). In one small study (n = 36), 90% of adults that had a diagnosis of TS given when they were a child were shown to still have some evidence of tics in adulthood (Pappert et al., 2003). In fact, out of the 20% that considered themselves to be tic free, only 50% had no tics (Pappert et al., 2003).

There are a few cardinal features of TS asides from tics. There is a sex bias of about 5.2:1 in childhood where the majority of affected individuals are male; in adulthood the bias is reduced to around 3.1:1 (Freeman et al., 2000). In addition, patients with TS rarely have just the one diagnosis. They are highly likely to suffer from aetiologically related disorders such as attention deficit hyperactivity disorder (ADHD), obsessive compulsive behaviours and obsessive compulsive disorder (OCD) where there is 60%, 27% and 32% prevalence in individuals with TS respectively (Freeman et al., 2000).

1.2.2 Current Treatment

Current treatment for tics in TS comprises of two branches: pharmacological and behavioural. The recommended first line of treatment for children with mild and infrequent tics is behavioural treatment (Verdellen, van de Griendt, Hartmann, & Murphy, 2011). Two commonly used therapies include habit reversal therapy ([HRT], sometimes delivered with behavioural therapy in 'comprehensive behavioural intervention for tics') or exposure and response prevention therapy (ERPT). The context for behavioural therapy for tics is considering a tic as an overlearned habit where a stimulus (PSP) predicts the response (the tic) and develops ways reducing the contingency between the PSP and tic (Haselgrove & Hogarth, 2013). HRT tries to achieve this by teaching a competing response to use in response to the urge to tic (Azrin & Peterson, 1988). A competing response is a response that is incompatible with the tic, e.g subtle movement using antagonistic muscles. For instance, for a tic such as wrinkling the nose a competing response would be to depress the upper lip (Azrin & Peterson, 1990).

The alternative, ERPT, has shown to be equally effective (Verdellen, Keijsers, Cath, & Hoogduin, 2004) and essentially teaches patients to habituate to the PSP.

Through exposure and prevention of the tic, a reduction of the severity of PSP is achieved which thus reduces the occurrence or necessity of the tic (Verdellen et al., 2008). In a recent study, tic frequency at home was reduced by at least 30% in 89% of patients treated with ERPT and in 72% patients treated with HRT (Verdellen et al., 2004).

Whilst behavioural therapy has been shown to reduce tic occurrence to a large extent, only 25.9% of patients in a recent survey had received behavioural therapy; this is in contrast to 54.7% of whom had taken medication for their tics (Hollis et al., 2016). Commonly used medication to treat tics are α_2 adrenergic agonists (clonidine and guanfacine), atypical dopamine antagonists (risperidone and aripiprazole), typical dopamine antagonists (haloperidol and pimozide), $GABA_A$ receptor agonist clonazepam and $GABA_B$ receptor agonist baclofen. Young patients with TS have rated aripiprazole as the most useful medication for treating their more severe tics with the least affective side effects (Hollis et al., 2016). Sadly, the pharmacological treatments employed in TS are only used to ameliorate tic symptoms and not cure them (Roessner, Plessen, et al., 2011).

In rare treatment-refractory cases of TS in adults with severe harmful tics (‘malignant’ tics), a surgical intervention called deep brain stimulation (DBS) has been used. DBS targets specific structures within the basal ganglia: for instance the globus pallidus interna, thalamus and the nucleus accumbens (Baldermann et al., 2016). In a recent systematic review, in 57 eligible studies DBS resulted in a significant improvement of 52.68% in Yale Global Tic Severity Scale (YGTSS) scores (Baldermann et al., 2016). The thalamus appears to be the target of preference with 78 of the 156 cases using thalamic stimulation, however, there appeared to be no

significant difference between any of the targets used. I will discuss the role of the basal ganglia and thalamus in TS in **1.3.2**.

Of the treatments discussed, only behavioural therapy carries no side effects. The most commonly observed side effects in tic medication are sedation and weight gain (Waldon, Hill, Termine, Balottin, & Cavanna, 2013), whilst other problematic perceived side effects include a differing sense of self (Hollis et al., 2016). In DBS there are of course more adverse events due to the invasive nature of neurosurgery including transient visual symptoms, low mood, dysarthria, apathy and weight gain (Baldermann et al., 2016). Furthermore, whilst there are ethical considerations for using DBS in young patients with TS (due to the numerous risks involved in neurosurgery, limited evidence base and nature of TS which means symptoms may ameliorate in adulthood) there is some evidence that it works better in younger patients (Baldermann et al., 2016). There is thus, unsurprisingly, a drive for developing non-invasive treatments for young individuals with TS with no or limited side effects.

Research into other brain stimulation techniques that target the cortex (and thus can be non-invasive and applied outside the scalp) is popular and repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) have both been used as a therapeutic intervention with the aim of reducing tics. This could be helpful, perhaps, as an adjunctive to behavioural therapy. By far, the most popular site for non-invasive brain stimulation in TS is the supplementary motor area (SMA) (Hegde, Bose, Kalmady, & Reddy, 2015; Kahl et al., 2017; Kwon et al., 2011; Landeros-Weisenberger et al., 2015; Le, Liu, Sun, Hu, & Xiao, 2013; Mantovani et al., 2006; Wu et al., 2014). I will come back to the role of the SMA in TS in **1.4**, nevertheless, stimulating this region using inhibitory electromagnetic or

electrical stimulation seems promising in reducing tics. Sadly, there is a shortage of placebo (sham) controlled trials. In the two available, active is seemingly no effective than placebo (Landeros-Weisenberger et al., 2015; Wu et al., 2014). However, this could be due to the chosen stimulation parameters and amount of stimulation. SMA rTMS has been shown to reduce blood-oxygen-level dependent (BOLD) signal in motor networks moreso in the active group versus placebo (Wu et al., 2014), which suggests some promise for the treatment.

1.2.3 Genetics

Whilst it is clear that TS is heritable, there is no simple candidate gene for the expression of TS. Rather, TS has a highly polygenic pattern of heritability (Scharf et al., 2013). In a recent review, the population-heritability estimate of TS claimed to be 77% (where 10% is low heritability and 90% is high heritability) (Robertson et al., 2017). A highly polygenic disorder indicates that many genetic variations dispersed throughout the genome may each contribute to the overall risk of developing TS. The first risk loci that have surpassed genome-wide significance thresholds for TS have been identified as deletions in Neurexin 1 (NRXN1) and duplications in CNTN6 that explain TS in 1% of sufferers (Huang et al., 2017). Whilst other genetic variations have been found that may relate to an increased risk for TS, for example SLITRK1 (Abelson et al., 2005; Deng, Le, Xie, & Jankovic, 2006; Miranda et al., 2009; Zimprich et al., 2008) and CNTNAP2 (Verkerk et al., 2003; Wright et al., 2006), these have not survived GWAS (genome-wide association study) significance level, and their involvement remain controversial.

Neurexins are synaptic adhesion molecules, located at presynapses. They communicate to neuroligins on the postsynapse to stimulate synaptogenesis and synapse maturation (Cao & Tabuchi, 2017). Just slight alterations to neurexin

function can cause huge disruption to signalling within neural networks (Südhof, 2008); it is thus unsurprising that NRXN1 has been implicated in both TS and Autism spectrum disorder (ASD) (Cao & Tabuchi, 2017). Furthermore, in a study explored the clustering ability of NRXN-1β on neurotransmitter receptors it was observed that NRXN1 is able to induce the formation of $GABA_A$ and n-methyl-d-aspartic acid (NMDA) receptors (Graf, Zhang, Jin, Linhoff, & Craig, 2004). NRXN1 thus has an important role in neurodevelopment and is important for the balance of inhibition and excitation from GABAergic and glutamatergic neurotransmission; which, as I will explain later in this chapter, appears to be very important in TS. Like NRXN1, CNTNAP2 is a candidate gene for ASD (Kourtian et al., 2017) and is important in neuronal migration and myelination of axons (Peñagarikano et al., 2011).

Finally, de novo coding variants have been explored in a sample of 325 disorder trios (i.e. 325 trios including both parents and affected child). The advantages of looking at de novo coding variants are that they are extremely rare and thus when found have a large signal to noise ratio (Willsey et al., 2017). They estimated that de novo damage (i.e. damage that occurs to the gene after conception) in 400 genes are risk factors for around 12% of patients with TS and they found 4 risk genes for TS (in unrelated families) including WWC1, CELSR3, NIPBL and FN1. All four genes are brain-expressed and WWC1 (the gene with the highest association for TS) is known to have an important role in regulating AMPA receptors (a type of glutamate receptor) and contributes to the regulation of synaptic plasticity (Cacchio et al., 2009).

These results overall suggest that genetic deviants in TS may result in suboptimal brain development in infancy which result in tics, associated behaviours and comorbidities with TS. Whilst the research is far from comprehensive at this

stage, exploring the genetics of TS may later help disentangle the biological underpinnings of TS.

1.3 Neurobiological Basis for TS

1.3.1 Abnormalities of neurotransmission – evidence from postmortem studies and animal studies

The pathophysiology for TS is complex and there is no specific substrate that has been confirmed as the cause for tic behaviours. There is, however, a range of evidence that suggests that dysfunctional corticostriato-thalamo-cortical (CSTC) pathways are involved in the presentation of TS: namely from postmortem, animal, genetic and neuroimaging studies. Furthermore, various neurotransmitters or neuromodulators have been implicated in the pathological mechanisms for tics: for instance, dopamine, γ-amino butyric acid (GABA), glutamate, histamine, acetylcholine, serotonin and noradrenaline (Robertson et al., 2017; Singer, 2005).

1.3.1.1 Dopamine

The dopamine hypothesis for TS results from the efficacy of dopamine antagonists on tic symptoms. Regulated dopaminergic innervation is essential to normal basal ganglia function and it has been suggested that tic behaviours result from a dysfunctional dopaminergic system (Buse, Schoenefeld, Münchau, & Roessner, 2013). Postmortem studies have shown increased dopaminergic uptake sites in the caudate and putamen (Singer, Hahn, & Moran, 1991), increases of dopamine transporters, dopamine (D1,D2) receptors and α_2-adrenergic receptors in the PMC, premotor cortex, dorsolateral prefrontal cortex (DLPFC) and lateral orbitofrontal cortex (OFC) (Minzer, Lee, Hong, & Singer, 2004; Yoon, Gause, Leckman, & Singer, 2007). In fact, it has been suggested that dopaminergic

abnormalities were more marked in the DLPFC than in the ventral striatum, caudate and putamen (Minzer et al., 2004).

Whilst it is appealing to interpret this research as TS being the result of abnormal dopaminergic transmission within the brain, it is important to keep in mind these studies were each only conducted in 3 paired brains (one TS and one matched control). Deaths may have been complex and neuroleptics were likely to have been taken within their life. It is far more likely that a range of neurotransmitters and genes important for neurodevelopment play a part in the development of TS (Nespoli, Rizzo, Boeckers, Hengerer, & Ludolph, 2016; Udvardi, Nespoli, Rizzo, Hengerer, & Ludolph, 2013). Dopamine, glutamate ([Glu], the major excitatory neurotransmitter of the brain) and GABA (the major inhibitory neurotransmitter of the brain) interact closely within the CSTC pathways and an imbalance of excitation and inhibition may result in disinhibition of the pathways, resulting in the onset of tics (G. M. Jackson, Draper, Dyke, Pépés, & Jackson, 2015).

1.3.1.2 GABA

Several postmortem and animal studies support the concept of altered GABAergic function in TS and are especially convincing. In the caudate and putamen, fast-spiking parvalbumin positive (PV+) interneurons have been found to be significantly reduced in the brains of patients that were afflicted with TS (Kalanithi et al., 2005; Kataoka et al., 2010). PV+ (a globular protein that binds to calcium) interneurons are GABAergic (Cowan, Wilson, Emson, & Heizmann, 1990) and this evidence could be taken to reflect a reduction in the inhibitory signalling within the striatum leading to disinhibition throughout the pathway.

Perhaps the most compelling evidence, however, is the evidence from animal models in non-human primates and rats. Microinjections of $GABA_A$ antagonist bicuculline or picrotoxin have been delivered to the posterior putamen and dorsal anterior striatum and by targeting different spatial regions, tic-like or compulsive behaviours are expressed (Bronfeld, Yael, Belelovsky, & Bar-Gad, 2013; McCairn, Bronfeld, Belelovsky, & Bar-Gad, 2009; McCairn, Iriki, & Isoda, 2013; Worbe et al., 2009, 2013). The different spatial locations tap into different neural circuits that persevere from the striatum to the cortex via the thalamus. Simple motor tics resulted from 60% of bicuculline injections in the striatum whilst saline injections had no effect. Importantly, 0% of injections outside of striatum resulted in motor tics (McCairn et al., 2009).

The evidence does not stop here, there is a range of other evidence that suggest altered GABAergic transmission in TS. However, the evidence is from transcranial magnetic stimulation (TMS) and imaging studies (e.g. using magnetic resonance spectroscopy [MRS]). I will talk more about these in **Chapter 2.**

1.3.2 Neuroimaging in TS

1.3.2.1 The Basal Ganglia

Neuroimaging research in TS is dispersed with studies with small sample sizes and inadequate control for motion. The most recurring imaging finding that has been found to date is reduced caudate volume in patients with TS (M. H. Bloch, Leckman, Zhu, & Peterson, 2005; Hyde et al., 1995; Peterson et al., 2003). Early evidence comprises of poorer quality images; however, a large volumetric imaging study in 127 patients with TS (both adults and children) has been conducted (Peterson et al., 2003). Key findings from this study are as follows: caudate volume is reduced in adults with TS, children with TS have smaller caudate volumes compared

to the lenticular nucleus, patients taking typical neuroleptics show increased caudate volume (in comparison to patients with TS and healthy controls) and finally smaller lenticular nucleus volumes were significantly associated with adults with TS and those with comorbid OCD. Importantly, caudate volume measured at childhood has been shown to predict symptom severity in adulthood (M. H. Bloch et al., 2005). Whilst it has been argued that caudate does not relate to tic severity at time of scanning, it is of note that the relationship between tic severity and caudate volume at time of scanning (in childhood) was at least of trend value ($p = 0.08$). In another paper using the same cohort the authors report a significant positive relationship between tic severity at time point 1 (childhood) and time point 2 (adulthood) (M. H. Bloch et al., 2006). It is not incomprehensible that caudate volume simply relates to tic severity.

Both studies above use the same cohort and imaging data and thus approached motion artefact in the same way. Motion artefact severity was rated blindly on a 6-point ordinal scale and they reported that the artefact was found to be no different between the scans included for analysis for the TS and control group. However, only the scans of the poorest quality were excluded from analysis: out of 0-5 (no motion to severe motion) only scores of 4 and 5 were excluded. This is highly problematic given the results of a recently published study looking at the effect of motion artefact on volumetric imaging (Pardoe, Kucharsky Hiess, & Kuzniecky, 2016). The take home message of their study is that qualitative exclusion of scans based on visual motion artefact is inadequate; in fact, they found that grey matter volume in regions including caudate nuclei and thalamus were inversely related to motion (increased motion was related to lower grey matter volume). This was only not the case if a strict exclusion criteria was taken allowing only scans with a quality rating equivalent to 0 in Peterson et al.'s (2003) paper. This raises some doubt on the

findings of reduced caudate volume in TS. Given that tic severity negatively correlates with caudate volume, an alternative hypothesis would be that caudate volume reductions are a result of increased motion inside the scanner. A need for a robust index of motion that can be used as a covariate in imaging studies or prospective motion correction that is immune to tics is necessary for further research (Pardoe et al., 2016). In fact, when a stricter motion exclusion criteria was appropriated (assumed from the large exclusion rate due to visible motion artefact, minimum 27.25%) reduced caudate volume was not found in a large sample (109) of children with TS (Greene, Williams, Koller, Schlaggar, & Black, 2016).

Other structural abnormalities that have been found in TS include increased grey matter in the midbrain in a study of 31 adults with TS (Garraux et al., 2006), increased grey matter volumes in bilateral ventral putamen and decreased grey matter in left hippocampal gyrus in a study of young males with TS (Ludolph et al., 2003). Greene et al. (2016) further found reduced white matter in regions in the putamen and insula in children with TS relative to healthy children. Whilst the authors did not find decreases in grey matter in any regions (which would be consistent with motion artefact) they did find increases in grey matter, for instance in the thalamus which is consistent with another paediatric TS study (Lee et al., 2006) but contradictory to an adult study (L. Wang et al., 2007). There have been at least two studies in children, however, that have shown no differences in volume or shape of any of the basal nuclei (Roessner et al., 2009; Williams et al., 2013).

Supporting evidence for dysfunction of the basal ganglia comes from a range of perfusion/ metabolic imaging studies conducted in the 1990s using positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Reduced perfusion and metabolism has been found in the basal ganglia,

with most studies pointing to reduced flow and metabolism in the caudate and thalamus (Braun et al., 1993; Eidelberg et al., 1997; Klieger, Fett, Dimitsopulos, & Kurlan, 1997; Moriarty et al., 1995). A more recent SPECT study that investigated the effects of DBS on perfusion in the brain (Haense et al., 2016), explored metabolism in the basal ganglia prior to surgery. Whilst its sample size is very small and unrepresentative of the general TS community (5 adults – 3 women and 2 men), the experimenters eliminated motion errors by using an anaesthetic during scanning. The anaesthetic in this case was given after the tracer and thus did not affect perfusion results. In a scan prior to neurosurgery they found reduced perfusion in somatosensory cortex, motor cortex, premotor, SMA, DLPFC, parietal lobe and increased perfusion in the cerebellum. This certainly does not replicate previous results finding reduced perfusion or metabolism in the basal ganglia.

There is a great discrepancy between neuroimaging studies, which is not entirely surprising due to the heterogeneity of TS, medication taken, other therapies, comorbidities, amount of motion in the scanner, different field strengths used, different scanners and centres, different imaging parameters used, different sample sizes and different cohorts used (adults or children, males and females or males). Larger well-controlled studies, using high resolution 7 Tesla magnetic resonance imaging (MRI) that take into account the issues with imaging children and patients with movement disorders are needed in order to create an image of consensus involving neuroimaging with TS.

1.3.2.2 Cortical Structures & Cerebellum

Imaging findings related to TS are not restricted to subcortical structures. As I have already detailed, the CSTC circuitry is the nearest researchers have come to a neurobiological substrate for TS; the basal ganglia are part of a network that leads

from the striatum, via the thalamus to the cortex. Cortical structures have thus been implicated in TS. Various methods have been utilised to look at this: methods to look at the morphometry of these structures, cortical thickness analysis and imaging techniques used to assess the white matter microstructure (diffusion tensor imaging [DTI]).

Imaging studies in both children and adults with TS show in general: cortical thinning and reduced grey matter volume of the sensorimotor and prefrontal cortices in comparison the healthy controls (Draganski et al., 2010; Draper et al., 2014; Muellner et al., 2015; Sowell et al., 2008; Tisdall et al., 2016; Worbe et al., 2010). Whilst these regions are often implicated within the neuroimaging literature as abnormal in TS, the breadth of cortical structures identified are not consistent across papers; for instance only inferior frontal gyrus thinning was found in one paper (Tisdall et al., 2016), which also had one of the largest percentage of exclusion rates for removing imaging data due to motion artefacts (19.7%). Whilst thinning of prefrontal cortex (PFC) has been found, hypertrophy of frontal areas has also been found in children with TS (Peterson et al., 2001). In addition, increases in white matter in the right frontal lobe (Fredericksen et al., 2002) and decreases in white matter have been found in the left frontal lobe (Kates et al., 2002). I cannot easily construe these findings but I will suggest that more work needs to be done in order to clarify the role of the cortex in the pathology of TS. Whilst the most consistent findings seem to be related to widespread cortical thinning, particular of frontal and sensorimotor areas, we should be cautious of this result given that widespread cortical thinning, across the whole cortex, has been shown to be related to increased movement in the scanner (Pardoe et al., 2016); except in the occipital cortex and some regions in the somatosensory strip where thickness is increased.

Before I move on to discussing connectivity in the brain, the cerebellum has gained some interest at being involved in the pathophysiology of TS, due to its role in action and language. A few studies have implicated the cerebellum as having a pathophysiological role in TS. The cerebellum has been shown to have an increased metabolic state in unmedicated adults with TS compared to controls (Pourfar et al., 2011) and two studies have shown reduced grey matter in cerebellum: one particularly large study (n = 163) of adults with TS (Tobe et al., 2010) and one moderately small study (n = 19) of male children with TS (Hong et al., 2002).

1.3.2.3 The Corpus Callosum

A range of evidence has revealed differences in connectivity across the corpus callosum (CC) in patients with TS, particularly in the transcallosal motor regions. These specific findings however, are far from unequivocal and appear to be different for adult and children populations. Early imaging studies measured structural, two-dimensional qualities of the CC from T1 anatomical images of the brain. Measurements have been taken from the CC to quantify its size, i.e. two-dimensional area (from a mid-sagittal slice), length of the center line, circumference, bending angle, and curvature, including measures from sub-regions within the CC. Peterson et al. (1993) conducted one of the first studies that focused on the CC in TS. He conducted a small study and concluded that the CC was smaller in a group of adults with TS (14 subjects) than controls in. They found relative to controls adults with TS had a reduced area (17.7%), circumference (10%), center line length (5.3%) with an increased bending angle (by 5%) and increased mean curvature (11%). The group looked at 5 subregions within the CC and found that all were equally reduced. Various measures were positively correlated with sub-cortical regional volumes: the globus pallidus, putamen, caudate, lenticular nucleus, striatum and entire basal ganglia. The reverse has been found in children and adolescents in a study using a

similar method (Baumgardner et al., 1996); those with TS had significantly increased areas in 4 out of 5 subregions of the CC and an increased circumference, length and bending angle.

In a large morphometric study conducted by Plessen et al. (2004) in 158 TS subjects and 121 controls they found that there was a strong interaction of diagnosis with age in relation to the CC area. However, their findings were the reverse of the earlier studies: adults (n = 45) had a larger mean CC area and children (n = 66) had a smaller area. Interestingly, there was an age-by-sex interaction that was a strong predictor of CC size where there was a steeper negative slope with age and CC area in the female group. Whilst this final finding further conflicted an earlier study (Mostofsky, Wendlandt, Cutting, Denckla, & Singer, 1999) that found females with TS did not show any change in CC characteristics, if there truly is an interaction between age and sex this may shine light on the fact the females with TS are less likely to 'grow out' of their tics than their male counterparts. In this large sample, they were able to tease out the influence of medication and comorbidities and found no influence of such.

Importantly for our understanding of compensatory mechanisms for tic control, Plessen et al. (2006) found evidence of a relationship between CC size and motor tic severity (when controlling for whole brain volume). CC size had strong relationships with other regional volumes in the brain; the CC inversely related with the DLPFC in both control subjects (CS) (R=-0.25) and TS (R=-0.49) in the OFC in TS (R=-0.25) but not CS (R=-0.03) and it positively correlated with the premotor area in both TS (R = 0.41) and CS (R = 0.3). Given the shortcomings of the earlier studies in their sample size, imaging technologies at the time and the advancement of preprocessing techniques this study is certainly more convincing. Despite this,

studies since have not replicated these findings. Roessner et al. (2011) found a larger callosal motor region in medication-naïve children (9.6-15.1 years) with TS in a large study of 50 patients and 44 controls.

In addition to neuroimaging, TMS is able to probe at the integrity of the CC. To the best of my knowledge, only one study to date has been conducted in adults with TS; Bäumer et al. (2010) measured interhemispheric inhibition (IHI) between motor regions, which can give an index of the inhibitory effective connectivity across the CC and found that whilst there was no difference in right-to-left connectivity there was a significant difference in left-to-right connectivity; where those adults with TS demonstrated reduced inhibition. They detected an asymmetry in the patient group that was absent in healthy adults. This is reminiscent of some of the early imaging studies that hint at some sort of asymmetry of subcortical structures being integral to the pathology of TS (Peterson et al., 1993; Singer et al., 1993). However, the evidence for this is few and far between.

1.3.3 Pathological Connectivity Abnormalities

1.3.3.1 White matter connectivity

Looking at connectivity within the brain and treating the neural circuitry as networks is adept to understanding the neurobiological underpinnings of TS. We know that the pathology of TS likely involves CSTC circuitry. The genes involved point to abnormalities of the synapse and axons through development. There are various studies identifying that the largest brain structure to comprise of white matter fibre bundles, the CC, as involved in the pathology and perhaps adaptive in TS. Research within the realm of white matter connectivity, assessed by diffusion tensor imaging (DTI) is therefore an excellent choice for assessing TS pathology and the resultant adaptive compensation.

DTI allows the indirect measure of white matter microstructure by assessing the diffusion of water molecules in different directions, which is thus sensitive to the bundles of axons that white matter encompasses simply because water molecules will not diffuse perpendicular to these bundles. White matter microstructure integrity can be quantified by different tensor parameters: fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD) and mean diffusivity (MD). By far the most common finding in TS is widespread reduced FA, increased RD and increased MD (Müller-Vahl et al., 2014; Neuner et al., 2010; Wen et al., 2016; Wen, Liu, Rekik, Wang, Chen, et al., 2017b). In addition, FA within the CC has also unanimously been shown to be reduced: this has been found in children and adolescents (S. R. Jackson, Parkinson, Jung, et al., 2011; Plessen et al., 2006), adults with pure TS (Neuner et al., 2010) and in a case study compared an affected monozygotic twin to his non-affected sibling (Cavanna et al., 2010).

I would err on the side of caution regarding these findings, however, as a study explicitly testing head motion on measures of anisotropy and diffusivity in DTI images found apparent decreases in FA and increases in RD accompanying head motion (Yendiki, Koldewyn, Kakunoori, Kanwisher, & Fischl, 2014). Not one of the articles that has shown reduced FA that I have referenced above have adequately disclosed head motion parameters, used them as covariates or even demonstrated that they had excluded data due to excessive motion. One paper (Wen, Liu, Rekik, Wang, Chen, et al., 2017b) used support vector machine (SVM) learning to categorise scans into TS or control group to find the most discriminating aspect of WM in TS and found that RD was the most predictive one variable, which could simply reflect excessive motion being characteristic of TS. One other study assessing CC microstructure in children for instance did not find reduced FA and in fact found reduced AD and MD in TS (Wolff et al., 2016); however, the other two

articles that do not show reduced FA either show increased AD (Y. Liu et al., 2013) or absolutely no differences (Jeppesen et al., 2014). This may be explained by a longitudinal study that found reductions in AD and RD over time in children with TS (but not healthy controls) suggesting that variations in the sample may lead to these differences (Debes et al., 2014). It is clear that future studies need to at the very least generate head motion descriptors for individual datasets that they can use as a covariate.

White matter connectivity is not only assessed by quantifying anisotropy and diffusivity within specific regions; one can use a method called probabilistic tractography to quantify the connectivity between specific structures within the brain. The two papers that have done this in TS have both treated motion with care. One paper reported the head motion variables for the group and showed that there were no differences between each group and motion in any plane or rotation was less than 1mm (Wen, Liu, Rekik, Wang, Zhang, et al., 2017); whilst the other did not report these variables they had a stringent motion correction procedure and high exclusion rate of 18.33% due to motion (Worbe et al., 2015). Both papers had commendable sample sizes, compared to most research within this field in TS, testing 44 children and 49 adults with TS respectively (Wen, Liu, Rekik, Wang, Zhang, et al., 2017; Worbe et al., 2015). Wen, Liu, Rekik, Wang, Chen, et al. (2017b) used graph theory to characterize aspects of the network within the small world white matter connectivity in children with TS. The children with TS showed that they had similarly distributed hubs within the brain (i.e. nodes that are more well-connected than other nodes where the most information flows through, well-organised networks use hubs) However, they found that the TS group displayed reduced global and local efficiency, reduced normalized shortest path length, increased normalized clustering coefficient and increased small worldness. Finally, in specific nodes, efficiency was reduced in

the children with TS in frontal regions (left inferior frontal gyrus), temporal (left hippocampus and fusiform gyrus), occipital regions (bilateral inferior occipital gyri) and parietal regions (left supramarginal gyrus, inferior parietal gyrus and bilateral superior parietal gyri). The differing parameters that described the neural network for the TS and control group were then able to discriminate between both groups to high accuracy (86.47%) using SVM learning. This study therefore found reduced efficiency within white-matter networks in children with TS despite the network being increased in small worldness.

Worbe et al. (2015) used a different approach in adults with TS and had divergent findings; they found that adults with TS had abnormally enhanced connectivity accompanied by increased FA and reduced RD in CSTC pathways. Specifically, this was found between the striatum and thalamus and various cortical sites (including anterior cingulate cortex (ACC), fusiform gyrus, inferior parietal cortex, OFC, frontal areas, supplemental motor area, primary sensory and motor cortex, superior parietal cortex) with additional increased connectivity between the thalamus and putamen bilaterally and mildly augmented connectivity in putamen and globus pallidus connections in the left hemisphere. Interestingly, they found that in their female participants the enhanced connectivity was stronger and there was no effect of medication. Finally, in their study they found that tic severity scores positively correlated with the connectivity between striatum/ thalamus and the inferior frontal gyrus, PMC, SMA and negatively with cingulate cortex and positively with the connectivity between thalamus and putamen. This suggests that enhanced connectivity relates to increased tic severity except in the cingulate cortex where it is beneficial to have increased connectivity. This altered white matter structure likely reflects abnormal structural maturation.

1.3.3.2 Functional connectivity

Asides from looking at the structural connectivity within the brain, one can assess the functional connectivity by looking at resting state functional MRI (rsfMRI) and quantifying how brain activity within regions correlate with each other and thus plausibly communicate to each other. There are several methods of assessing functional connectivity including a network-type analysis as discussed above in the structural connectivity section (using graph theory), by looking at the correlation coefficients between areas or finally, by assessing amplitude of low-frequency fluctuation (ALFF) and fractional ALFF (fALFF), i.e. how the signal intensity in brain regions spontaneous fluctuate over time, with the latter reducing the influence of cardiac or respiratory noise (Zou et al., 2008). rsfMRI data is an important source of information of brain-wide connections in TS that can both add and complement structural data. As with white matter connectivity, characterisations of rsfMRI have been used to classify data into TS and control groups which demonstrates the aspects of connectivity that are most distinctive in TS (Greene, Church, et al., 2016; Wen, Liu, Rekik, Wang, Chen, et al., 2017a).

Two of the most cited studies looking at rsfMRI in TS demonstrate organisation within brain networks that appear to be immature both in children (Church et al., 2008) and adults with TS (Worbe et al., 2012). Whilst we have to be cautious when interpreting these results as neither papers have evidence of stringent head motion controls the take home message is interesting, if valid. They suggest that patients with TS show a delay in maturation within key brain networks. In adolescents, the correlation coefficients of connections within control networks was assessed and compared to a large study of development in the brain with 210 healthy participants (Fair et al., 2007). Age curves were generated for the participants aged 7-31 and the 33 children with TS (aged 10-15) tested were

assessed comparatively to these age curves. Interestingly, the indices of the control networks taken from the patients reflected much younger healthy children, i.e. the older children from the TS sample appeared to have functional connectivity that resembled controls aged 7-9 years. For instance, the dorsal ACC and mesial superior frontal cortex were situated in the fronto-parietal network in the children with TS. In data following normal development, however, these regions have been shown to be in transition to the cingulo-opercular network (where it would reside in adulthood). Furthermore, in mature adult-like networks we would expect a shift from short-range (or local) to long-range (or distant) connectivity (Fair et al., 2009). Contrastingly in children with TS there were more local connections within posterior regions of the brain compared to children without TS.

The work in adults (Worbe et al., 2012) largely supports the white matter connectivity paper from the same group insofar as adults with TS have undergone an anomalous trajectory of brain maturation characterised by aberrant connectivity within the brain. When assessing functional connectivity using graph theory, the group demonstrated that adults with TS had an absence of hubs within their network; i.e. no nodes were significantly more connected than all nodes together and thus characteristic of a noisier network. Furthermore, there were an increased amount of stronger functional connections among regions within the CSTC pathways (i.e. stronger local connectivity) with many of the functional abnormalities within cortical and deep brain sites being related to tic severity and complexity, e.g sensori-motor cortex, thalamus, putamen and insula. This reflects the work in children showing immaturity within the adult brain.

Whilst this interpretation is appealing, more work needs to be done to assess connectivity in TS research. For instance, one more recent rsfMRI study using graph

theory was conducted in 29 children with TS who were scanned close to onset of the disorder (between 0 - 4 years after onset) (Wen, Liu, Rekik, Wang, Chen, et al., 2017a). These certain children showed many alterations within indices for connectivity within the network: they showed significantly decreased strength across the network, reduced clustering coefficient, reduced global and local efficiency and increased shortest length path. They found that there was a partial overlap in the hub distributions with brain networks, i.e. the group with TS did not show absence of hubs unlike Worbe et al. (2012)'s paper. Rather, there were subtle differences and the position of nodes within a hierarchy of control was altered (indicated by betweeness centrality). Some nodes had increased influence of the network and some had reduced. Frontal regions and insula had an increased influence, as did the hippocampus and fusiform gyrus and parietal. Occipital lobe either had increased or decreased and left globus pallidum had reduced. Furthermore, the index of how a node is able to influence the system was positively related to tic severity in various regions (except in the right inferior frontal gyrus where it was negatively related).

The indices of network efficiency were further able to discriminate between the TS and control group to an accuracy of 88.79%. These results are not entirely complementary to the above papers and the changes across the network appeared more subtle with more variance. This may be related to the particularly young age of the sample (3-16 years), some of the patients were scanned less than a year after tic onset which of course would not be sufficient for a diagnosis according to the DSM-V. Sadly the authors did not explore the relationship between age, or time since diagnosis, and functional neural network indices. It would be interesting to see whether cross-sectionally there are any differences in the developmental trajectory between groups. This would certainly be helpful to assess whether there is in fact aberrant development of functional connectivity across childhood in TS.

1.4 Tic Generation and Suppression

Tics have been described as a result of an overextended habit learning system (Graybiel, 2008). Neuroanatomically, the pathological root of the disorder is commonly understood to involve dysfunctional CSTC circuitry (Singer, 2005; Worbe et al., 2012). I have outlined the evidence that supports this claim above, and whilst not comprehensive the vast majority of research suggests CSTC dysfunction (even if different studies do not agree on the details). The general idea is that disinhibition occurs within CSTC circuits, leading to the inappropriate reinforcement of stereotyped behaviour (Albin & Mink, 2006).

I have already written about the proposed pathophysiological signature of TS and a common theme that recurs is dysfunction and disinhibition of the CSTC pathways. It is thought that disinhibition of these pathways leads to tic generation. Amongst the wide range of modalities used to investigate the origins of tics in TS, functional imaging has been particularly useful at inspecting the network of regions involved in generating tics. I will thus discuss theories of tic generation and suppression with a focus on key functional MRI (fMRI) and a few positron emission tomography (PET) studies.

It is clear that a widespread network is involved in the generation of tics. One fMRI study conducted in adults with TS looked at BOLD signal change in the moments leading up to tics in a paradigm that encouraged patients to tic if they felt one coming (Bohlhalter et al., 2006). Before tic onset, insula, putamen, thalamus, cerebellum, ACC, SMA, medial and lateral premotor areas and parietal operculum all had heightened activity. SMA, ACC, cerebellar and putamen activity persevered until tic onset whilst the other regions dampened and DLPFC, superior parietal lobules, somatosensory, motor cortex and substantia nigra were significantly activated at tic

onset. This pattern of findings is not unique to this particular study and similar networks have been shown to be involved in a [^{15}O]H_2O PET study with similar instructions (Stern et al., 2000).

A well-controlled albeit small fMRI study in 12 adults with TS looked at functional connectivity focused on a single right sided facial tic in a free ticcing versus imitation paradigm; controlled by a control group imitating the same type of tic in the scanner (Z. Wang et al., 2011). When tics were spontaneous rather than voluntary (i.e. when patients imitated their tic), stronger connectivity was observed between substantia nigra to caudate, substantia nigra to putamen, globus pallidum to SMA (via the thalamus), premotor to SMA and primary somatosensory cortex to PMC. When comparing tic imitation in patients to controls, there was a higher influence of PFC on secondary motor regions (premotor regions and SMA).

The SMA has been found to activate temporally close to tics time and time again in fMRI studies (Bohlhalter et al., 2006; Hampson, Tokoglu, King, Constable, & Leckman, 2009; Neuner et al., 2014) in a manner that is similar to the way it activates preceding (and proceeding) voluntary movement in healthy adults. The activation of which is stronger in the periods preceding and proceeding a tic versus movement onset, yet similar during onset of movement (or tic) (Hampson et al., 2009).

The involvement of SMA in tic generation is unsurprising. After all, the SMA is a major terminus of thalamus projections (Schell & Strick, 1984) and further has some of the densest projections to and from the PMC in non-human primates (Luppino, Matelli, Camarda, & Rizzolatti, 1993) and humans (Arai, Lu, Ugawa, & Ziemann, 2012; Johansen-Berg et al., 2004; Roshan, Paradiso, & Chen, 2003).

Electrical stimulation of the SMA has been shown to induce a sensation of urge to move or anticipation of movement (Fried et al., 1991) and high-frequency rTMS, which is considered facilitatory, of the SMA has been shown to induce echophemomena in healthy adults (Finis et al., 2013). Moreover, there is considerable evidence of SMA involvement in other basal ganglia disorders such as Parkinson's Disease (PD), Huntington's Disease (HD) and Dystonia.

Hypoactivity in the SMA is well established in PD (Brown, Ridding, Werhahn, Rothwell, & Marsden, 1996; Rascol et al., 1992; Tisdall et al., 2016), which may be specific to the rostral part of SMA with, in fact, overactivity in the caudal part of SMA (Tisdall et al., 2016). Interestingly, in dystonia the opposite appears to be true: overactivity has been shown in the rostral SMA whilst hypoactivity found in caudal SMA (Ceballos-Baumann et al., 1995). In HD, an increased influence of the SMA in pre-symptomatic patients (approaching disease onset) has been reported as a possible compensatory mechanism (Klöppel et al., 2009) and as involved in the pathological progression of the disease, where cortical thinning has been shown associated with symptoms of bradykinesia and dystonia (Rosas et al., 2008).

It is fascinating that in PD and HD, the SMA appears to not only be involved in pathology but appears to be implicated as adapting in a compensatory way in response to the disease (Klöppel et al., 2008; Pollok et al., 2013). This may also true in TS; whilst the SMA is implicated in tic generation it is also highly implicated in tic suppression and may very well be a structure that contributes to tic remission.

One hypothesis for the remission of tics in TS proposes that compensatory neuroadaptations develop over adolescence in response to an increasing ability to successfully suppress tics (S. R. Jackson, Parkinson, Jung, et al., 2011). An

alternative explanation is that whilst tics are a result of dysfunction of CSTC pathways with signatures of a developmental delay in maturation (Church et al., 2008; Worbe et al., 2012, 2015) the normal developmental process still ensues, i.e. synaptogenesis, synaptic pruning and myelination, and is largely responsible for the reduction of tics across adolescence (Plessen, Bansal, & Peterson, 2009). It may actually be a bit of both.

The PFC has the most protracted course for synaptogenesis and synaptic pruning when compared to sensorimotor, parietal and temporal cortices (Casey, Tottenham, Liston, & Durston, 2005) and thus has been in the spotlight as a possible region for compensation. In fact, if we view tic suppression as an important mechanism for tic remission it follows that the PFC and SMA have both been proposed as area that help regulate motor inhibition in TS in suppression of voluntary movement and tics. A network involving the communication between sensorimotor cortex (SMC), PFC and SMA has been shown to be more tightly coupled in TS during motor inhibition (Serrien, Orth, Evans, Lees, & Brown, 2005); in the absence of behavioural differences this is likely to be adaptive in nature. In support of this, stronger alpha connectivity has been shown between left SMA and PMC in motor inhibition (Niccolai et al., 2016). The role of SMA during motor inhibition in TS is likely to be adaptive in helping reduce tic suppression given that patients were no worse at inhibiting movement in the above studies. Interestingly, as well as during movement inhibition, an increased coupling between SMA and PMC has also been found during a voluntary finger extension task in the periods before and during the movement but not after (Franzkowiak et al., 2012); sadly no behavioural results were available to help tease out whether this is a sign of pathology or adaptive.

Recently, an increased concentration of GABA has been detected in SMA in adolescents with TS (Draper et al., 2014); this reduction was associated negatively with BOLD activation in SMA, MEP size ahead of a volitional movement and increased FA in the region of the corpus callosum that receives projections from the SMA. The increased GABA is possibly a reactive response to the aberrant excitatory signals being received by the SMA; the increased inhibitory tone can be viewed as homeostatic in nature. Finally, I have explained that the SMA is involved in the genesis and suppression of tics. The final support for this comes from evidence of a novel therapeutic intervention in TS. There have been several papers exploring the effect of rTMS in TS and the most convincing site of interest is the SMA, whereby 'inhibitory' low-frequency rTMS has been shown to reduce tics (Chae et al., 2004; Kwon et al., 2011; Landeros-Weisenberger et al., 2015; Le et al., 2013; Mantovani et al., 2006; Wu et al., 2014) and in a case study 'inhibitory' cathodal transcranial direct current stimulation of the SMA has also shown to be effective (Hegde et al., 2015). This is in contrast with other regions such as PMC and premotor cortex which have been shown to not be successful sites for rTMS in TS (Munchau et al., 2002; Orth, Kirby, et al., 2005; Snijders et al., 2005).

Returning to the PFC, it has been identified countlessly as a region that may help achieve tic suppression; this is partly due to various fMRI studies detecting that the PFC is hyperactive in patients with TS during cognitive control tasks and active tic suppression. In tic suppression engagement of right frontal cortex has been associated with increased activity in the right caudate and decreased activity in the globus pallidus in comparison to when patients were able to tic freely (Peterson et al., 1998). An MEG study showed that frontrostriatal activations were increased during motor inhibition of eye blinks (Mazzone et al., 2010). Changes in PFC white matter microstructure were related to enhanced cognitive control in a small group of

adolescents with TS (S. R. Jackson, Parkinson, Jung, et al., 2011). Finally, as discussed earlier, hypertrophy of the prefrontal cortices have been found in children with TS but not adults (Peterson et al., 2001).

It is important here to recognise the proposed role of prefrontal regions, particularly inferior frontal cortex and pre-SMA, in response inhibition (Aron, 2011). The art of suppressing tics could well rise from inhibiting movement once a signal, namely PSP, is detected; certainly this sort of method is utilised in behavioural therapy such as HRT (Himle & Woods, 2005). There is not, however, a great deal of evidence for this in TS. Evidence largely rests on the indirect evidence of hypertrophic prefrontal cortices which has not always been replicated.

There is of course a multitude of evidence looking at PMC excitability in TS and magnetic resonance spectroscopy (MRS) studies in TS, I will review these in **Chapter 2**.

Chapter 2: Transcranial Magnetic Stimulation and Magnetic Resonance Spectroscopy

2.1 Transcranial Magnetic Stimulation

2.1.1 The Basics

TMS is a non-invasive tool that allows the stimulation of the neural tissue in the cortex via electromagnetic induction. The principle on which TMS is based is Faraday's law of induction (Faraday, 1832); the TMS coil serves as an electromagnet in which upon discharge a time varying magnetic field is generated which is then able to induce an electrical field in a material which drives a current within the cortex. In a typical experiment, one would place the coil on top of the scalp, over the brain region of interest. The electrical current generated by the TMS stimulus can then stimulate the cortex and induce action potentials. This technique is advantageous compared to electrical stimulation techniques since the magnetic field is not resistant to scalp, skull and other tissue in between the coil and cortex. The strength of the magnetic field does, however, dissipate the further the distance from the coil. TMS is therefore generally only used to stimulate sites located in cortex rather than deep brain structures; however, deep brain TMS is in the midst of being developed as a therapeutic technique to access structures deeper in the brain (Y. Bloch, Arad, & Levkovitz, 2014).

Exactly how TMS affects neuronal tissue in the brain is not well understood. Several recent publications have made the argument that TMS stimulates neuronal axons rather than cell bodies (Ridding & Rothwell, 2007; Siebner, Hartwigsen, Kassuba, & Rothwell, 2009; Volz, Hamada, Rothwell, & Grefkes, 2015). Neuronal axons have the lowest threshold for the brief TMS pulse to cause an action potential (Ridding & Rothwell, 2007).

Importantly the shape of the coil has implications for how focal the stimulation is. A circular coil is comprised of copper wire wound in a circular ring, typically with a diameter of 70mm. Such a coil induces an electrical current underneath the entire coil with no current induced in the centre of the coil. This may be beneficial for targeting larger sites (for example, the bilateral occipital cortex). In order to achieve more focal stimulation a figure-of-eight coil would be used. This arrangement of wire induces a weak current beneath the 'wings' of the coil and a sum of the current at the centre (twice of that at the wings). This is good for stimulating smaller regions such as the hand area of motor cortex.

2.1.2 Safety & Contraindications

TMS is generally thought of as a safe non-invasive technique. However, there are contraindications for TMS due to risk of seizure. Stringent screening procedures for TMS experiments have been developed from guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation in 1996 (Wassermann, 1998). A responsible investigator would enquire if participants had any of the following: migraines, neurological problems, epilepsy, a family history of epilepsy, or those taking epileptogenic medication (such as selective serotonin reuptake inhibitors) and would exclude participants if necessary. Safety screening and better equipment has dramatically reduced incidence of seizure. Although the risk is generally thought to be associated with repetitive TMS (rTMS) there has been at least one case of seizure in single-pulse TMS (sp-TMS), however, the participant in this case had bipolar disorder and mild contraindications (a modest dose of lithium, chlorpromazine, and a brother with epilepsy) (Tharayil, Gangadhar, Thirthalli, & Anand, 2005).

2.1.3 The Physiology of Motor Threshold in Health and Disease

MT is defined as the minimum intensity of stimulation required to reliably induce an MEP of a specific amplitude in a target muscle, either at rest (RMT), or when the muscle is partly activated (active motor threshold [AMT]). Motor threshold (MT) gives us important information about the state of neuronal tissue, i.e. how biased the neurons are to fire. Moreover, it is important as it allows stimulation of comparable intensities to be delivered to all participants within an experiment. In patient studies, where there may be a group difference in MT, ensuring all stimulation is referenced to MT is particularly important. When the intensity of TMS machine output is referenced to MT the experimenter is confidently stimulating the same neuronal population in each individual.

Unsurprisingly, RMT has been found to be highly correlated with skull-to-cortex distance (Herbsman et al., 2009; Kozel et al., 2000). The magnitude of MT is dependent on several processes. In between the delivery of the TMS pulse (the input to the system) and the motor evoked potential (the output), a range of processes occur. An electrical current is induced, and trans-synaptically stimulates corticospinal neurons; this then evokes a descending volley through the corticospinal tract, through to the spinal cord and down to the muscle (Amassian, Cracco, & Maccabee, 1988; Di Lazzaro, Ziemann, & Lemon, 2008). MT is dependent on the membrane excitability of cells in the PMC, motor neurons within the spinal cord, neuromuscular junctions and muscle (Kobayashi & Pascual-Leone, 2003).

Pharmacological studies have been used to distinguish what physiological processes underpin MT. MT has been shown to reflect voltage-gated sodium channel (VGSC) function, which is responsible for the rising phase of an action potential (Catterall, 2000).(Catterall, 2000). Evidence comes from drug studies where

common antiepileptic drugs carbamazepine, phenytoin and lamotrigine (whose routes of action are blocking VGSCs) have been shown to increase MT (for review see Ziemann et al. [2015]). MT appears to not be dependent on voltage-gated calcium channels (VGCC), which has been implicated in sustained action potential firing and not initiation (Catterall, 2011). Neither has MT shown to be mediated by γ-aminobutyric acid (GABA), dopamine (DA), noradrenaline (NA), serotonin or acetylcholine (Stagg, Bestmann, et al., 2011; Ziemann et al., 2015). Interestingly, however, drugs that manipulate non-NMDA glutamatergic receptors (such as fast-acting ionotropic AMPARs) have been shown to influence MT. Ketamine, an NMDA antagonist that paradoxically enhances non-NMDA glutamatergic transmission, reduces MT in a dose-dependent manner (Di Lazzaro et al., 2003).

In addition to the influence of corticospinal fibres' excitability on MT; research using DTI to explain MT variability has shown the importance of cortico-cortico connectivity. Klöppel et al. (2008) found regional fractional anisotropy (FA) to be inversely associated with MT; i.e. increased FA was linked with lower RMT. FA is considered to reflect white matter integrity, coherence of fibre bundles, axon diameter, packing density, membrane permeability and degree of myelination (Beaulieu, 2002). The measure of FA in this instance seemingly reflects an index of efficiency of communication between brain regions. The brain regions with FA that significantly related to MT include the PMC, premotor areas, caudal prefrontal cortices, corona radiata, genu of the internal capsule, cerebral peduncle and the trunk and splenium of the corpus callosum (Klöppel et al., 2008).

MT has been shown to be altered in a range of neuropsychiatric disorders. It is thus likely that MT indexes an important quality of neuronal excitability that is relevant to the understanding of disease pathology and/or neuroadaptations in the

face of disease. Both reductions and increases in MT have been associated with various disorders. OCD (Greenberg et al., 2000) and Alzheimer's Disease (Di Lazzaro et al., 2004), two disorders that have been linked with altered glutamatergic function, have been associated with reductions in MT. By contrast, increases of MT have been reported in the literature; for example in untreated patients with epilepsy, which could potentially signify some sort of protective mechanism against seizure spread (Delvaux et al., 2001; Pawley et al., 2017).

MT can be used over and above looking at group differences; the symmetry of threshold bilaterally could be important in health and disease (Di Lazzaro et al., 1994). Importantly, MT can be used to monitor disorders that change over time (both neurodevelopmental and neurodegenerative). In support of this, longitudinal increases in threshold have been demonstrated in motor neuron disease which likely tracks the degeneration of neurons (Tisdall et al., 2016). This is despite established reductions in MT at onset of the disorder (Brown et al., 1996; Mills & Nithi, 1997).

2.1.3.1 Motor Threshold in TS

The literature regarding MT in TS is conflicting. The majority of studies have been conducted in adults with TS and there is seemingly no difference in both RMT and AMT between patients and controls (Bäumer et al., 2010; K. F. Heise et al., 2010; Orth, Amann, et al., 2005; Orth et al., 2008; Suppa et al., 2011). One adult study, however, found that in comparison to healthy controls patients with TS had a significantly higher RMT in a group of 29 unmedicated adults with TS (Orth & Rothwell, 2009); it is likely no coincidence that this study has the largest sample size (compared to sample sizes of 6-20 where there were null results).

In adolescents, no differences have been found in MT in TS (Draper et al., 2015; C. A. Heise et al., 2008). Of note, however, change in RMT has been demonstrated in a group of children with TS in response to repetitive TMS (rTMS) delivered to the SMA in a sample of 25 children with TS (Le et al., 2013). Children were given rTMS 5 days a week for a month and the results found symptom improvement that persevered for 6 months, which correlated with an increase in RMT in both the left and right hemisphere (Le et al., 2013). Whilst this is only one study in TS, it hints at a useful clinical use of MT to assess treatment, whether or not MT is different at a group level for a disorder.

2.1.3.2 'Gain' in CSE

A key theoretical construct is 'gain' in CSE. This can be defined as the rate at which CSE increases. This construct can be operationalised in several ways but is most often measured as the slope of the TMS recruitment curve or the rate at which MEPs increase in amplitude ahead of a volitional movement. TMS recruitment curves are assessed by using a stimulus-response TMS technique, where the intensity of TMS is systematically increased from RMT in order to measure the intrinsic capability of the motor cortex to ramp up global excitability in the resting muscle. Gain of motor excitability can also be measured during the preparation of a volitional movement in a resting muscle, where the TMS intensity is not altered, but the time in which the TMS pulse is given is altered. Typically, the closer to onset of the movement, the greater the MEP amplitude signalling that gains are made in motor excitability during movement preparation.

2.1.3.2.1 Gain in CSE Measured at Rest: Recruitment (IO) Curves

The IO curve is a useful physiological probe that taps into largely excitatory circuitry governed by inhibitory mechanisms (Stagg, Bestmann, et al., 2011; Ziemann

et al., 2015): it is often taken as a measure of global excitability. It is important to measure a range of stimulator intensities since certain drugs have been known to selectively affect specific components of the resultant curve that comes with testing a range of stimulator intensities. IO curves have been shown to be altered by a large range of pharmacological substances that I will summarise here. Positive allosteric modulators (i.e. indirect enhancers) of $GABA_A$ receptors have been shown to reduce both slope and plateau repeatedly (for example benzodiazepines lorazepam and midazolam [Boroojerdi, Battaglia, Muellbacher, & Cohen, 2001; Schönle et al., 1989]). Non-competitive NMDA antagonist (and glutamate enhancer) ketamine increases MEP amplitude (Di Lazzaro et al., 2003). Dopamine (DA) agonist cabergoline selectively reduces high-amplitude MEPs (Korchounov, Ilić, & Ziemann, 2007; Nardone et al., 2006). Various neuromodulators increase IO slope, for instance NA agonists (for example d-amphetamine [Boroojerdi et al., 2001] and reboxetine [Plewnia et al., 2002]) and serotonin (5-HT) agonist sertraline (Ilic, Korchounov, & Ziemann, 2002).

Interestingly, despite being hailed as having high sensitivity in evaluating motor excitability in health and disease (Boroojerdi et al., 2001), there are relatively few studies looking at IO curves in neurological or neuropsychiatric disorders. It is clear, however, in the few studies that have been conducted that IO curves measure an important physiological phenomenon that is clinically (i.e. can be related to symptoms and pathology) and behaviourally relevant. For example, in stroke patients the metrics of the IO curve have been related to both clinical symptoms and task-related BOLD activity. Ward et al. (2006, 2007) conducted research in patients with subcortical strokes. They showed that IO curve slopes were positively associated with peak maximum hand grip of the affected hand and were negatively related to

task-related BOLD activity (dynamic hand gripping with impaired hand) in bilateral premotor areas and contralesional cerebellum.

Within the context of TS, Orth et al. (2008) found that adult patients had significantly shallower IO slopes than a control group and that slope correlated positively with tic scores. Those with shallower slopes had fewer tics than those who were more like the control group, which is indicative of a compensatory mechanism in TS that reduces the occurrence of undesired movements. The evidence from pharmacological and patient studies validates the use of IO curves in exploring motor excitability within a disorder and how it may or may not relate to symptomatology.

2.1.3.2.2 Gain in CSE Measured During Motor Execution

Studies have been conducted to look at changes of MEP amplitude (as a measure of CSE) at different time-points during a volitional movement in TS (Draper et al., 2015; K. F. Heise et al., 2010; S. R. Jackson et al., 2013). Whilst controls, as expected, had much higher MEP amplitudes close to the start of the movement; those with TS did not ramp up MEP amplitude to the same extent despite there being no difference at the start of movement preparation. In addition, Draper et al. (2015) showed that the variability of MEPs did not reduce at the end of movement preparation as it does in healthy controls. Both reduction in motor excitability and increased variability during movement execution suggests that a consistent neuronal firing pattern is not maintained close to movement onset as it is in the healthy population.

Just as IO curves have been shown to be related to symptomatology in TS; gain of motor excitability whilst executing a movement has also been shown to relate to tic symptoms. K. F. Heise et al. (2010) demonstrated that the MEP amplitude in

the later stages of movement execution was negatively related to measures of finger tics, complex tics and phonic tics but not the YGTSS (Goetz, Pappert, Louis, Raman, & Leurgans, 1999; Leckman et al., 1989) or Modified Rush Video Scale.

2.1.4 Paired pulse Transcranial Magnetic Stimulation

Paired pulse TMS (pp-TMS) is a paradigm that involves two pulses being rapidly emitted from the coil to a single cortical site. An initial conditioning stimulus (CP) that is usually but not always sub-threshold is quickly followed by a supra-threshold test stimulus (TP). Given that the CP tends not to elicit a response in itself, the conclusion from these studies in the motor cortex is that the response from the TP is altered through a neural change rather than a change at the spinal chord. The CP has been show to alter the response to the TP even if the coil is positioned in a more anterior or posterior position to the where the TP is delivered (Kujirai et al., 1993) this further supports the suggestion that the alteration occurs at a cortical rather than spinal level.

The results of pp-TMS studies are therefore taken to hint at the underlying neurophysiology at the level of the cortex. Three of the most reported measures are: SICI, LICI and ICF. SICI is measured by a sub-threshold CP followed by a supra-threshold TP 1-5ms afterwards. ICF is measured using a similar protocol to SICI pp-TMS with a longer ISI of 7-20ms. LICI uses two supra-threshold pulses with an ISI of 50-200ms.

The origin of the SICI response is suggested to be intracortical and pharmacological studies show that it appears to be dependent on GABAergic interneurons (Ziemann et al., 2015), in particular it appears to be enhanced by $GABA_A$ receptors and inhibited by GABA re-uptake inhibitors. There is evidence that

1ms SICI ($SICI_1$) and SICI with longer ISIs are distinct physiologically. Whilst pp-TMS typically measures synaptic inhibition or excitation, $SICI_1$ likely also has some relation to the axonal refractory period; i.e. the period following a stimulus and action potential where the axon is unresponsive to further stimulation (Fair et al., 2009; Fisher, Nakamura, Bestmann, Rothwell, & Bostock, 2002).

LICI appears to be a long-lasting inhibition, distinct from SICI. LICI is the result of slow IPSPs mediated by $GABA_B$ receptors (Stagg, Bestmann, et al., 2011); this is supported by the fact that the protocol uses a long ISI and pharmacological studies have shown that $GABA_B$ agonists increase the LICI effect (McDonnell, Orekhov, & Ziemann, 2006; Salavati et al., 2017). In contrast, non-selective positive allosteric modulators of $GABA_A$ have no effect but zolpidem, a selective agonist (of a $GABA_A$ receptor benzodiazepine site), has been shown to increase the LICI effect (Mohammadi et al., 2006).

The physiology of ICF is less clear, however, it appears to be somewhat dependent on a large sum of facilitation and a weak sum of inhibition; $GABA_A$ agonists and NMDA antagonists have resulted in an increased ICF response (Ziemann et al., 2015).

Pp-TMS measures are helpful in trying to decipher alterations of neurotransmission at the cortex in neurological and neuropsychiatric disorders, which could help lead to better pharmacological or stimulation interventions. Understanding what is happening at the cortex has important implications for therapeutic interventions that can alter cortical excitability (rTMS or transcranial direct current stimulation [tDCS] for example).

2.1.5 Paired-pulse TMS in Tourette Syndrome

TMS has been used time after time in TS to investigate synaptic function at the PMC, the greatest output of the CSTC. Pp-TMS takes various measures that when taken together can give us a broad picture of PMC excitability; two important measures used in TS are termed short interval intracortical inhibition (SICI) and ICF. SICI is an index of $GABA_A$ receptor action that has been consistently found to be reduced in TS (Gilbert et al., 2004; K. F. Heise et al., 2010; Orth, Amann, et al., 2005; Ziemann et al., 1997). The evidence concerning ICF is inconsistent (Orth & Rothwell, 2009); in one study patients with TS had increased ICF whereas others reported no effects (Gilbert, Sallee, Zhang, Lipps, & Wassermann, 2005; Ziemann et al., 1997). The effect of ICF results from a net facilitation that partly comprises the an inhibitory post-synaptic potential mediated by $GABA_A$ receptors (Ziemann, 2013). NMDA receptor antagonists and $GABA_A$ agonists reduce ICF; whilst glutamate agonists/ Noradrenaline agonists increase it (Ziemann et al., 2015). ICF is a notoriously variable measure to acquire (Orth, Snijders, & Rothwell, 2003), possibly due to the varied neurotransmitters it is dependent on. Given the heterogeneous nature of TS and difficulty obtaining reliable ICF measures, it is unsurprising that the evidence surrounding ICF in TS is inconsistent.

2.1.6 Dual site TMS

Ds-TMS is a protocol that was first used by Ferbert et al. (1992) that can be used to assess the functional or ‘effective’ connectivity between two brain sites. As with pp-TMS, ds-TMS involves two rapid magnetic pulses, however, instead of these being fired over the same site the first pulse will be emitted from one coil over one area and the second pulse will be emitted from another coil over a different area. The principle is that if the first pulse effects the response from the second pulse in a way

that cannot be achieved from a coil in an unrelated area these two areas are somewhat effectively connected. Further, one can investigate the time course of the interactions between two desired regions.

Using this protocol, we can make inferences from the extent of the interaction to how these regions may be structurally connected and whether in some groups of people this is different to others.

Boorman, O'Shea, Sebastian, Rushworth, & Johansen-Berg (2007) conducted a study looking at connectivity between the premotor cortex (PMd) and PMC from both a functional and structural perspective. They looked at ds-TMS measures during a choice-reaction task and DTI. They found that these two measures correlate. Specifically, the FA, only in specific regions involved in the task (dorsal premotor area and PMC), was highly correlated with the ds-TMS measures. Further supporting this idea, probabilistic tractography conducted on these highly correlated regions showed that these regions were those that projected to and from the dorsal premotor area, PMC and other regions related to the action choices.

When studying the motor system ds-TMS has made important contributions to our understanding of areas that are effectively connected. Studies have shown that a CP over the ventral premotor area or pre-SMA can affect the response to the TS in an excitatory or inhibitory manner (Arai et al., 2011, 2012; Mars et al., 2009).

There are important practical considerations to take in mind when conducting an experiment using ds-TMS. When one is not accustomed to using the TMS kit they may look over the blinding practical obstacles of conducting a ds-TMS experiment over certain regions. TMS coils are generally not small (averaging 70mm wing-to-wing diameter). The coils have handles, which although can be configured in a

branding iron arrangement, are more commonly placed perpendicular, on the same plane as the coil. This makes it particularly difficult to arrange two coils on a head when the desired sites are close together. It is a well-documented finding that orientation of the coil has an impact on response, possibly due to orientation of pyramidal axons (Fox et al., 2004). This fact makes the specific way the coils are arranged very important, causing potential experiments to be impossible. For example, Chouinard & Paus (2010) explain that it would be impossible to conduct a ds-TMS SMA-proper to PMC experiment. Despite this, several groups have attempted this in adults (although the conditioning site is usually closer to pre-SMA rather than SMA-proper). One group (Arai et al., 2011), describe that they could only use participants if they could place the SMA coil less than 40mm from the vertex (with the PMC coil in place). They were not transparent about how many individuals were excluded because of this. This, of course, is a great shame for probing effective connectivity in children and adolescents with TS.

Although this problem could potentially be overcome by using two small coils instead of the standard 70mm coils, there are intrinsic problems with smaller coils. They have a smaller distance of magnetic field penetration, being used more commonly for peripheral nerve stimulation, and because of this one tends to use a larger percentage of maximum stimulator output when stimulating a subject, which causes the coil to quickly overheat. Despite this, at least in terms of the motor system, ds-TMS measures have a lot to offer concerning effective connectivity.

2.1.7 Summary

TMS can give us insight into the cortical excitability within the motor cortex; which may be particularly useful when considering movement disorders and can in fact give us finer information regarding the imbalances of inhibitory and excitatory

systems within that area. It can further give us information of the effective connectivity between PMC and other areas that project to it. This again may be useful when trying to define what is happening at the level of the cortex in both those with movement disorders and also in healthy individuals. This essay has demonstrated that TMS can be paired with neuroimaging techniques (for example, DTI and ^{1}H-MRS) in order to build a better picture about brain structure and the neurochemistry of those areas in pairing with TMS measures. There are practical considerations when using TMS that are easy to overlook that must be considered when designing an experiment. However, TMS is an important technique within psychology research, which has the potential to lead to important therapeutic interventions in neuropsychiatric disorders such as Tourette syndrome.

2.2 Magnetic Resonance Spectroscopy

^{1}H Magnetic resonance spectroscopy (^{1}H-MRS) is an imaging technique which permits the non-invasive *in vivo* quantification of neurochemicals within pre-specified volumes of interest (VOIs) or across the whole brain. ^{1}H-MRS is an important tool in measuring alterations in various neurometabolites in neurodevelopmental disorders; it is likely that these biochemical changes may occur before structural changes and may provide important and sensitive information about changes within tissue structures that are associated with TS and other disorders.

2.2.1 Magnetic Resonance Spectroscopy in Tourette Syndrome

Since 2005 there have been eight published ^{1}H-MRS experiments looking at neurometabolite alterations in TS. Seven papers used 3T MRI scanners whilst one used a 7T. Voxels of interest that have been explored include putamen, thalamus, caudate, premotor cortex, SMA, SMC, PMC, visual cortex and ACC. Both reduced GABA (Puts et al., 2015) and no alterations in GABA (Draper et al., 2014; Tinaz et

al., 2014) have been demonstrated in SMC; Puts et al. (2015) further found a negative relationship with GABA concentration and motor tic severity. In the ACC, reduced GABA has been found (Freed et al., 2016) in young patients with TS compared to healthy controls, which did not relate to clinical symptoms. Freed et al. (2016) did not find any differences in Glx concentration (a composite of glutamate and glutamine) which is supported by three other studies that found no differences in Glu or Gln in ACC (Fan et al., 2017; Kanaan et al., 2017; Naaijen et al., 2017). Despite this correlations have been found between glutamate levels in the ACC and obsessive compulsive symptoms in TS (Naaijen et al., 2017) and glutamine levels and motor tic severity (Fan et al., 2017), suggesting dysfunction Glu/Gln cycling may be involved in the ACC in TS.

Striatal and thalamic VOIs have been of interest in TS ^{1}H-MRS studies. A reduction in creatine in the thalamus has interestingly been demonstrated in TS (DeVito et al., 2005; Kanaan et al., 2017) and reduced N-acetyl aspartate (Naa) (DeVito et al., 2005) which has not been replicated. In striatal VOIs; one early study demonstrated reduced Naa, choline and creatine in putamen and a reduction in creatine in the caudate (DeVito et al., 2005). In a more recent study that used two striatal VOIs (one left and one right), but did not separate the varying structures did not find the same pattern of results; however, they did show reduced Glu, Glx and Gln/Glu in patients with TS compared to healthy controls; interestingly this reduction of Glu and Gln normalised when medicated with aripiprazole to levels comparable to controls (Kanaan et al., 2017).

Unlike the studies of GABA in 3T, the one study at 7T did not find a reduction of GABA in SMC (Draper et al., 2014). Draper et al. (2014) did not find any alterations of GABA in a PMC VOI or visual cortex VOI in TS; however, they did find

an increased concentration of GABA in the SMA. The increase of GABA related to increased FA in the region of the CC that projects to bilateral SMA; a reduction of BOLD in PMC during task-related fMRI and motor excitability measured by TMS-induced MEP. Whilst it did not directly relate to motor tic severity, motor tics did positively relate to FA suggesting that the increased GABA is potentially reactive to increased tic severity.

Whilst there is not a great consistency across the ^{1}H-MRS studies already conducted in TS it has been shown that there are alterations in GABA, Glu and Gln in the CSTC pathways that needs to be further explored with high resolution scanning (7T MRI) in core VOIs. Using 7T to acquire ^{1}H-MRS greatly improves the signal-to-noise ratio (SNR) and increases chemical shift dispersion. The SNR increase means that 7T ^{1}H-MRS is highly sensitive to detecting metabolites, this greatly advantages the detection of metabolites that have intrinsically low concentrations such as GABA. Due to spectral overlapping, detection of GABA, Glu and Gln is challenging in ^{1}H-MRS at the typical field strengths used (3T or below). 7T thus has the advantage of producing a visually discernible GABA-peak without the need for an edited protocol, therefore being susceptible to motion artifacts (Wittfoth et al., 2012). Furthermore, the Glutamate and Glutamine peak can be distinguished (rather than acquiring the composite Glx). These advantages are important advancements that allow us to acquire more sensitive measures of neurometabolite concentrations - an imperative for relating findings to behaviour or pathology.

^{1}H-MRS measures chemical composition within a prespecific VOI across tissues and is thus not sensitive to different pools of neurotransmitter and rather captures the neurochemicals existing within the VOI as a whole. Small alterations in synaptic neurotransmission, given that they are phasic and transient, may not be

captured to the same extent as large pools of neurotransmitter/ neurometabolites that may differ in result of disease. It is important, therefore, to quantify functionally what ^{1}H-MRS is capturing in order to conclude with confidence what alterations of neurochemicals in neurodevelopmental disorders reflects or signifies.

Chapter 3: Investigation of Corticospinal Excitability in Young People with Tourette Syndrome at Rest and During Motor Execution

3.1 Introduction

3.2 **Experiments 1** and **2** use Transcranial Magnetic Stimulation (TMS) to track the excitability of the primary motor cortex (PMC) both at rest and ahead of volitional motor execution during a simple Go/ No-Go task in a sample of young people with Tourette syndrome and a group of sex and age-matched healthy controls. In **Chapters 1** and **2** I have given an overview of theories of the pathology of TS, tic generation, suppression and remission and explored the use of TMS in TS. In **Experiment 1,** I investigate Resting Motor Threshold (RMT) and TMS Recruitment Curves (IO Curves); in **Experiment 2** I use single pulse TMS (sp-TMS) to track the rise of motor excitability during volitional motor execution in a behavioural task (Go/ No-Go task).

The primary aims of **Experiment 1** and **2** are to explore PMC excitability, indexed by TMS-induced motor response, both at rest and whilst engaged in the execution of a volitional movement. In the light of previous research, it appears that there are alterations in the neural circuitry of PMC which either: 1) play a causal role in TS or 2) act as some sort of adaptive compensatory mechanism that may help to control tics. Previous literature largely (but not exclusively) concerns adults with TS, which are a small unique group of patients whose tics typically worsen through to adulthood rather than ameliorate like the majority of patients with TS. These experiments will investigate CSE in young people with TS to determine whether the patterns of results that we see in adults also holds for younger patients. This group

will allow me to focus on how both development and tic severity affect PMC excitability.

I would expect to see alterations in these core measures of PMC excitability that reflect both the dysfunction in CSTC pathways that plays a causal role in TS and the compensatory adaptations that are acquired as a response to the disorder. Healthy developing children show increased RMTs and reduced IO curve slopes that normalise by early to mid adolescence (Garvey et al., 2003; Garvey & Gilbert, 2004; Garvey & Mall, 2008). Previous neuroimaging literature demonstrating delays in the maturation of key brain networks in TS (Church et al., 2008; Worbe et al., 2012). If motor networks are immature in young people with TS I would expect to see increased RMT and shallower IO curves in our patients compare to controls. Whilst the evidence surrounding RMT is inconclusive in TS; shallower IO curves have been shown before in adults with TS (Orth et al., 2008).

Reduced gain during the execution of volitional movements has been shown in children (Draper et al., 2015) and adults with TS (K. F. Heise et al., 2010) and thus I expect to find reduced gain during a volitional movement. If reduced gain is related to the pathology of TS there may also be adaptive compensatory mechanisms involved in the regulation of motor excitability during volitional movement. If this is the case, I should see a negative relationship with tic severity whereby increases in gain are related to reduced tic severity; this has been found previously in a studies with a small sample size (Draper et al., 2015; K. F. Heise et al., 2010).

3.2 Method

3.2.1 Participants

Seventeen young people with Tourette Syndrome (TS) were recruited to take part in **Experiment 1** and **2** (age range = 11.9-21.6 years, mean (M) = 16.47 years ± 3.17, 3 females). The sum of motor and phonic tic scores ranged from 3 to 44, M = 22.6 ± 11.0. Seven participants in the TS Group suffered from additional comorbidities besides TS. Three had an additional diagnosis of OCD, one had ADHD and three participants were diagnosed with ASD. See **Table 3.1** for additional details of tic scores, comorbidities and medication. Seventeen sex and age-matched controls (CS) (age range = 11.9 - 21.8 years, M = 16.59 ± 3.18, 3 females) also took part.

Table 3.1: Clinical and biographical characteristics of the participants with TS that took part in the study including sex, age, tic severity, any comorbidities the participant has and medication

TS ID *(n = 17)*	Sex	Age (Years)	YGSS	Motor tic Score	Phonic tic Score	Impairment Score	Comorbidity	Medication
TS006	M	21.6	37	15	17	5	None	Clonidine
TS013	M	18.3	13	13	0	0	None	Clonidine
TS018	M	19.3	13	13	0	0	None	None
TS028	F	19.0	51	13	13	25	OCD	Fluoxetine
TS030	M	17.0	23	12	11	0	None	None
TS031	M	18.5	47	16	11	20	None	Fluoxetine
TS034	M	15.4	3	3	0	0	None	None
TS048	M	16.0	19	8	6	5	None	Clonidine
TS055	M	17.0	25	15	0	10	ADHD	None
TS069	M	12.6	13	9	4	0	None	None
TS071	M	13.6	67	19	18	30	OCD	None
TS081	F	12.8	46	17	9	20	OCD	Clonidine, Melatonin, Aripiprazole
TS082	M	11.9	17	12	0	5	None	None
TS084	F	21.6	45	15	10	20	ASD	Citalopram
TS088	M	12.4	58	18	20	20	ASD	Clonidine
TS092	M	14.2	38	12	11	15	ASD	None
TS103	M	18.9	64	22	22	20	None	None

3.3 TMS Protocol

An unpaired Magstim Bistim 2 machine and a 70mm figure-of-eight coil were used to deliver single-pulse TMS (sp-TMS) to the motor hotspot of the first dorsal interosseous (FDI) muscle in the right hand. First the RMT was found as the intensity required to elicit an MEP of at least 150-200μV in 5 out of 10 trials. The location of the motor hotspot was continuously tracked throughout both experiments using the BrainSight 2 neuronavigation system.

Two BrainSight trackers were used: one was adhered to the participants' foreheads and the other to the TMS coil. A camera and software that aligns specific points on the subject's head to a virtual on-screen head, and the use of automatic curvilinear reconstruction, allowed the experimenter to ensure that the TMS coil was always placed directly over the target region. Once the motor hotspot was located, the TMS coil was stabilised using a Manfrotto arm. The coil was continuously observed by the experimenter and adjusted whilst the trials were delivered to ensure that the coil was positioned over the target. In order to record the muscle twitch from the FDI muscle, disposable electromyography (EMG) electrodes with a diameter of 5mm were placed on the FDI muscle in a standard belly-tendon configuration. BrainVision Recorder software was used to record EMG responses to the TMS protocol and recorded at a sampling rate of 5000 Hz and with a sampling interval of 200 μS.

3.3.1 Experiment 1: Recruitment Curves (IO Curve)

The participant rested their chin on a chin-rest whilst receiving sp-TMS at incremental percentages of each individual's RMT. The TMS intensities used to acquire the IO curve were between 95 – 130% of the individual's RMT in increments of 5%; 10 trials were delivered at each intensity with 80 trials in total. A fixed inter-

trial interval (ITI) of 5 seconds was utilised. TMS stimuli were pseudo-randomised and organised into 8 blocks. After each block, the experimenter checked that the participant was tolerating the procedure well and would readjust the coil position if necessary.

3.3.2 Experiment 2: sp-TMS with a Go/No-Go task

The participant sat with their chin placed on the chin rest, 50cm away from a 17-inch monitor where the stimuli were displayed. The task was a simple Go/No-Go task in which the presentation of a green circle signalled the participant to make a button press with their right hand as quickly as possible and red circle signalled the participant to withhold their response. The stimuli were organised into 12 blocks of 9 trials. Each block consisted of a ratio of 1:8 No-Go to Go trials. The trial on which the No-Go appeared within the block was randomised within each set of 9 trials.

Prior to the experiment, 36 practice trials without TMS were used to familiarise the participants with the task and to calculate an initial median response time (RT). This initial time was used in the first block of the sp-TMS Go/No-Go task in order to calculate when to trigger the TMS stimulator. sp-TMS were triggered (in the main experiment) at 25%, 50% and 75% of RT as calculated by the median RT during the practice trials and then by an updated median RT which was calculated every 9 trials. Each trial was either terminated by a button response or was programmed to time out after 2s. A graphical representation of one trial during the Go/No-Go task can be seen in **Figure 3.1**. TMS was triggered at 100% of RMT throughout the study.

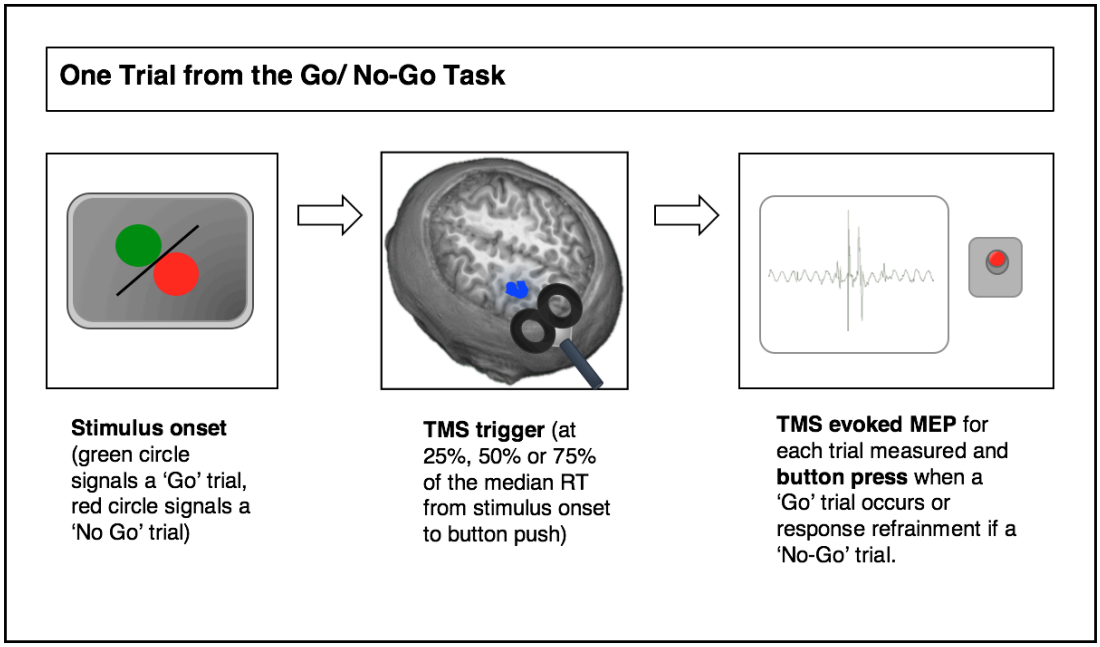


Figure 3.1: A graphical representation of a single trial in the Go/No-No task

3.4 Results

The EMG signal recorded throughout the experiment was analysed using EEGLAB in MATLAB. The peak-to-peak amplitude of the MEP was measured for each trial in both experiments. Data from each trial were visually inspected. If the trial was contaminated by any other activity (a contracted muscle for example), the trial was excluded. All participants took part in both experiments.

3.4.1 Experiment 1: Input-Output Properties and Gain Changes of CSE

3.4.1.1 Group differences in Resting Motor Threshold

RMTs for the TS group (M = 48.00, *standard deviation* (SD) = 10.45) were higher than those for the CS group (M = 37.60, SD = 5.20). Thresholds for both groups were normally distributed and a Levene's Test for Equality of Variances highlighted that variances were not homogenous. An unpaired student's t-test

(corrected for heterogeneous variance) confirmed this difference was significant ($t(23.45) = 3.677$, $p<0.01$).

The differences in RMT that I observed appeared more pronounced in the younger participants. To investigate this further a stepwise regression was conducted to examine if age contributed to a linear model that included group as a predictor. Two variables were entered (group and age) and no variables were removed. The model was statistically significant, ($F(2, 31) = 17.03$, $p<0.0001$) and explained approximately 50% of the variance ($R^2 = 0.524$, Adjusted $R^2 = 0.493$). Group (beta = 0.536) was weighted more heavily than age (beta = -0.476) and was thus the primary predictor in the model. The unstandardised and standardised coefficients, their Pearson's correlation with RMT, squared semipartial correlations, and their structure coefficients are presented in **Table 3.2**.

Table 3.2: Stepwise Regression model using age and group to predict RMT. This table presents for each factor: unstandardised and standardised coefficients, Pearson's correlation with RMT, squared semipartial correlations, and their structure coefficients.

Model	b	SE-b	Beta	Pearson r	sr2	Structure Coefficient
Constant	62.114	6.590				
Group	10.232	2.369	0.536	0.545	0.286	0.753
Age	-1.475	0.384	-0.476	-0.487	0.227	0.673

In summary, age is predictive of RMT, particularly in the TS Group, where RMT are dramatically higher in the younger group. Younger patients in the TS Group require a higher intensity of stimulation to produce similar MEP sizes to both older adolescents with TS and controls that are of the same age. Please refer to **Figure 3.2**.

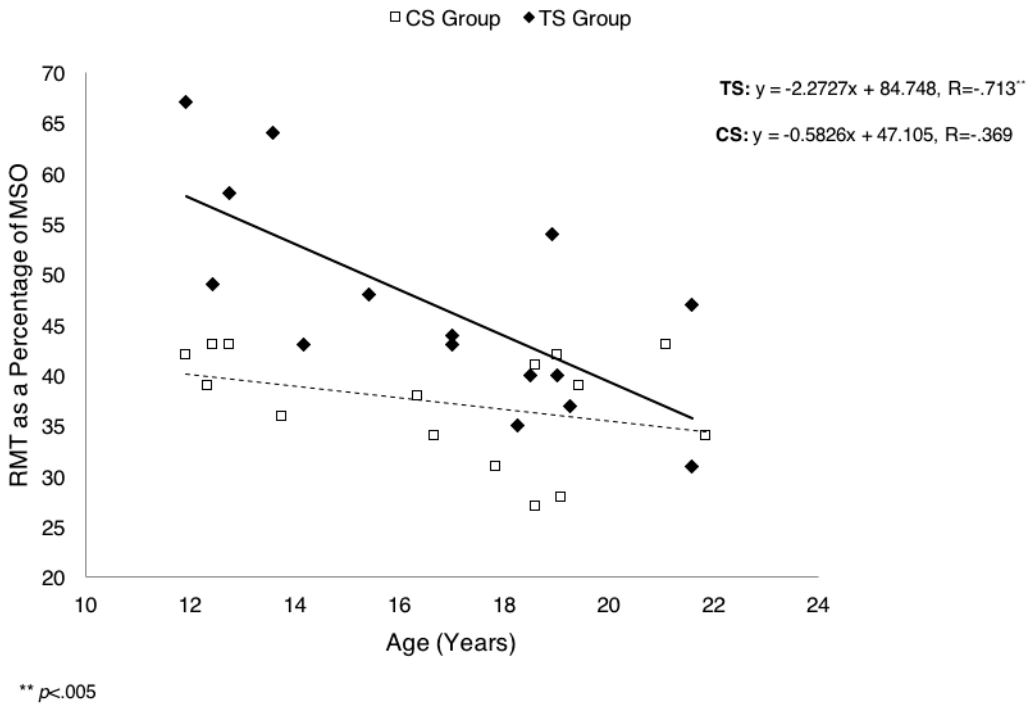


Figure 3.2: Resting Motor Threshold (RMT) calculated as percentage of maximum stimulator output (MSO%) to elicit a motor evoked potential (MEP) of between 150-200μV plotted against the age of participant. A stepwise regression was conducted and showed that both group and age was predictive of RMT ($F(2, 31) = 17.03$, $p<0.0001$). These two factors explained roughly 50% of the variance ($R^2 = .524$, Adjusted $R^2 = .493$).

3.4.1.2 Group differences in global excitability indexed by TMS input-output curves

Raw MEPs were log-transformed to a base of 10 in order to conform values towards normality and homogenise the data's variance. This allowed us to compare groups using parametric statistics without violating the assumptions underlying these analyses. The transformed MEP data was collapsed for each participant into mean MEPs at each intensity (percentage of MSO%).

To calculate each individual slope, percentage of MSO% was plotted against log-transformed MEP values collected for that intensity. A sigmoidal curve, Boltzmann curve, with the following equation (taken from Houdayer et al., 2008) was fitted to each individual curve.

$$\mathrm{MEP}_S = y_0 + \frac{\mathrm{MEP}_{\mathrm{MAX}}}{1 + e^{(\frac{S_{50}\text{-}S}{K})}}$$

In this equation $\mathrm{MEP}_{\mathrm{MAX}}$ is the maximum MEP amplitude measured, S_{50} is the MSO% needed to produce 50% of the maximum MEP, 1/K is the gradient of the maximum steepness of the curve. y_0 is the minimal MEP response, which as in Houdayer et al.'s (2008) paper was set to 0.

The median coefficient of determination (R^2) for the fit of each individual slope was 0.96. There was no significant difference between the slopes' coefficient calculated between groups (p = 0.847). Refer to **Figure 3.3** for an example of an input-output curve from the data.

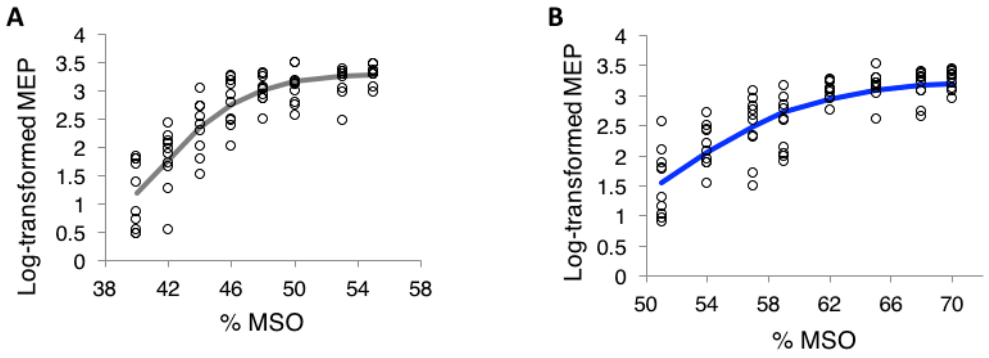


Figure 3.3: An example from the control (CS) group (**Figure 3.3A**) and Tourette Syndrome (TS) group (**Figure 3.3B**) of a Boltzmann Curve (using the equation from Houdayer et al. 2008) fitted to log-transformed MEP data (to a base of 10).

Group differences in CSE were investigated by using a two-way mixed ANOVA on log-transformed mean MEP data from all intensities used to collect the IO curves. Age was entered as a covariate. As expected there was a significant within-subject effect of intensity (F(3.1, 95.7) = 2.924, p<0.05 (Greenhouse-Geisser corrected)). There were neither interactions between intensity and age (p = 0.45) nor

between intensity and group when using a Greenhouse-Geisser correction (p = 0.10). There was a significant between-subjects main effect of group ($F(1,31)$ = 6.606, p <.05), however, the Levene's test of equality of error variances showed differences between 105-130% and so this should be interpreted on the side of caution. **Figure 3.4** shows the mean log-transformed IO curves for each group.

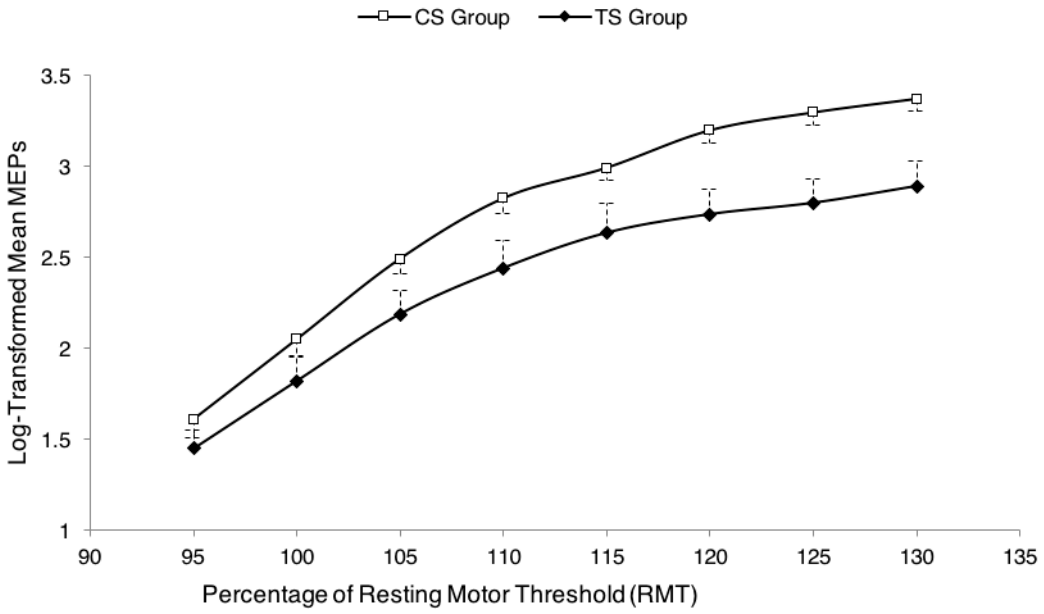


Figure 3.4: Input-Output Curves were collected for each participant with 100% resting motor threshold (RMT) defined as the intensity needed to elicit an MEP between the sizes of 150-200μV in 5/10 trials. This graph shows the mean log-transformed motor evoked potentials (MEPs) for the control (CS) group and Tourette Syndrome (TS) Group. For the higher intensities there appears to be a global reduction of cortico-spinal excitability (CSE) in the TS group. A two-way mixed ANOVA with age entered as a covariate shows a within-subject main effect of intensity ($F(3.1, 95.7) = 2.924$, $p<0.05$) and a between-subject effect of group ($F1,31) = 6.606$, $p<0.05$).

3.4.1.3 Individual Variability within IO Curves

To investigate differences in variability between the groups, I computed the coefficient of variation (CoV) of log-transformed MEPs for each individual and for each level of TMS intensity. Relevant data is presented in **Figure 3.5**. A mixed ANOVA was used to explore individual variability further with Group (TS vs. CS) entered as a between-subject factor, TMS intensity (95–130% of RMT) entered as a within-subject variable, and Age entered as a covariate. The ANOVA revealed a

significant main effect of Group ($F(1,31) = 5.806$, $p<0.05$) and a marginal of Age ($F(1,31) = 3.474$, $p = 0.07$). MEP variability was larger in the TS group and decreased with age in both groups. The main effect of TMS intensity was not significant ($p = 0.305$). In addition, there was no Group × TMS intensity interaction effect ($p = 0.772$). In contrast, there was a significant TMS intensity × Age interaction ($F(2.25,62.99) = 3.737$, $p<0.01$).

Overall, these findings confirm that the TS group exhibited more variability, as indexed by CoV, in their MEP responses than matched typically developing controls and that for both groups, MEP variability decreases with age. Importantly, the rate of this decrease in variability is comparable across the groups.

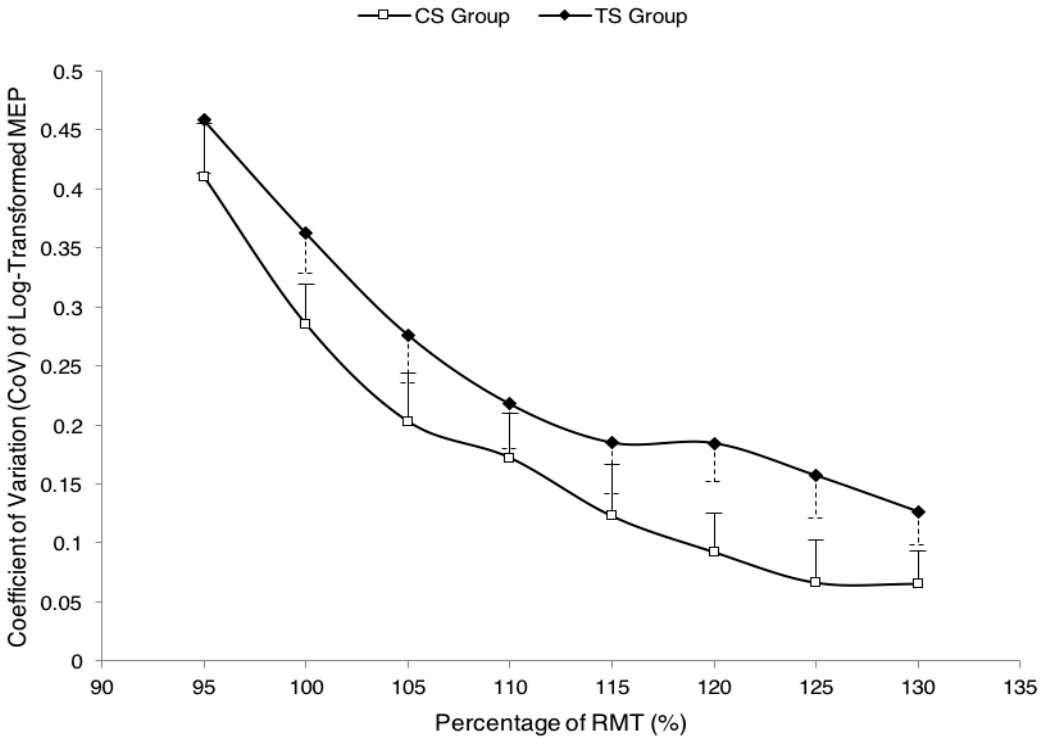


Figure 3.5: Mean coefficient of variation (CoV) values for log-transformed MEP values recorded in the FDI muscle when stimulated by a Transcranial Magnetic Stimulation (TMS) machine at different percentages of Resting Motor Threshold (RMT) (which is defined as the intensity needed to elicit an MEP between the sizes of 150-200μV in 5/10 trials). CoV dramatically reduces as participants are stimulated with higher intensities of TMS.

3.4.1.4 Relationship between IO Slope, Age and Tic Severity

To investigate the relationship between IO slope and tic severity, IO slope was calculated using the method devised by (Peterchev, Goetz, Westin, Luber, & Lisanby, 2013). The authors suggested that a sensitive measure approach to look at individual differences in CSE is to multiply the slope by the RMT. This is to take into account the inverse relationship between slope and RMT.

Table 3.3: A Linear Regression model using age and group to predict IO Slope. This table presents the each factor's: unstandardised and standardised coefficients, Pearson's correlation with IO Slope, squared semipartial correlations, and their structure coefficients.

Model	b	*SE-b*	Beta	Pearson *r*	sr^2	Structure Coefficient
Constant	8.569	5.139				
Motor tics	-.546	.202	-.541	-.473	.287	-.317
Age	.680	.283	.481	.481	.227	.604

A linear regression revealed that both age and motor tics in the TS Group significantly contribute to a model predicting the I-O Slope ($F(2, 14) = 5.742$, $p<0.05$) and explained approximately 40% of the variance (R^2 =.451, Adjusted R^2 =.372). Motor tics were weighted highest as primary predictor. The unstandardized and standardised coefficients, Pearson's correlation with slope, squared semi-partial correlations, and structure coefficients are presented in **Table 3.3**. **Figure 3.6**, however, demonstrates that the contribution that motor tics gives to the model is driven by an outlier.

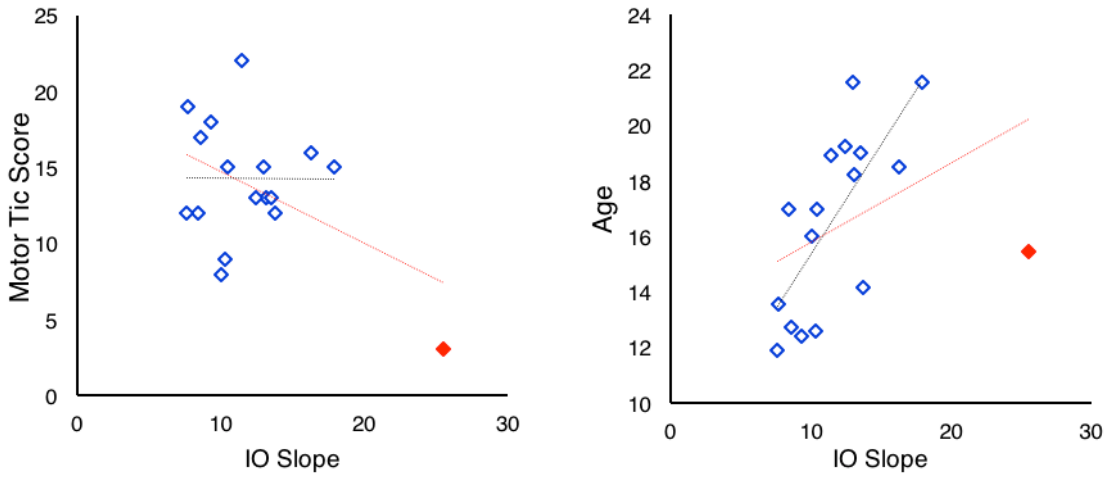


Figure 3.6: In red is subject TS034 who has a very low tic score which outlies all other motor tic scores and IO slope. The trend lines for motor tic scores and age scores have been represented with and without the outlying data point. It is clear that the relationship between motor tics and IO slope in particular is driven by this data point.

It is impossible to predict whether the outlying data point contributes meaningfully to this analysis. I was hesitant on excluding the value given that both the participant's motor tic score and IO slope outlies the normal distribution for all other motor tic scores and IO slope values. This patient has a very low motor and phonic tic score, 3 and 0 respectively, despite a diagnosis of Tourette Syndrome. It is likely that the patient is growing out of their tics which is indicative of beneficial neuro-compensatory adaptations. It is quite possible that if the dataset was larger and there were more patients with low tic scores a similar trend could occur. I have chosen to be cautious with this finding given the deviance of the outlier. It is worth noting that this finding is in direct opposition with the relationship between tic severity and IO curve in a group of adult patients (Orth et al., 2008).

3.4.2 Experiment 2 – sp-TMS Go/ No-Go Task

3.4.2.1 Behavioural Data

Behavioural data was first analysed to check for differences between groups. RT estimates for correct GO trials were based upon button press responses.

However, since EMG data was also collected from the responding hand on all trials, including No-Go trials, reaction time effects can also be verified using the EMG defined onset of movement. This might allow for an earlier, and potentially more sensitive measurement of RT on GO trials, and the identification of self-corrected error responses on No-Go trials, which is not possible from analysis of button-press responses. A two-tailed independent groups t-test was conducted for RT for correct GO trials. This revealed a significant difference between groups with the TS group responding more slowly on average ($t(32) = -2.319$, $p<0.05$).

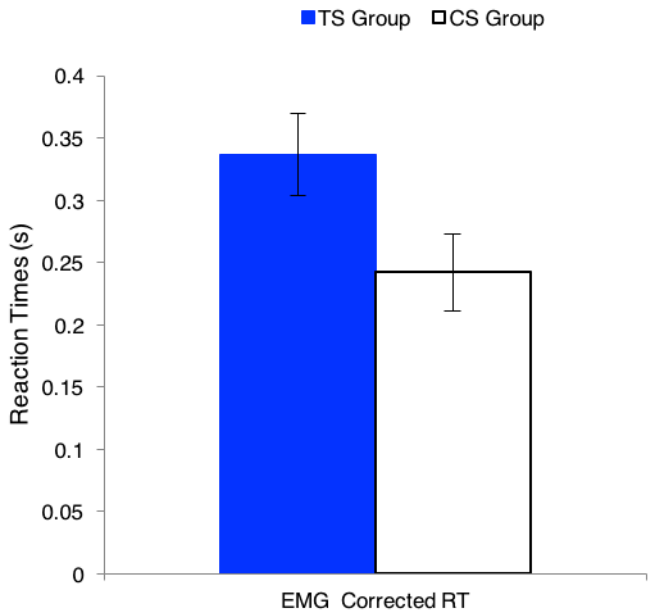


Figure 3.7: Go trial response times in seconds for the Tourette syndrome (TS) and control (CS) Group with standard error bars. An unpaired t-test revealed that the TS Group performed significantly slower than their matched controls (t(32)=-2.319, $p<0.05$).

By contrast, there were no differences in accuracy measures. Specifically, both groups performed at 100% accuracy for GO Trials and there were no between group differences in errors made on No-Go Trials. This was the case when looking at correct button-press responses and also when I used the EMG recordings to identify movement-contaminated (i.e., self-corrected movement) trials (two-tailed *t* tests, $p = 0.634$, $p = 0.371$ respectively).

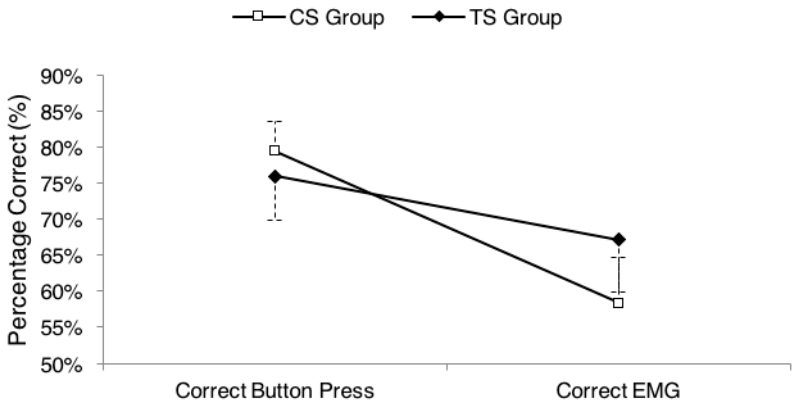


Figure 3.8: This graph shows the percentage of 'No-Go' trials that were correctly responded to in the TS Group and matched controls. The values in the first column are percentage of trials the participant correctly inhibited a button press to and the second column are trials that movement was entirely inhibited (by observing the EMG recording).

3.4.2.2 The Gain of Motor Excitability Ahead of a Volitional Movement (MEP Data)

The peak-to-peak amplitude of the MEP was measured for each trial. Each trial was individually inspected for contamination by any other muscle activity and spoiled trials were excluded. Each MEP was time-stamped as the percentage of the response time of that trial that the TMS was triggered. MEPs were then binned into four different timings of movement preparation according to their time stamps: 0-29%, 30-49%, 50-69% and 70-100% of response time. Median MEPs (measured in micro volts) for each bin were calculated for each individual and then averaged for each group.

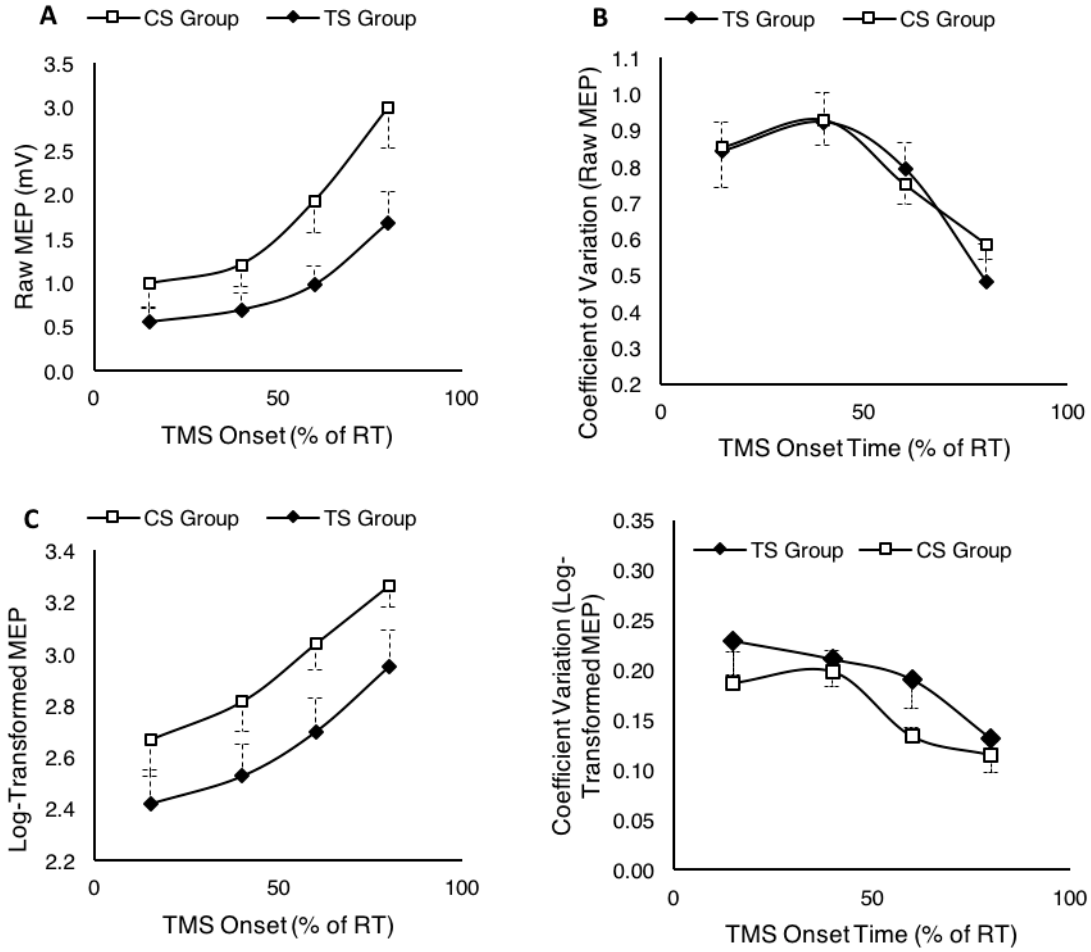


Figure 3.9: Figure 3.9A) Median MEPs were calculated for 4 different binned timings of movement preparation (0-30%, 30-50%, 50-70% and 70-100% of response time (RT)). **Figure 3.9B)** For each binned data, mean of individual coefficient of variation (CoV) values were calculated and plotted for each group. **Figure 3.9C)** Since MEPs are not normally distributed, mean of individual log-transformed MEPs were calculated for each bin of data. **Figure 3.9D)** Instead of CoV, for log-transformed data individual variance for each bin was captured by calculating coefficient of variations (CoV) for each bin of log-transformed MEPs. For **Figure 3.9A-D**, errors bars show group standard error of the mean (SEM) at each bin.

MEPs were log-transformed to a base of 10. Both raw MEP data and log-transformed data are presented in **Figure 3.9**.

A two-way mixed ANOVA was conducted on the log-transformed MEP data with age entered as a covariate. The ANOVA revealed that there was no main effect of TMS onset time ($p = 0.24$), no significant interaction of TMS onset time and age ($p = 0.91$) and no significant interaction of TMS onset time and group ($p = 0.36$, all p values are Greenhouse-Geisser corrected). Contrary to this, there was a significant

main effect of age ($F(1,31) = 11.36$, $p<0.005$) and a significant main effect of group ($F(1,31) = 5.77$, $p<0.05$).

In addition, I calculated the coefficient of each individual's corticospinal excitability (CSE) gain as a function of percentage of movement preparation for each individual and investigated whether the slope was different between groups. I modelled a linear function, fitted to raw MEPs. An independent one-tailed t-test demonstrated that that there was a trend for the CS group ($M = 33.06$, $SD = 30.5$) to have a steeper slope than the TS group ($M = 16.71$, $SD = 13.76$): $t(22.25) = 2.015$, $p<0.05$ (equal variances not assumed).

These data confirm that ahead of volitional movements, motor excitability is reduced compared to age-matched typically developing controls. To examine the effect further a stepwise regression was conducted. A model which included age and group was statistically significant at predicting MEP amplitude just head of the execution of a volition movement ($F(2, 31) = 6.628$, $p<0.005$) and explained between 25-30% of the variance ($R^2 = .299$, Adjusted $R^2 = .254$. Age and group had equal weights. The unstandardised and standardised coefficients, the Pearson's correlation coefficients, squared semipartial correlations, and their structure coefficients are presented in **Table 3.4**.

Table 3.4: A Stepwise Regression model using age and group to predict MEP amplitude between 70-100% of movement preparation. This table presents each factor's: unstandardised and standardised coefficients, Pearson's correlation, squared semipartial correlations, and their structure coefficients.

Model	b	*SE-b*	Beta	Pearson *r*	sr^2	Structure Coefficient
Constant	2.645	.490				
Age	.067	.025	.398	.405	.158	.740
Group	-.382	.156	-.368	-.375	.135	-.686

When grouping the data by age, it becomes clear that the differences in motor excitability are driven primarily by the younger participants. This can be seen by dividing the data into two sub-groups: adolescents (i.e. under 18 years, N = 10) and young adults (aged 18 years or over; N = 7). Please see **Figure 3.1Figure 3.10** for the relevant data. On inspection of the figure, whilst there is a large difference between MEP amplitudes in the youngest participants, there is no group difference in the young adult group. This suggests that it is the adolescents rather than the adults with TS that exhibit a marked reduction in motor excitability during motor preparation.

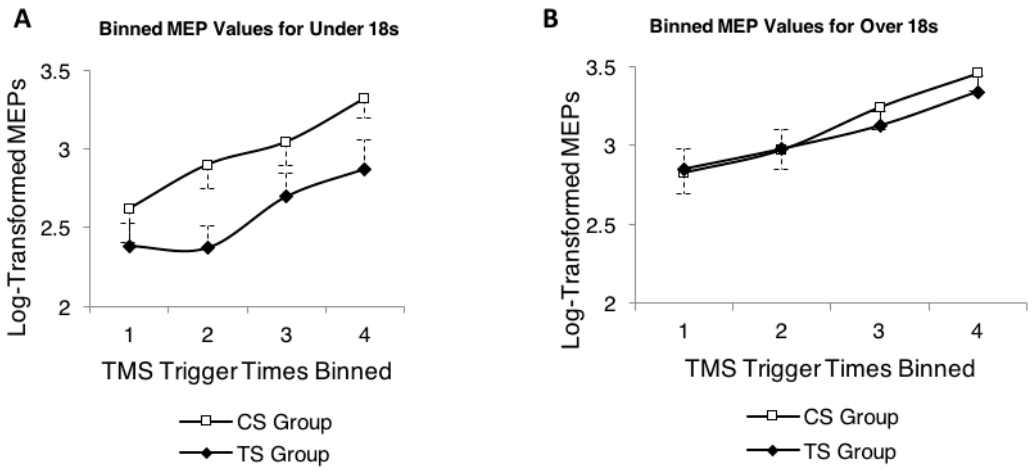


Figure 3.10: Log-transformed MEP values (to a base of 10) were binned into 4 stages of movement preparation and means were plotted. Error bars are standard error of the mean. Data was split into two groups: **Figure 3.10A)** those under 18 (n = 20) and **Figure 3.10B)** those 18 years or above (n = 14). It is evident that the difference in age (between groups) and group revealed by the two-way mixed ANOVA conducted (F(1,31)-11.36, $p<0.005$ and F(1,31) = 5.77, $p<0.05$ respectively) is driven by a big difference in the younger group of TS.

Tic scores were not significantly related to CSE measured during Go Trials during the Go/No-Go paradigm.

3.4.2.3 Individual MEP Variability Throughout Movement Preparation

To investigate differences in variability between the groups I computed the CoV of the log-transformed MEP data for each individual at each TMS onset time bin, see **Figure 3.9D** for the corresponding graph. These data were entered into a

mixed ANOVA with Group (TS vs. CS) as a between-subject factor, TMS onset time (0–29%, 30–49%, 50–69%, 70–100% of RT) as a within-subject variable and Age as a covariate. The ANOVA revealed a main effect of age ($F(1,30) = 7.837$, $p<0.01$). By contrast, there were no other significant main effects. Unlike CoV reduction during gain during TMS intensity, there was no effect of TMS onset time ($p = 0.277$, G-G corrected). Neither was there a significant main effect of Group, Group × TMS onset time interaction nor Age x TMS onset (minimum $p = 0.250$). These findings confirm that for both groups, TMS variability measured during movement preparation decreases with age.

3.4.2.4 Relationship between Input-Output Slope and Go/No-Go Slope

In both groups there is a close relationship between individual Input-Output slopes at rest (when calculated as a linear function) and the slope of CSE gain as a function of movement preparation. Those with TS have a global reduction of CSE during movement preparation in a Go/No-Go task the group and a global reduction of CSE at rest. A Pearson's Correlation shows that in both groups these two measures are strongly positively correlated (CS Group: $R=.792$, n = 17, $p < .0001$, TS Group: $R=.643$, n = 17, $p < .01$). See **Figure 3.11** for visual depiction of group correlations.

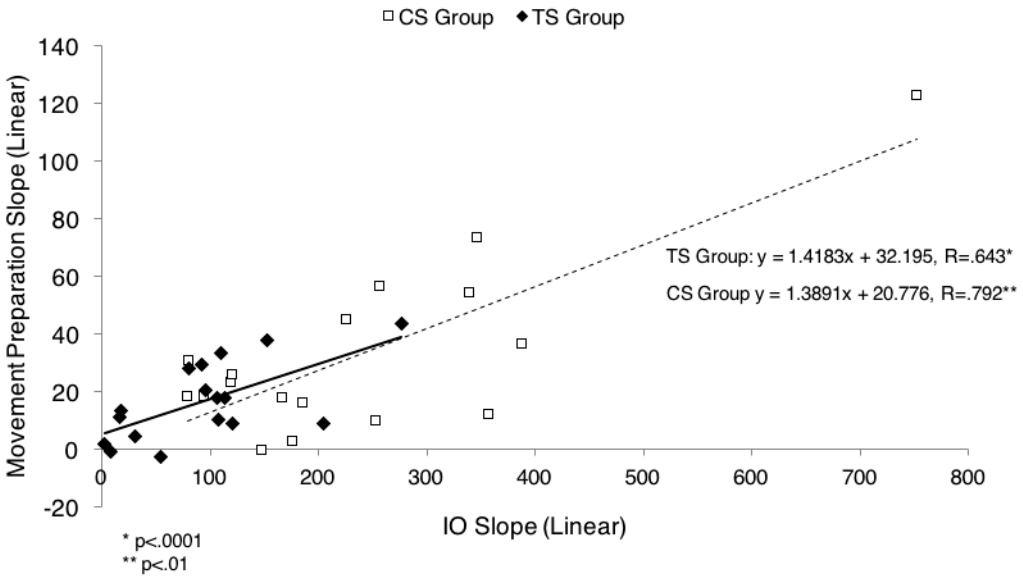


Figure 3.11: Linear slopes were calculated as a function of Input-Output Curves and as a function of movement preparation during Go trials in a Go/No-Go Task. These two measures are strongly positively correlated (CS Group: R=.792, n = 17, $p < .0001$, TS Group: R=.643, n = 17, $p < .01$) in both groups.

3.5 Discussion

Core physiological measures of motor excitability using TMS were measured in a group of young people with TS and a group of age and sex-matched controls. RMT, IO Curves and MEP gain during a volitional movement were measured. The key results that I have found are as follows:

1. Young TS patients exhibited increased RMT, reduced gain in motor excitability when indexed by IO Curves or ahead of volitional movements, and had higher MEP variability at rest.
2. All TMS measurements of motor excitability, including variability, were modulated by age; the differences in RMT and gain of the motor system were most pronounced in the youngest patients.
3. Behavioural task accuracy was comparable across patients and healthy controls, however, patients with TS were slower (higher RTs).

4. A strong positive relationship was found between IO curve linear slope and linear slope ahead of a volitional movement.

I will now discuss each of these findings in correspondence to adult studies with TS and postulate as to what significance the results regarding physiological measures of motor excitability may mean for young people with TS.

As discussed previously, TMS studies have demonstrated that MT is not usually affected in adults with TS. This is true in the vast majority of previous studies (Bäumer et al., 2010; K. F. Heise et al., 2010; Orth, Amann, et al., 2005; Orth et al., 2008; Suppa et al., 2011). One study, however, that looked at a large group of unmedicated adults with TS that presented with increased RMT (Orth & Rothwell, 2009). In my experiment, RMTs were similar to the healthy controls in the older subgroup, which is in keeping with most adult studies that report no threshold differences.

Perhaps surprisingly, other TMS studies assessing younger samples did not find an increase in RMT (Draper et al., 2015; C. A. Heise et al., 2008), despite the similarity of the range of age studied (11-20 and 10-15 years respectively). Neither experiment explicitly looked at the influence of age to the TMS assessed motor excitability and so it is uncertain whether there was any difference of threshold in the youngest patients. It is worth noting, however, that the method of calculating RMT was very different in both these studies. Draper and colleagues (2015) used a visible muscle twitch in the FDI as their criterion and C. A. Heise and colleagues (2008) used the maximum intensity to yield an MEP less than 50μV in the right abductor digiti minimi (ADM) muscle. Firstly, in both cases, the average MT across groups measured was much higher than in the current study (M = 52.5% and M = 66.8%

respectively) which could suggest that the thresholds were less sensitive. Secondly, the use of the ADM muscle is significant as it has been demonstrated in other diseases such as motor neuron disease that there are differential effects in FDI and ADM where the ADM is more likely to be spared and does not show threshold changes (Kuwabara et al., 2008). Additional evidence suggests that there may be differences in membrane excitability in the FDI and ADM (Jong et al., 2009). One final note of importance is to state that the sample size was larger in this study; this is important due to the heterogeneous profile of TS.

In keeping with my results, several studies conducted in adults and children, have demonstrated reduced gain in the motor system of patients with TS. This has been established using both IO curves and sp-TMS ahead of a volitional movement and is true of adults and children with TS. In one previous study, when patients with TS execute volitional movements, the increase in motor excitability prior to movement onset has been shown not to be accompanied by the reduction in variability that occurs in the healthy population (Draper et al., 2015; Klein-Flügge, Nobbs, Pitcher, & Bestmann, 2013). The typical reduction in variability is thought to be related to increasingly consistent neuronal firing rates in the PMC closer to movement onset, which is plausibly due to inputs from secondary motor areas (Klein-Flügge et al., 2013). **Experiment 2** showed that whilst there were increases in variability in the TS group; this was during rest and not whilst executing a volitional movement. This suggests that whilst there may be less evenly distributed excitability in the PMC at rest (Orth et al., 2008) this is compensated for during volitional movement in patients with TS.

Moreover, whilst SICI, a pp-TMS measure of physiological inhibition marking $GABA_A$ receptor function has been shown to be reduced at rest in adults with TS,

during movement preparation SICI normalises to a range comparable to healthy controls (K. F. Heise et al., 2010). K. F. Heise et al. (2010) argued that when executing a volitional movement patients with TS exert top-down control to suppress cortical hyper-excitability, recruiting help from distant brain regions such as the PFC and higher motor areas (K. F. Heise et al., 2010; S. R. Jackson, Parkinson, Jung, et al., 2011).

The results from this experiment do not show any evidence of hyperexcitability of the PMC in the measures taken, although reduced MEP amplitude certainly does not suggest more inhibition or less excitation. Despite this, the seemingly suppressed motor excitability does not appear to be an active suppression in the face of a state of hyperexcitability at rest. In contrast, in this study the state of reduced motor excitability appears to be a state of norm in the TS group. Although this state could be a mechanism that controls for the occurrence of tics; this would not explain why this reduction is most apparent in the younger patients.

Reduced gain could be resultant of tonic inhibition in the PMC mediated by either 1) local neurochemical alterations or 2) a brain region that outputs to the PMC and thus influences its excitability. A good candidate for 2 would be the supplementary motor area (SMA); the SMA has been shown to have increased levels of GABA as measured by 7T magnetic resonance spectroscopy (^{1}H-MRS) in adolescents with TS (Draper et al., 2014), which the authors suggest reflects tonic GABA rather than synaptic phasic GABA. The SMA has been implicated in the neurogenesis of tics and is one of the major outputs from the basal ganglia; it is conceivable that the tonic inhibition is reactive to the garbled messages it receives from subcortical structures.

My results are most compatible with the proposal that there is a delay in development of the motor network for children that have tics (Church & Schlaggar, 2014; Worbe et al., 2015). This is not necessarily incompatible with the idea that the SMA's increased inhibitory tone may influence PMC excitability given that this could be reactive to an 'immature' disinhibited motor network; in fact, if reactive it is likely that the inhibitory tone would be greatest when the network is most disinhibited and deviant from an optimum state. In addition, this evaluation of my results could explain why adults with TS have been found to have reduced measures of CSE (shallower gain in IO curves for example). The perseverance of severe tics into adulthood most likely relates to either 1) a failure for the motor networks to catch up with typically developed adults or 2) a failure to develop beneficiary adaptive mechanisms to outgrow tics (resulting in 'normal' motor output).

There are two key fMRI studies that indicate that the sensorimotor network in TS is functionally 'immature' in patients with TS: one study was conducted in children and one in adults. A resting-state connectivity fMRI study conducted in children compared the connectivity of key regions in two control networks: 1) the fronto-parietal network that is involved in attentional control and specifically rapid adaptive trial-by-trial control and 2) cingular-opercular network that is argued to be more involved in sustained 'set-maintenance' across a task and tonic alertness (Church et al., 2008; Dosenbach, Fair, Cohen, Schlaggar, & Petersen, 2008). They assessed maturity of the connectivity of regions by comparing the coefficient of connectedness to a large dataset looking at the development of these control networks in children (Fair et al., 2007). Coefficients of connectedness in TS control networks mirrored networks in lower aged children. Connected posterior regions in TS tended to be between short distant regions, rather than long-range connections that were adopted in the more 'mature' network configurations.

The resting-state fMRI study in adults used graph theory to characterise CSTC pathways: specifically, sensori-motor, associative and limbic networks (Worbe et al., 2012). They found that the degree (number of connections linking a node to the network) and strength of connections were higher and characteristic path length (average shortest path between all nodes in a network) were lower in patients with TS. Furthermore, there were no 'hubs' detected in patients with TS unlike in the control group where the pallidum, thalamus, hippocampus and amygdala were all identified as hubs. Children tend to have higher numbers of connections, lower average path lengths and higher efficiency of local transfer between subcortical and cortical region compared to adults – this was more akin to the data found in the TS group whom also have stronger connections. Interestingly, in this study efficiency of local information transfer in the left PMC was negatively associated with tic scores; suggesting that those with more efficient local connections and higher robustness had reduced tic severity.

A complementary experiment looking at structural connectivity from the same group assessed white matter connectivity (Worbe et al., 2015). They found that connections in the left PMC were positively associated with tic severity (i.e. higher connections were associated with more severe tics). This curious discrepancy may distinguish between cause and compensation; whereas the increased and perhaps noisy WM connections to the PMC may contribute to the occurrence of tics, when actively suppressing tics in the scanner those with highly efficient local connections to the PMC are at an advantage and have lower tic scores. It is worth considering that higher variability may be indicative of more noisy inputs, whilst reduced gain could be related to close regions exerting tonic inhibition on the PMC. In the majority of adolescents with TS that tics decline into adulthood, additional or distinct

compensatory mechanisms may be in place that we do not see in the often-studied niche group of adults with TS.

The results from the simple Go/ No-Go task supports the idea that young people with TS in fact do not have an impairment of inhibitory control particularly in simple cognitive control tasks (G. M. Jackson et al., 2015). Performance here was not compromised by TS, except slowed response times. These results are consistent with other studies using similar tasks with both children and adults with TS. Several experiments using different adaptations of Go/No-Go tasks have shown either no difference in performance indexed by response time or accuracy (adults: [Biermann-Ruben et al., 2012; Hershey et al., 2004; Serrien, Orth, Evans, Lees, & Brown, 2005], children: [Roessner, Albrecht, Dechent, Baudewig, & Rothenberger, 2008]). Other studies that have employed similar tasks have found the same results as in this current experiment: slowed response times but no differences in accuracy (adults: [Thomalla et al., 2014], children: [Eichele et al., 2010; Shephard, Jackson, & Groom, 2016]).

The neurophysiological states that accompany the slowed response times, namely reduced gain of motor excitability, is likely to be related to this. In a previous study exploring MEP variability during action preparation, trials in which a faster decrease in variability occurred were associated with faster response times (Klein-Flügge et al., 2013).

In summary, the neurophysiological findings of increased RMT, reduced gain at rest, reduced gain whilst executing volitional movements and increased variability of TMS-induced MEPs which normalise with age over adolescence is likely due a developmental delay in the maturation of key motor networks within the brain. This is

consistent with recent neuroimaging studies that assess functional and structural connectivity and other electrophysiological studies in patients with TS. These physiological differences may result in behavioural consequences, such as the slowed response times I found in the TS group.

Chapter 4: Investigation of Corticospinal Excitability in Young People with Tourette Syndrome during a Bimanual Motor Planning and Execution Task

4.1 Introduction

Preparation of action involves several processes occurring in distinct regions in the brain that all feed into the fluid execution of an appropriately planned and correctly selected action. TMS-induced MEPs reflect both local intracortical excitatory and inhibitory processes at the level of the PMC and processes occurring across the cortex which directly or indirectly mediates the output of the PMC (Bestmann & Krakauer, 2015). We can observe two important characteristics of TMS-induced MEPs to provide us information about what the PMC and other areas involved within the task are doing – namely peak-to-peak amplitude and trial-by-trial MEP amplitude variability. MEP variability may reflect the fluctuations in motor excitability at the level of the cortex and it is thought that anything that biases pools of neurons to fire within the PMC will reduce variability (Kiers, Cros, Chiappa, & Fang, 1993; Klein-Flügge et al., 2013). Measuring these two factors (MEP amplitude and variability) throughout a time-course during action preparation can thus be informative about the sorts of processes that may be involved in planning, selection and execution of an action.

Using TMS to look at task-related MEPs is a particularly useful technique for exploring PMC excitability in a sample of young people with Tourette Syndrome for several reasons. Given that MEP data can provide us with rich (albeit nonspecific) information about what is happening at the level of the PMC and connected regions, we can explore a changing time-course of global motor excitability not only during the execution of an action, as in **Experiment 2**, but to look at the preparation of action (which may involve de-selecting competing actions) and the stopping of action. We

can therefore look at the functional consequences of basal ganglia dysfunction and compensatory cerebral plasticity and hypothesise what compensation may be going on to prevent the occurrence of tics throughout daily life. For instance, looking at MEPs during a period that a participant is required to prepare a competing response or where the participant must withhold a response that was already prepared may give us clues about active tic suppression and 'unconscious' control mechanisms for the suppression of tics. This of course lies on the assumption that we can draw parallels between tics and voluntary action and tic suppression, reactive stopping and selective inhibition.

There are several hypotheses about compensatory mechanisms that may underlie tic control in Tourette Syndrome, allowing the majority of children with TS to remit from tics by early adulthood. The intention of this experiment is to provide evidence that will enable us to tease apart these theories. The underlying theory that tic suppression may be implemented through enhanced control of motor output will be tested and the resulting MEP data may shed light to how this may come about. Is it likely that the recruitment of prefrontal areas is responsible for the enhanced control of motor output that is evidenced in TS? If, for instance, differences in motor excitability are specific to the execution of action or are driven by baseline differences, it is unlikely that frontal areas are involved in the regulation of motor output in our sample of patients with TS. There is a wide range of evidence that supports the idea that prefrontal regions are involved in specific processes associated with action such as reactive stopping of an action (Aron, 2011). Thus, assessing motor excitability during a period where a participant must withhold an already prepared action may be informative of whether prefrontal areas (namely rIFC or pre-SMA) influence motor output in a functionally important manner in patients with TS; over and above that in healthy controls. Furthermore, one can assess motor

excitability during action preparation and selection of a correct response whilst inhibiting an inappropriate response and impulse control (preparation of a response whilst guarding for premature response).

In this experiment, I will assess motor excitability in a group of young people with TS and sex and age-matched controls in an adapted Go/No-Go task. The task involves the participant to select a response (either left or right index finger button press) following a preparatory cue and then during the execution of an action after an imperative Go signal or the withholding of a pre-potent response following a 'No-Go' or stop signal. Left-hemisphere TMS-induced MEPs will be collected at various time points in each different condition which allows the exploration of motor excitability during a preparatory, executory or stopping phase in a selected or non-selected effector. I predict that I will find evidence of reduced motor gain in patients with TS as in **Experiment 1** and **2**. I will explore whether there is evidence of differential modulation of CSE during a period of movement preparation without execution; patients with TS will show an increased gain of motor excitability (greater MEP amplitude) during movement preparation in the absence of motor execution if frontal or higher order secondary motor areas (such as the SMA) are engaged to a greater extent that controls. If greater gains in motor excitability are accompanied by reduced tic severity it is likely that increased recruitment of frontal/secondary motor areas are compensatory.

4.2 Method

4.2.1 Participants

Thirteen young people with TS and twelve sex and age-matched controls took part in **Experiment 3** with a minimum age of 10.90 and a maximum of 24.30 years (M = 16.41 ± 3.84 and 17.00 ± 3.50 respectively, one female in each group).

YGTSS scores were collected in the TS group; motor scores ranged from 11 to 23 with a mean of 14.77 ± 3.586 and phonic scores ranged from 0 to 19 with a mean of 9.23 ± 7.485. Three volunteers with TS had current diagnoses: ADHD, oppositional defiant disorder (ODD) and ASD and no volunteers were on neuropsychiatric medication at the time of the study. Due to time constraints, 3 of the control subjects had missing WASI scores. The remaining WASI scores had a mean of 116.89 ± 8.223 and 111.08 ± 16.89 for the CS and TS group, respectively. Please refer to **Table 4.1** for demographics of the participants that volunteered for this research experiment.

Table 4.1: Participant demographics for participants that took part in **Experiment 3**.

ID	Sex	Age	EHI Score	WASI	Comorbidity	Motor Tic Score	Phonic Tic Score	RMT	TP
CS080	M	19.3	60	-				40	47
CS110	M	17.4	75	97				34	45
CS114	M	15.4	-100	125				33	38
CS131	M	14.3	85	120				34	38
CS132	M	17.4	-70	116				43	50
CS146	M	12.3	60	119				42	54
CS157	M	13.3	60	124				41	48
CS160	M	14.9	85	115				37	42
CS182	M	15.0	80	120				45	51
CS225	M	19.5	-40	116				34	37
CS400	M	24.3	75	-				46	52
CS401	F	20.9	95	-				33	38
TS006	M	24.1	100	84		15	19	41	48
TS028	F	21.4	80	96		17	18	44	47
TS031	M	20.1	85	111		21	16	35	41
TS034	M	17.1	100	118		13	0	44	48
TS043	M	17.5	80	123		15	14	55	64
TS048	M	18.6	100	118		11	8	53	62
TS055	M	15.0	100	87	ADHD	23	16	45	53
TS069	M	13.5	100	133		12	0	63	70
TS071	M	15.3	100	118	ODD	12	7	52	64
TS082	M	13.3	100	124		13	0	58	68
TS101	M	10.9	80	129		14	15	60	69
TS102	M	14.4	100	111		13	7	37	42
TS113	M	12.3	95	92	ASD	13	0	49	60

4.2.2 Procedure

Participants were sat at a table, on an adjustable seat with their chin resting on a chin-rest. Small bean bags were placed under participants' hands to achieve a relaxed hand position to maximise comfort and minimise movement when responding with the button box.

4.2.3 Transcranial Magnetic Stimulation

A Magstim Bistim 2 machine was used to deliver single-pulse TMS (sp-TMS) in an unpaired configuration with a figure-of-eight coil that had a wing diameter of 70mm. BrainSight neuro-navigation software (Rogue Resolutions) utilised two trackers with reflective spheres and the Polaris camera to monitor the participants head and coil position. A T1 weighted anatomical brain scan was obtained prior to the study for all participants except two (one from each group). When an anatomical scan was available an automatic 3D curvilinear reconstruction of the participant's head and brain were created, this allowed the participant's head to be mapped on to their virtual head through a calibration procedure using a tracker secured to the participant's head and pointing with a tracked pointer tool at various landmarks that were marked on the virtual head in reality. The landmarks used were the tip of the nose, nasion and both inter-tragal notches of the ears; these landmarks allowed good registration of the participant's actual head to their virtual head model.

Once the participant was registered to their image on BrainSight a check was performed to guarantee good registration; the pointer was used to point at various positions on the scalp to confirm that actual scalp position was never more than 3.0 mm from the model scalp position. Good registration ensured two important details: firstly, we could track where the coil was in relation the participant's scalp accurately

and secondly, we could monitor where the participant moves to, allowing an accurate online correction for coil position.

The left hemisphere 'hand area' was located using anatomical landmarks (specifically the omega shape in the motor strip) and targeted on the neuro-navigation software. A virtual circular grid was superimposed over the anatomical target on the surface of the cortex with targets spaced 5 mm a part. This was then used to find the motor hotspot for the FDI muscle in the right hand. The coil was moved to each target systematically and five sp-TMS pulses were given, the coil was then moved to the next position and the targets that produced the MEPs with the largest peak-to-peak amplitude, with the least variability were sampled and tested again until the best sample was chosen for the rest of the study.

If a T1 anatomical scan was not available, the participant's head was calibrated to a model head on BrainSight and the navigation software was only used to track the participant's head and coil. The motor hotspot was found using a trial-and-error method moving the coil around in 5 mm intervals once a position was found to induce an MEP in the FDI muscle.

Two initial measures were taken; firstly, the resting motor threshold (RMT), defined as the lowest percentage of maximum stimulator output (MSO%) that was required to induce an MEP $\geq 50\mu V$ in 5 out of 10 trials using sp-TMS. Secondly, the MSO% was calculated for a test pulse (TP), to use during a behavioural task, which was required to induce an MEP $\approx 750\mu V$ at rest, over 10 trials (or maximum MEP if $750\mu V$ was unattainable). The stimulator was then set to the intensity found for the TP was the duration of the behavioural task.

4.2.3.1 Electromyography

Ag-AgCl surface electrodes were used with conductive gel and were placed in a belly-tendon montage over the FDI muscle on both the right and left hand. A ground was only placed on the right hand (over the ulnar styloid), given that MEPs were only measured from the right hand. Brainamp ExG and BrainVision Recorder software (Brain Products GmbH) were used to amplify, digitize, record, preprocess and visualise the EMG signal with a sampling rate of 5000 Hz with a sampling interval of 200 µS. Behavioural Task

4.2.3.2 Behavioural Task

The behavioural task used was an adaptation of a cued Go/No-Go Task. A white fixation cross was presented on a grey background for a random amount of time between 2000-3000ms, followed by stimulus 1 (S1) - a blue or yellow square – which was displayed for 500ms. Then, following a 1000-3000ms inter-stimulus interval stimulus 2 (S2) was presented - a green or red circle - for 500ms followed by a period of 2000ms where the participant could respond using a button box. S1 cued the participant to prepare either a left or right hand response (blue denoted left hand, yellow right hand), whilst S2 cued the participant to Go (green, respond with the correct hand) or No-Go (red, withhold a response). There were 168 trials in total with an equal number of left hand/right hand trials. 3/5 trials were Go (108) and 2/5 trials were No-Go (60). The monitor which the stimuli were displayed on was placed circa 80cm directly in front of the chin-rest.

Sp-TMS was delivered either in the interval between S1 and S2 (Period 1) or after S2 (Period 2); i.e. only one sp-TMS pulse was delivered per trial. This was to prevent pulses in close proximity affecting CSE in a paired-pulse TMS configuration. Pulses were delivered at 30/50/70% intervals of the period between S1 and S2 or at

30/50/70% intervals between the end of S2 and average RT, re-calculated after every trial. 50% of sp-TMS pulses fell in Period 1, whilst 50% of sp-TMS pulses fell in Period 2. Thus, CSE could only be probed in 27 Go trials per hand and so participants were instructed to only respond after S2 had disappeared from the screen in order to avoid trials that the sp-TMS pulse may have affected processing of the stimulus; TMS was only delivered after S2 had been displayed. Sp-TMS was only delivered to the left hemisphere primary motor cortex (PMC), the intensity used was the MSO% recorded for the TP at rest. Every 15 trials the participant was asked if they were comfortable and after 90 trials a longer break was offered if needed.

Prior to the experimental task, a block of 36 practice trials were completed without TMS where feedback was given to ensure participants did not respond too quickly and that they understood the task well. An initial average RT was calculated from the practice trials to compute when sp-TMS was to be delivered in Period 2. For a visual depiction of the task please refer to **Figure 4.1.**

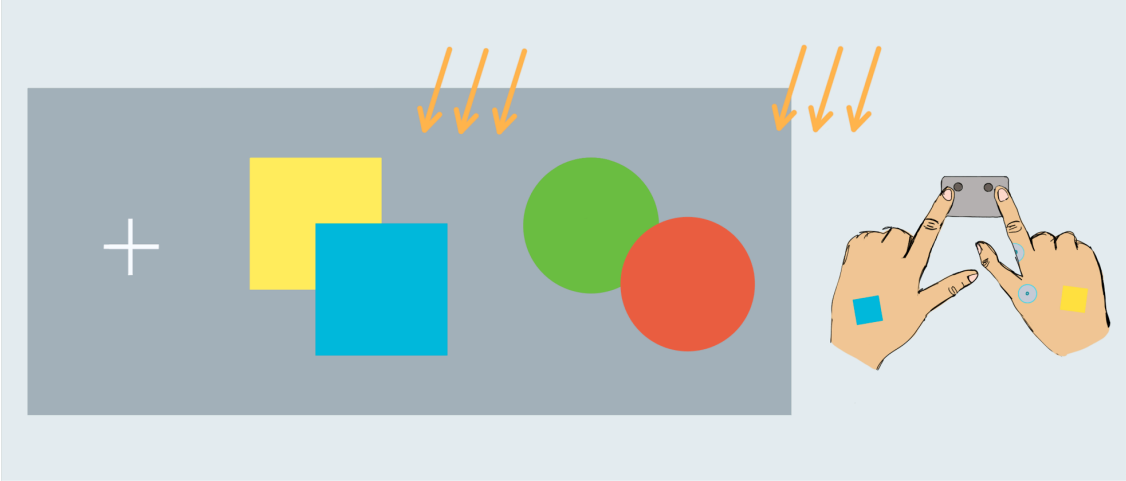


Figure 4.1: A graphical representation of the combined TMS and behavioural task. A fixation cross was presented followed by stimulus 1 (S1) which cued the participant whether they would be using their left hand (blue) or right hand (yellow) to press a button following stimulus 2 (S2) if it was a 'Go' trial (green circle), participants were to withhold a response if they saw a 'No-Go' trial (red circle). On each trial TMS was either delivered once between S1 and S2 or after S2 at 30/50 or 70% of an updated individual response time (RT).

4.2.4 Electromyography Data Pre-processing

As in **Chapter 3** MEPs were log-transformed to base 10 for the same reasons as I detailed in the previous chapter. Grand means were calculated in separate bins, separately for each hand, binned times were as follows: Bin 1 (0-35% of Period 1), Bin 2 (36-50% of Period 1), Bin 3 (51-100% of Period 1), Bin 4 (0-40% of Period 2), Bin 5 (41-80% of Period 2) and Bin 6 (81-100% of Period 2).

As well as calculate peak-to-peak MEP amplitude, I quantified the amplitude of the peak-to-peak signal in a 50ms window prior to TMS stimulation for each trial, and I found a positive relationship between the average amplitude of that signal (indicating muscle activity) and MEP amplitudes collected during the task even when excluding trials based on stringent outliers (outside 2 SDs from the mean of log-transformed data). Whilst there was no significant group difference between this baseline signal amplitude, assessed by a two-way t-test ($p = 0.178$), in order to take

this difference into account I took all MEPs as a ratio to the baseline noise. Example of relationship between baseline signal and MEP amplitude can be found in **Figure 4.2.**

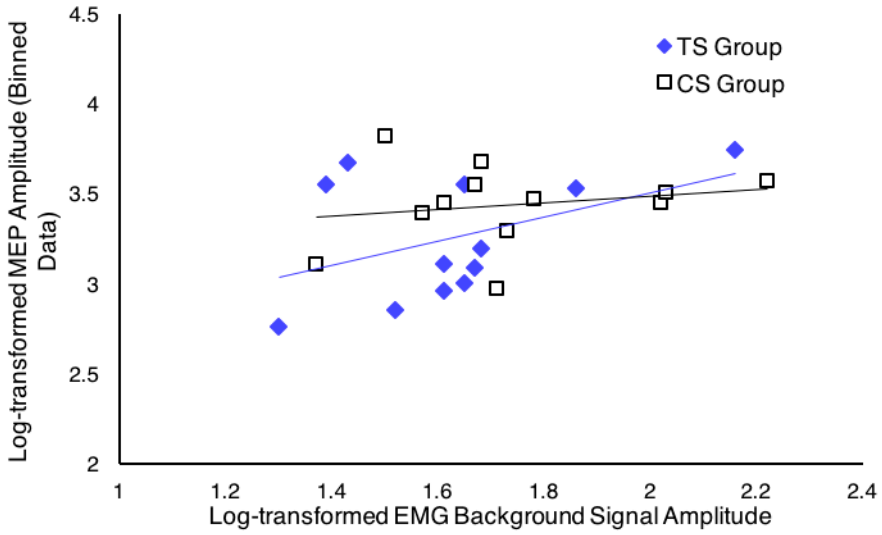


Figure 4.2: Scatterplot showing the positive relationship between EMG background signal and MEP amplitude in binned data collected during the behavioural task. TS Group in blue ($R = 0.44$) and CS Group in black ($R = 0.19$).

4.3 Results

Participant demographics were compared using independent t-tests to check that the groups were well matched. Student's t-tests revealed that there were no significant differences between age, sex or WASI score ($p = 0.336$-0.955). **Figure 4.3** depicts the spreads of age and WASI scores for both group.

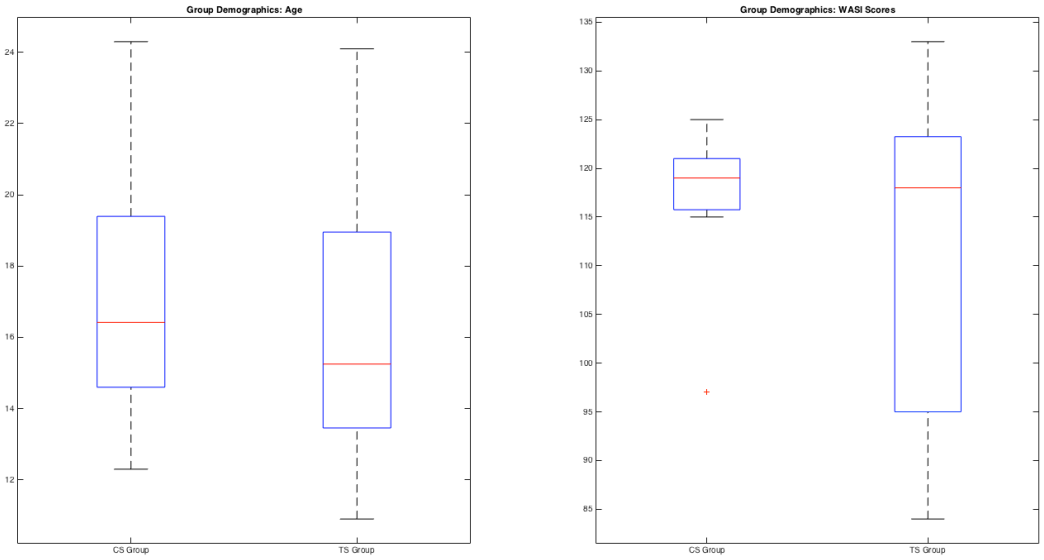


Figure 4.3: Participant Demographics shown as box plots, age and WASI scores

Edinburgh handedness inventory (EHI) scores (10 item and extended 15 item) significantly differed between groups, CS (M = 45.8, SD = 58.0) and TS Group (M = 94.1, SD = 8.8). A Mann-Whitney U test was employed as these scores deviated significantly from normality (assessed by a Shapiro-Wilk test) and the groups did not have equal variances (Levene's test), $U = 143$ and $U = 144$, $p<0.001$. In our sample, the controls had a more varied handedness score than the TS who grouped very close to 100 (i.e. very right handed), please see **Figure 4.4**.

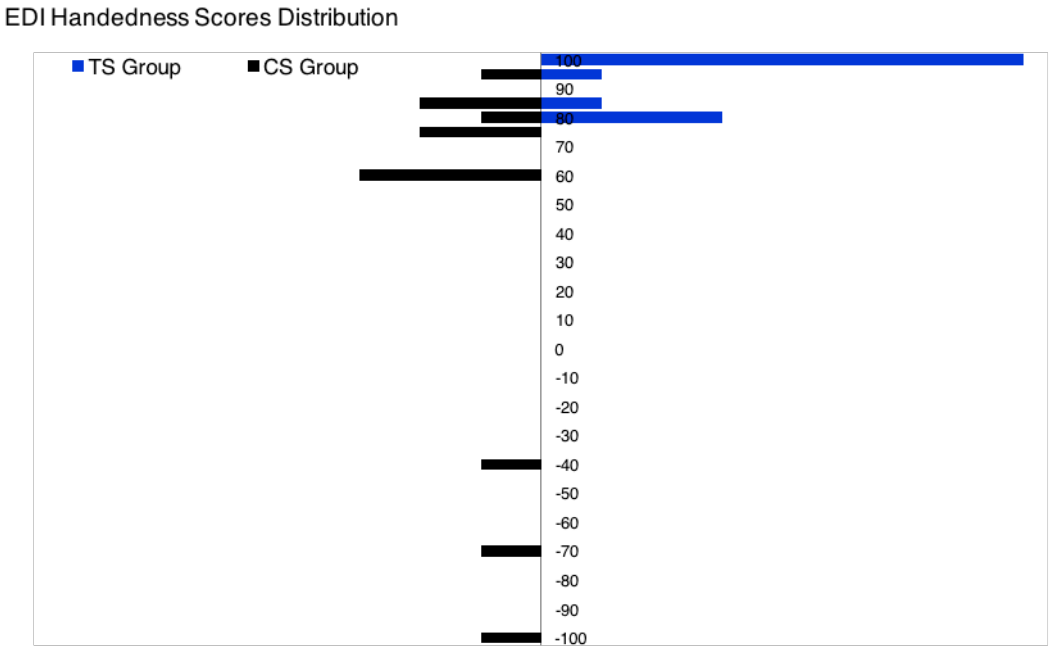


Figure 4.4: Edinburgh Handedness Inventory Score Distribution, standard items.

4.3.1 Clinical Scores

The motor and phonic scores from the YGTSS were used in statistical analysis to assess whether behavioural or motor excitability measures were related to clinical symptomatology in the TS group. Motor scores ranged from 11 to 23, median = 13 and phonic scores ranged from 0 to 19, median = 8. Please view **Figure 4.5** to visually assess individual and group phonic and motor tic scores.

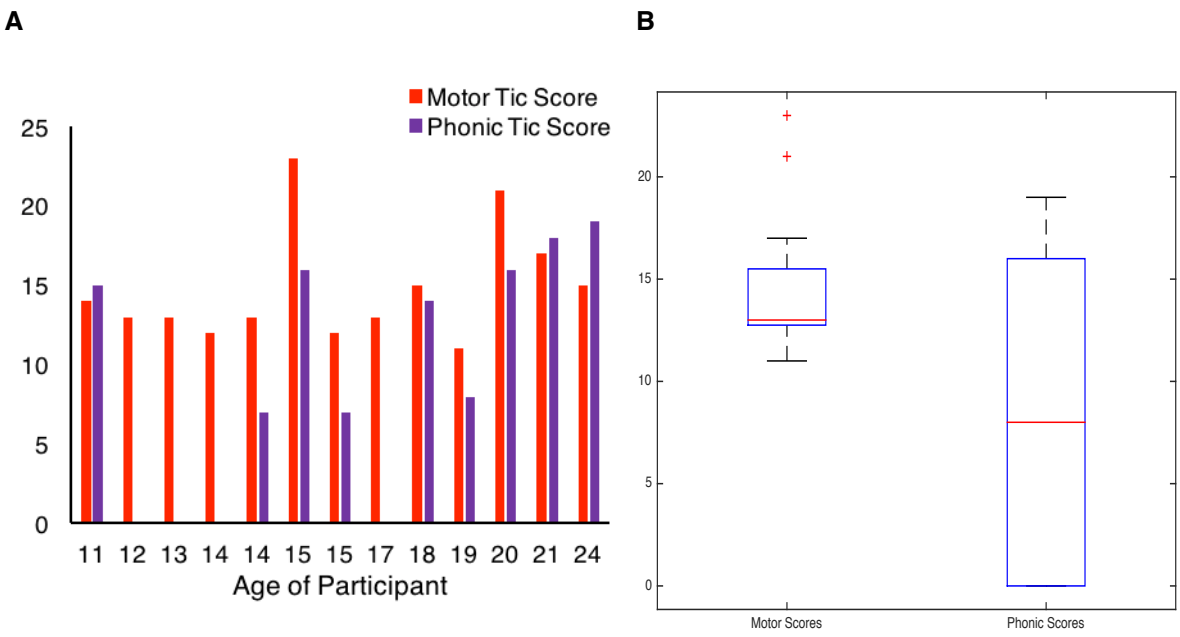


Figure 4.5: A) Motor tic scores and phonic tic scores for each individual with TS, organised by age. **B)** box plots to show distribution of motor and phonic tic scores. Red line is median, blue box is interquartile range, whiskers end on minimum and maximum values up to 2.71 standard deviations of the data, outliers are represented by red crosses.

4.3.2 Behavioural Results

4.3.2.1 Response Times

A mean response time (RT) was calculated for each participant in two ways. The first calculation is the time taken to press a correct button response from the time that S2 had finished presenting (500ms after onset) - bpRT. The second definition is the onset of EMG movement (emgRT) from termination of S2. To calculate this, the EMG signal was visually inspected for each trial (from onset of S1 until the time of the button press) and the onset of movement of the button press was selected in the signal and timestamped for each trial. **Table 4.2** shows details of bpRT and emgRT for right and left-cued trials for each group. Since participants were instructed to only press the button once the stimulus disappeared from the screen it was not expected that there were any differences between group mean responses.

Table 4.2: Mean response time (RT) ± SD for CS and TS group presented as RT from button press (bpRT) and from start of activity on the emg signal (emgRT) in milliseconds (ms) separated into right-cued and left-cued trials

	bpRT (ms) Right-cued trials	**Left-cued trials**	**emgRT (ms) Right-cued trials**	**Left-cued trials**
CS Group	331 ± 129	315 ± 136	88 ± 127	118 ± 117
TS Group	307 ± 98	324 ± 107	119 ± 90	185 ± 109

There are no significant differences between groups in right or left trials, irrespective of whether the RT was defined as a button press or EMG movement onset (p = 0.156-0.861). Groups were thus collapsed and there was a clear difference between bpRT and emgRT as one may expect. A paired samples t-test demonstrated that in the RH condition and the LH condition, bpRT had an onset significantly further in time than emgRT ($t(24) = 21.236$, $p<0.001$, $t(24) = 16.386$, $p<0.001$). This is unsurprising, however, surprisingly there was a significant difference found between RH and LH emgRTs ($t(24)=-4.941$, $p<0.001$, which was absent if one looked at bpRT (p = 0.897). Reassuringly, mean bpRT and emgRT were strongly correlated in LH and RH trials (r = 0.896-0.907, $p<0.001$). Furthermore, in each individual's data, emgRT and bpRT correlated well with a minimum r coefficient of 0.51 and a maximum of 0.97. This suggests that emgRT is a more sensitive way at assessing response times than bpRT.

In order to test if there was an effect of TMS stimulus on response time, trials were split into LH and RH trials, and TMS given prior to S2 or after S2. If TMS stimulus interfered with RT due to the sound emitted one may expect that responses made when sp-TMS was delivered after S2 would be delayed in comparison to when

sp-TMS was delivered before S2. There may be more interference with RH trials given that there is a muscle twitch induced with each pulse.

This is exactly what was found: paired sample t-tests demonstrate that when TMS is delivered in Period 1 (P1), RT is faster than when TMS is delivered in Period 2 (P2). This is not simply for right-cued trials but also left-cued trials. Whether you use emgRT or bpRT the result is the same – participants respond faster to right-cued GOs and left-cued GOs when TMS is delivered in P1 ($t(24)=-6.777$, $p<0.0001$; $t(24)=-3.688$, $p<0.01$). For details of mean RT in milliseconds from termination of S2 presentation (as emgRT) please see **Table 4.3**.

Table 4.3: Mean RT ± SD in milliseconds from termination of S2 presentation (as emgRT) separated by when TMS was delivered for which hand trial

	TMS in Period 1		**TMS in Period 2**	
	Right Trial RT	**Left Trial RT**	**Right Trial RT**	**Left Trial RT**
CS Group	18 ± 34	101 ± 31	157 ± 47	135 ± 41
TS Group	54 ± 116	138 ± 124	185 ± 89	232 ± 115
Across Groups	37 ± 114	120 ± 114	171 ± 124	186 ± 133

4.3.2.2 Accuracy

Participants made very few errors in the adapted Go/No-Go task. There were no differences between groups when analysing the distributions of error types across each group (Mann-Whitney U Test: minimum $p = 0.186$). However, the distributions across different error types made (incorrect hand go/ false positive with correct hand/ false positive with incorrect hand) were significantly different. A non-parametric Friedman's Two-Way ANOVA by ranks rendered a Chi-square value of 24.99, $p<0.001$. Whilst false positive (initiating a movement during No-Go trials) and false negative (omitting a response during Go trials) errors were negligible, errors were made where the incorrect hand was used to respond. The CS group made a mean

average of 1.92 errors in total and the TS group made a mean average of 3.07. For information about types of errors made please refer to **Table 4.4**.

Table 4.4: Error types separated by hand for each group (Mean ± SD)

		Incorrect Hand Go	False Positive Correct Hand	False Positive Incorrect Hand	False Negative	Total
CS Group	Right-cued	0.83 ± 1.27	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.29	0.92 ± 1.24
	Left-cued	0.75 ± 1.36	0.17 ± 0.39	0.00 ± 0.00	0.08 ± 0.29	1.00 ± 1.35
TS Group	Right-cued	0.77 ± 1.01	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.38	0.92 ± 1.04
	Left-cued	1.38 ± 1.89	0.15 ± 0.55	0.15 ± 0.55	0.46 ± 0.77	2.15 ± 2.27
Average across groups	Right-cued	0.80 ± 1.12	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.33	0.92 ± 1.12
	Left-cued	1.08 ± 1.66	0.16 ± 0.47	0.08 ± 0.40	0.28 ± 0.61	1.60 ± 1.94

4.3.3 Cortical Excitability

A one-way independent samples t-test was employed to see whether there were differences between RMT. As expected there was a significant difference between the CS group (M = 38.5, SD = 4.9) and the TS group (M = 48.8, SD = 8.8). $t(19.7)=-3.697$, $p<0.01$. A Levene's test revealed unequal variances and thus the df was reduced from 23 to 19.048). Refer to **Figure 4.6** for mean RMT and information about the distribution of group RMT.

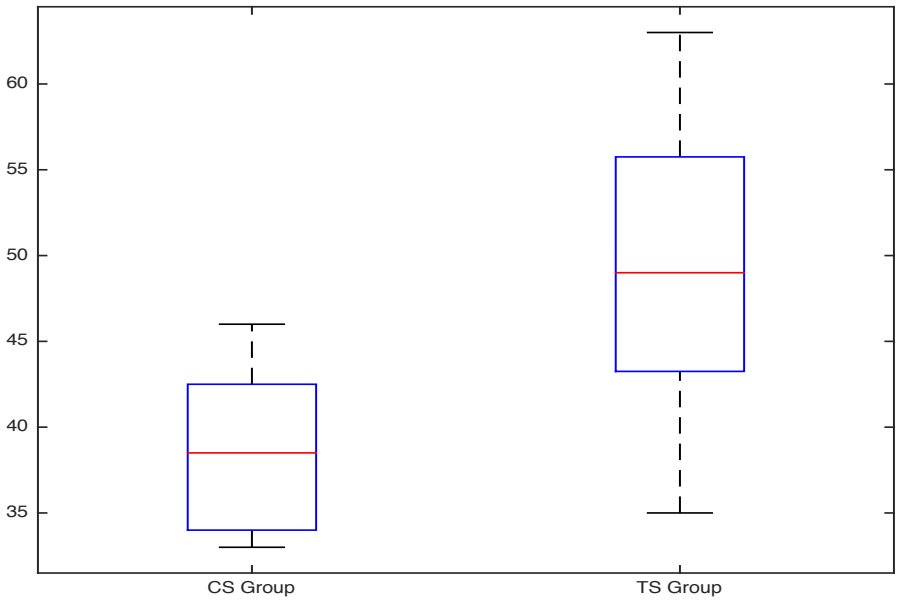


Figure 4.6: Box plots of each groups' average RMT with red line as median RMT, the blue box as interquartile range and the whiskers as minimum and maximum values for the group.

The TP used during the experiment was a mean of 116.25% of RMT, this did not differ between groups (TS M = 115.66±4.67, CS M = 116.89±6.83).

MEP data collected during the behavioural task were analysed using grand means of individually binned mean log-transformed MEP data depending on time of stimulation. Individual variability was also characterised by creating CoV for log-transformed data for each bin. Data was binned as follows: in period 1 (the period after S1 but before S2 presentation) MEPs were binned into three bins: 0-35% (bin

1), 36-50% (bin 2) and 51-99% (bin 3) of the time interval between presentations. Data after S2 presentation were binned as follows: 'No-Go', 0-40% of RT to Go trial (bin 4), 41-80% (bin 5) and 82 -100% (bin 6). Data was separated for left-cued trials and right-cued trials. All trials are thus as follows: right-cued period 1 (rhP1), left-cued period 1 (lhP1), right-hand 'Go' (rhGo), left-hand 'Go' (lhGo), right-cued 'No-Go' (rhNo-Go), left-cued 'No-Go' (lhNo-Go). Bins 1-3 were from period 1, Bins 4-5 were from Period 2 ('Gos'). All MEPs were collected from right hand and all stimulation was at the left hemisphere PMC.

Table 4.5: Grand Mean ± SD are presented for CS and TS group for log-transformed MEP data collected during a Go/ No-Go behavioural task. Period 1 is the period between S1 and S2 where S1 cues either the left hand or right hand to make a movement (or withhold a movement) after presentation of S2. Period 2 is the period between S2 ('Go' or 'No-Go') and individual response time. MEPs are binned dependent on time of TMS delivery and are separated into left-cued trials and right-cued trials.

		Period 1			Period 2			
		Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6	No-Go
CS Group	*Left*	1.96 ± 0.08	1.98 ± 0.10	1.95 ± 0.09	1.88 ± 0.08	1.91 ± 0.09	1.97 ± 0.10	1.92 ± 0.08
	Right	2.01 ± 0.08	1.99 ± 0.09	2.01 ± 0.08	2.16 ± 0.09	2.23 ± 0.10	2.26 ± 0.11	1.94 ± 0.08
TS Group	*Left*	1.97 ± 0.07	1.93 ± 0.08	1.93 ± 0.08	1.93 ± 0.09	1.94 ± 0.09	2.04 ± 0.11	1.87 ± 0.08
	Right	2.01 ± 0.07	2.01 ± 0.09	2.04 ± 0.08	2.23 ± 0.08	2.40 ± 0.10	2.47 ± 0.09	1.95 ± 0.08

Table 4.6: Individual MEP variability (Mean of binned individual CoV) ± SD are presented for CS and TS group for log-transformed MEP data collected during a Go/ No-Go behavioural task. Period 1 is the period between S1 and S2 where S1 cues either the left hand or right hand to make a movement (or withhold a movement) after presentation of S2. Period 2 is the period between S2 ('Go' or 'No-Go') and individual response time. MEPs are binned dependent on time of TMS delivery and are separated into left-cued trials and right-cued trials.

		Period 1			Period 2			
		Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6	No-Go
CS Group	*Left*	0.08 ± 0.04	0.07 ± 0.03	0.09 ± 0.05	0.09 ± 0.06	0.08 ± 0.05	0.08 ± 0.08	0.09 ± 0.04
	Right	0.07 ± 0.03	0.07 ± 0.05	0.07 ± 0.03	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.05	0.08 ± 0.04
TS Group	*Left*	0.10 ± 0.04	0.11 ± 0.08	0.12± 0.05	0.10 ± 0.06	0.09 ± 0.05	0.08 ± 0.04	0.13 ± 0.06
	Right	0.12 ± 0.05	0.12± 0.07	0.11 ± 0.05	0.09 ± 0.04	0.05 ± 0.03	0.04 ± 0.03	0.12 ± 0.05

4.3.3.1 MEPs measured between S1 and S2 (Period 1) during a response preparation

MEPs were all measured from the right hand, however, during this phase participants had either been cued their left hand or their right hand. Data was thus initially split into left-cued trials and right-cued trials, and will be referred as lhP1 and rhP1 trials, and analysed for each participant using EEG Lab in Matlab and an in-house Matlab script to measure peak-to-peak MEP amplitude in response to a TMS stimulus. Mean log-transformed MEP data were calculated, first in bins of their corresponding time after S1 presentation (refer to **Figure 4.7**). Data presented in **Figure 4.8** shows the MEP amplitudes for each group binned, for LH and RH trials.

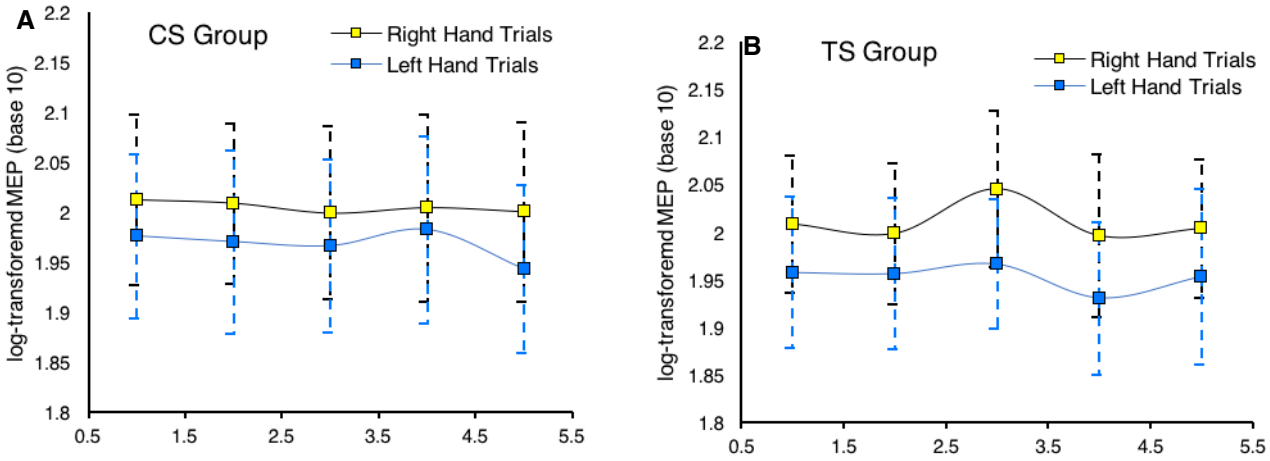


Figure 4.7: Right hand mean log-transformed MEPs were calculated for five different bins after S1 onset (i.e. during Period 1). The first bin was between 300-600ms after S1 onset, the next 601-900ms, then 901-1100ms, then 1101-1499ms and final 1500-2120ms. Error bars are SEM, filled blue squares are cued left hand trials and filled yellow squares are cued right hand trials and **A** is trials for the CS group and **B** depicts trials for the TS group.

The purpose of this was to sensitively assess whether there were functional fluctuations in CSE in response to being presented the S1 cue and awaiting S2 presentation. MEPs were also binned according to when they were delivered as a percentage of the time between S1 and S2.

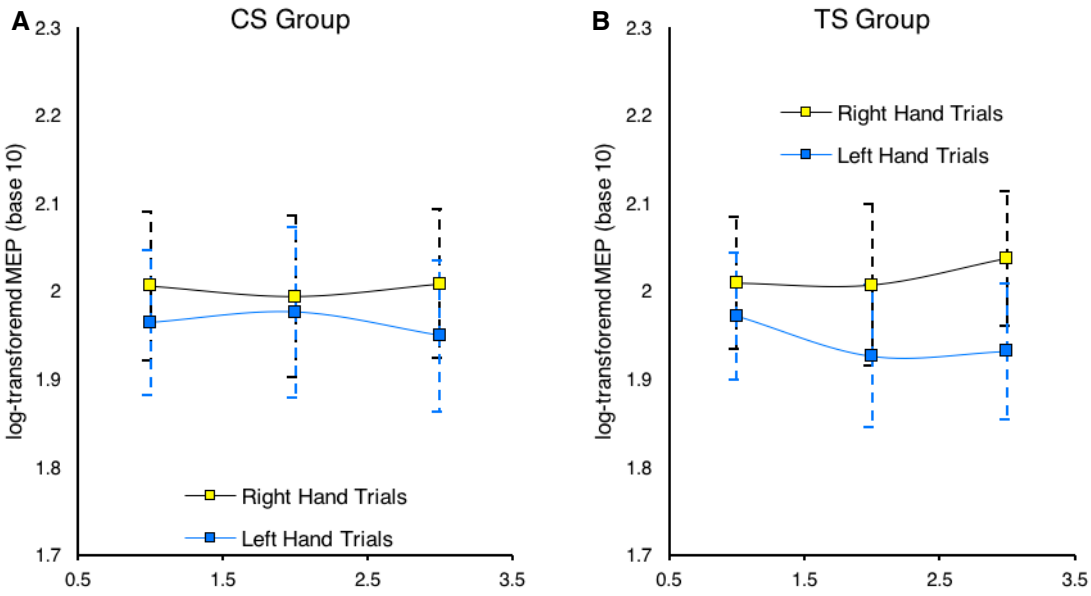


Figure 4.8: Right hand mean log-transformed MEPs were measured in three equal bins across the time course between presentation of S1, prior to onset of S2 (in the attention period). Error bars are SEM, filled blue squares are cued left hand trials and filled yellow squares are cued right hand trials and **A** is trials for the CS group and **B** depicts trials for the TS group.

Although this is a cruder measure it may be more sensitive to differences between trial type and groups since the data is grouped in a way to maximise amount of data in each bin. Statistical analysis was conducted on both five-binned and three-binned MEP data. Five-binned data did not result in any differences in results and so is not reported.

4.3.3.1.1 Do MEPs that originate from the right-hand PMC differ between right-cued and left-cued trials in period 1?

A two-way repeated measures ANOVA was used to assess whether RH MEPs (left PMC CSE) differed when preparing a LH or RH response. This was used in the CS and TS group separately. In P1, there was a significant main effect of hand in both the CS and TS group (CS: $F(1, 11) = 5.156$, $p<0.05$ and TS: $F(1,12) = 7.899$, $p<0.05$). In each group, as can be seen in **Figure 4.8**, MEP amplitude was greater in right hand trials. Importantly, there was no effect of bin in either group (minimum $p = 0.348$). Neither was a significant interaction between bin and hand found in either group (minimum $p = 0.135$). Motor excitability was thus increased in left hemisphere

PMC when preparing for a contralateral response vs. an ipsilateral response. This is despite any explicit execution of a movement.

4.3.3.1.2 Are there group differences between MEPs collected during left-cued trials in period 1?

A two-way mixed-design ANOVA was used to explore whether there were any differences between the CS and TS group in the lhP1 MEPs. No significant effects were found, i.e. no between subjects effect of group ($p = 0.856$), no main effect of bin was found ($p = 0.268$) and lastly no interaction effect between bin and group ($p = 0.240$).

4.3.3.1.3 Are there group differences between MEPS collected during right-cued trials in period 1?

A two-way mixed-design ANOVA was used to explore whether there were any differences between the CS and TS group in the rhP1 MEPs. No significant effects were found, i.e. no between subjects effect of group ($p = 0.894$), no main effect of bin was found ($p = 0.395$) and lastly no interaction effect between bin and group ($p = 0.747$).

4.3.3.1.4 Are there group differences between variability of MEPs collected during Period 1?

To answer the question if there were differences in the intrinsic left hemisphere CSE variability, CoV of the logged MEPs was calculated for each rhP1 bin for each individual. A two-way mixed design ANOVA was then used to compare the group means of CoV. This revealed a main effect of group ($F(1,23) = 7.037$, $p<0.05$. Patients showed increased MEP variability relative to controls. There was no effect of bin ($p = 0.712$, G-G corrected), nor an interaction effect between bin and group ($p = 0.584$, G-G corrected). Interestingly this effect changed when looking at lhP1 CoV values. When employing the same analysis there was no effect of group

(p = 0.093), no main effect of bin (p = 0.427). Again, there was no interaction effect (p = 0.674).

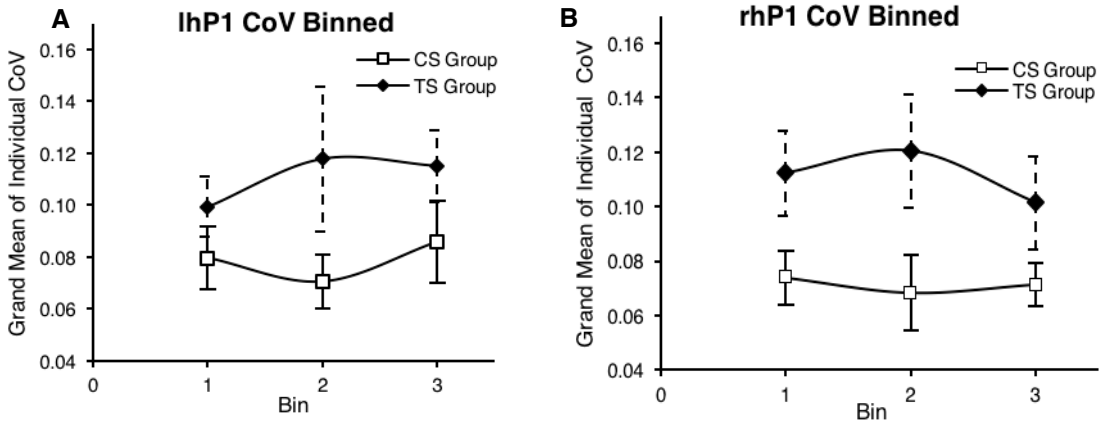


Figure 4.9: A shows mean of left-cued individual MEP variability (individual CoV) across period 1 (between S1 and S2) for CS group (empty squares) and TS group (filled diamonds) where movement is prepared but not executed. **B** shows mean of right-cued MEP variability (individual CoV) across period 1 (between S1 and S2) for CS group (empty squares) and TS group (filled diamonds) where movement is prepared but not executed.

4.3.3.1.5 Is there anything special about P1?

In order to explore whether there were any functional changes within the preparatory period over time, individual slopes were fitted to MEP amplitude by time of stimulation (in P1). Individual slope coefficients were calculated for LH trials and RH trials separately. Across groups, in rhP1 trials, slope coefficients ranged from -0.393 to 0.224 (M = -0.043, SD = 0.152). In lhP1 trials, slope coefficients ranged from –0.227 to 0.188 (M = -0.033, SD = 0.100). When investigating the coefficient of determination or correlation coefficients (R) it appears there is no evident linear relationship between rhP1 time or lhP1 time and MEP amplitude ([Right: R^2 – M = 0.029, SD = 0.026; R – M = -0.054, SD = 0.164]; [Left: R^2 – M = 0.028, SD = 0.030; R – M = -0.058, SD = 0.157]).

4.3.3.2 MEPs measured in Period 2 during execution of a voluntary motor response ('Go')

4.3.3.2.1 Is left hemisphere motor excitability modulated differently during execution of a right handed movement different than when preparing a left handed movement?

Left hemisphere TMS-induced MEPs were collected during the preparation of a right hand or left handed movement. To assess whether MEPs originating from the left hemisphere differed between left hand or right hand movements, these individual MEPs were log-transformed to base 10 and a two-way repeated measures ANOVA was used in each group separately. Firstly when looking at the CS group: there was a main effect of hand $F(1,10) = 18.681$, $p<0.01$, a main effect of bin ($F(2,20) = 8.098$, $p<0.01$). No interaction between bin and hand were found ($p = 0.556$, G-G corrected). Post-hoc bonferroni corrected t-tests confirmed that bin 6 (M = 2.111, SD = 0.093) was significantly increased compared to bin 5 (M = 2.072, SD = 0.093) and bin 6 was significantly increased compared to bin 4 (M = 2.033, SD = 0.086) but bin 6 was not significantly different to bin 5.

When assessing the same measures in the TS group a main effect of hand and bin were found ($F(1,11) = 81.428$, $p<0.0001$ and $F(2,22) = 11.132$, $p<0.001$ respectively). Interestingly, an interaction was also found between bin and hand that was absent in the control group ($F(2,22) = 4.965$, $p<0.05$). Post-hoc Bonferroni-corrected t-tests confirmed than bin 6 (M = 2.253, SD = 0.092) was significantly greater than bin 4 (M = 2.107, SD = 0.075) and bin 5 (2.206, SD = 0.080) was significantly greater than bin 4 but bin 6 was not significantly different to bin 5. The interaction term reflects that there was a greater ramping up of left hemisphere motor excitability in right hand motor execution compared to left hand motor execution.

4.3.3.2.2 Are there group differences in MEPs collected during left-cued trials in P2?

I entered 'No-Go', bin 4, 5 & 6 MEPs into a mixed x block ANOVA. The ANOVA revealed a significant main effect of bin (F(2.0,43.98) = 3.469, p<0.05). However, there was no significant main effect of group (p = 0.754), nor was there a significant interaction between bin and group (p = 0.439) indicating that there was a significant rise in left hemisphere motor excitability during left hand motor execution in both groups. Post-hoc Bonferroni corrected t-tests revealed that only bin 6 (M = 2.007, SD = 0.069) was greater than bin 4 (M = 1.915, SD = 0.059) and 'No-Go' trials (M = 1.903, SD = 0.056). No other significant differences were found.

4.3.3.2.3 Are there group differences in MEPs collected during right-cued trials in period 2?

I entered 'No-Go', bin 4, 5 & 6 MEPs into a mixed x block ANOVA. This showed a strong main effect of bin for left hemisphere MEPs during right hand cued responses (F(1.33, 27.981) = 58.956, p<0.0001, G-G corrected). In contrast, no significant effect of group (p = 0.251) was found, whilst a significant interaction effect between bin and group was revealed (F(1.33, 27.981) = 3.889, p<0.056, G-G corrected).

Post hoc bonferroni corrected t-tests demonstrated that there was a rise in motor excitability following S2. In both groups motor excitability was significantly increased compared to 'No-Go', however, in the CS group bin 4, 5 and 6 were not significantly different. (minimum p = 0.092, one-tailed Bonferroni corrected t-test). However, in the TS group MEP amplitude in bin 5 was significantly greater than bin 4 (t(11) = 4.271, p<0.01) and bin 6 was greater than bin 4 (t(12) = 4.271, p<0.01), but the effect plateaued and bin 6 was no greater than bin 5 (p = 0.89).

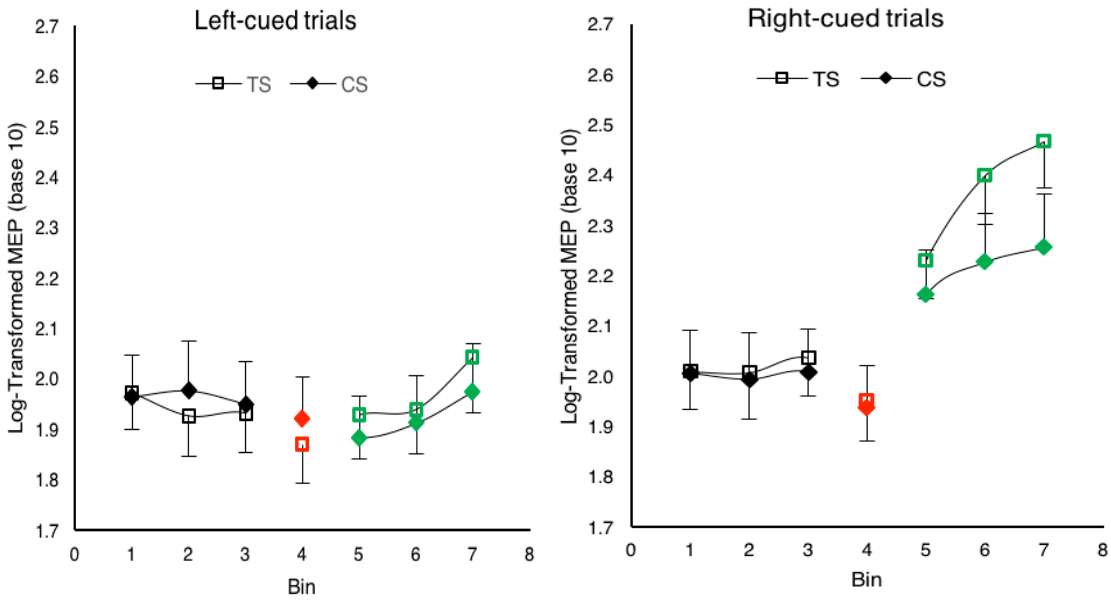


Figure 4.10: Left panel illustrates mean MEPs measured from the right hand during the planning and execution of left hand responses. Bins 1-3 (in black) refer to the movement preparation stage (following Cue 1). Bin 4 (in red) illustrates the mean MEP after the presentation of the No-Go cue (Cue 2). Bins 5-7 (in green) illustrate mean MEPs for Go trials, after presentation of Cue 2. Bins 5-7 also reflect relative proximity to movement onset. Right panel illustrates mean MEPs measured from the right hand during the planning and execution of right hand responses. Bins 1-3 refer to the movement preparation stage (following Cue 1). Bin 4 illustrates the mean MEP after the presentation of the No-Go cue (Cue 2). Bins 5-7 illustrate mean MEPs for Go trials, after presentation of Cue 2. Bins 5-7 also reflect relative proximity to movement onset.

4.3.3.2.4 Are there group differences in MEPs collected during right-cued trials in period 2 if we correct MEPs to baseline?

rhP2 MEPs were corrected to a lhP1 baseline. Means were calculated for each individual's lhP1 MEP amplitude and each rhP2 MEP was corrected by this baseline ($\frac{rhP2}{M(lhP1)}$). A mixed group x bin ANOVA was then used to assess whether there were any group differences in corrected rhP2 trials. Group and bin were entered as factors. There was a main effect of bin unsurprisingly ($F(1.35,28.30) = 61.243$, $p<0.001$, G-G corrected) and importantly a main effect of group revealed ($F(1,20) = 7.659$, $p<0.05$). The TS group surprisingly had a greater MEP rise than the CS group. A significant interaction between group by bin was found ($F(1.35, 28.298)= 4.400$, $p<0.05$, G-G corrected).

Independent samples t-tests were conducted to assess the interaction effect and were used to compare each rhP2 bin between groups. Post-hoc Bonferroni t-tests revealed that it was only in bins 6, i.e. closest to movement onset, that the TS group had a marginally higher MEP increase than the CS Group ($t(21)= 2.795$, $p<0.05$. Please refer to **Figure 4.11** for a graphical depiction of the increase in gain compared to baseline in the TS group. Groups' MEP amplitudes in bin 4, the furthest bin from movement onset, and 'No-Go' trials were no different. Interestingly, when corrected to baseline, it is evident that the TS group ramp up their motor cortex excitability by a greater degree than controls as they get closer to movement onset compared to a baseline state of general arousal and task engagement.

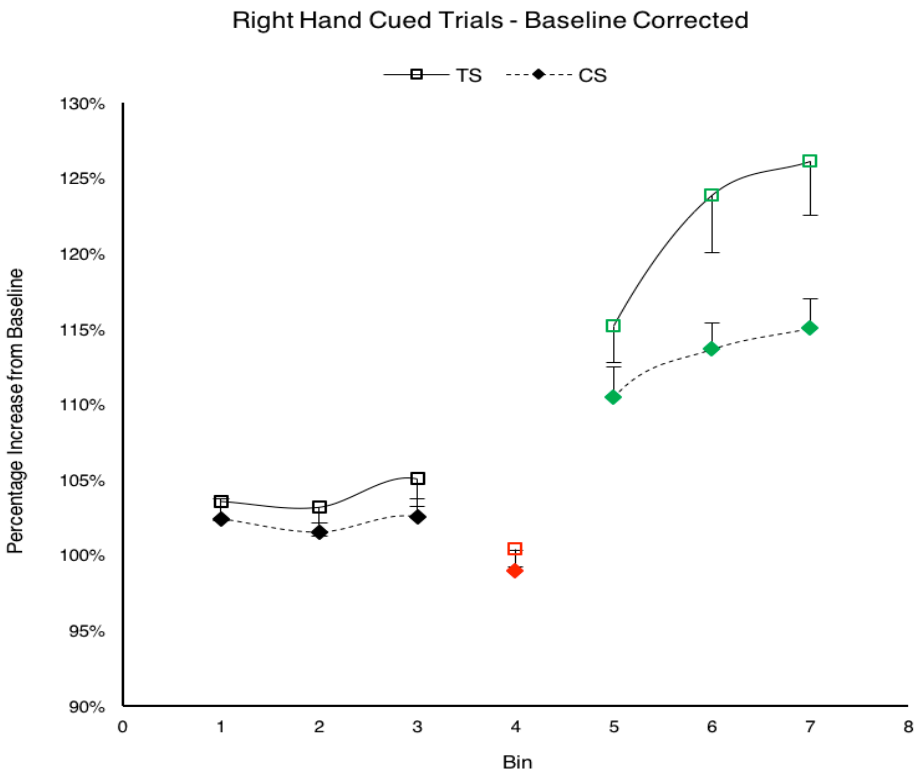


Figure 4.11: Illustrates change in Right hand MEP relative to baseline (MEP after Cue 1 for individual mean for LH Bin 1-3 trials). Bins 1-3 represent the period after Cue 1 (in black), bin 4 is the 'No Go' period (in red) while bins 5-7 represent the period after Cue 2 (in green).

4.3.3.2.5 Is there something special about MEPs collected during 'No-Go' trials?

An important question to consider is whether motor excitability during No-Go trials indexes the absence of motor preparation (whereby withholding a response is simply doing nothing) or whether something special is happening at the motor cortex, which may reflect a wider network of regions connected to the PMC. One way that one could probe this question is to ask whether the No-Go MEP is hand specific.

I first investigated this by entering hand and group into a 2x2 mixed ANOVA. The ANOVA confirmed that there were was an effect of hand-cued on 'No-Go' motor excitability, $F(1,23) = 7.547$, $p<0.05$. No other effects were found (minimum $p = 0.089$). Individual trial-by-trial MEP variability during No-Go trials did not differ dependent on hand; one other 2x2 mixed ANOVA was run and found no effect of hand, group or interaction between hand and group (minimum $p = 0.157$).

The processes involved in a 'No-Go' trial and in P1 are similar in that both involve visuomotor processes without motor execution and attention is directed at a specific effector. Is motor excitability different during this state versus when there is the explicit instruction to withhold a movement (response inhibition). In fact, No-Go MEPs do appear to be different to MEPs collected during P1. Bins were collapsed in P1 to create a mean of rhP1 and lhP1 for each individual.

I used a two-way ANOVA to compare left-hemisphere MEPs for right-cued trials in P1 and rhNo-Go trials. This revealed a significant effect of trial type (rhP1 vs rhNo-Go), $F(1,23) = 309.188$, $p<0.0001$, where MEPs collected during P1 had a significant greater amplitude than 'No-Go'. There was no significant interaction between trial type and group ($p = 0.998$) and no significant main effect of group ($p = 0.792$). A similar ANOVA was used to assess differences in trial-by-trial variability

collected within P1 and No-Go; variability did not differ between these trial types in right-cued trials *(*p = 0.229). There was a significant main effect of group, however, (F(1,23) = 5.386, $p<0.05$). The TS group had significantly greater MEP variability in right-cued trials (M = 0.117, SD = 0.011) compared to the CS group (M = 0.077, SD = 0.011). No significant interaction between trial type and group was found (p = 0.682).

This analysis was repeated for left-hemisphere motor excitability. Likewise, left-cued trials in P1 had greater MEP amplitude than lhNo-Go trials; the ANOVA showed a significant main effect of trial type *(*$F(1,23) = 15.201$, $p = 0.01$). In this case, however, there was no effect of group ($p = 0.748$). Neither was there a significant interaction effect between trial type and group ($p = 0.322$). Trial-by-trial variability was found to differ between P1 and No-Go trials in left cued trials (assessed by a similar ANOVA). A main effect of trial type was found ($F(1,23) = 8.361$, $p<0.01$). A marginal effect of group was found ($F(1,23) = 3.658$, $p = 0.068$). No interaction between group and trial type was found ($p = 0.587$).

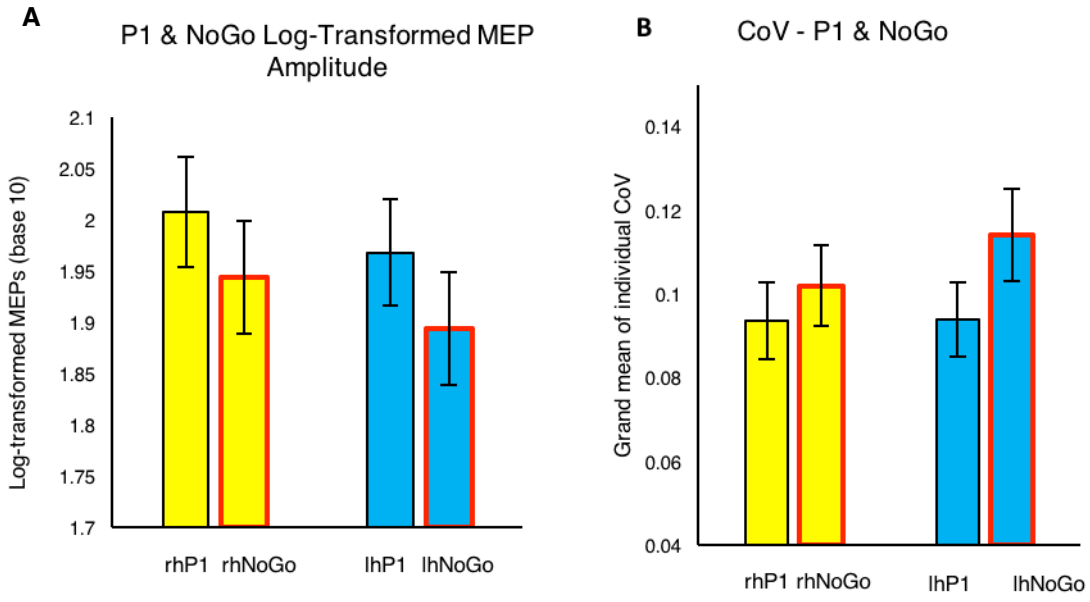


Figure 4.12: Averaged group data presented (TS and CS group are collapsed). In blue are left hand trials and in yellow are right hand trials. The bars highlighted in Red are 'No-Go' trials and those outlined in black are MEPs collected during Period 1 (preparing but not executing a movement). **A** showed MEP amplitude across trials whilst **B** shows MEP variability across trials.

4.3.4 Predictors of CSE

Age was found to be an important predictor of RMT in the TS group, which was a replication of my previous study. A stepwise regression was employed to see whether age, motor tic severity or handedness explained the variability in RMT in the TS group. Three variables were entered (age, motor tic severity and handedness) and two variables were removed, leaving only age. The model was statistically significant, ($F(1, 11) = 5.162$, $p<0.05$) and age explained approximately 30% of the variance ($R^2 = .319$, Adjusted $R^2 = .258$). The unstandardised and standardised coefficients, t statistic and *p* value are presented in **Table 4.7** and a visual depiction can be seen in **Figure 4.13**.

Table 4.7: Stepwise Regression model using age, handedness and tic severity to predict RMT. This table presents: unstandardised and standardised coefficients, t-statistics and *p* value.

Model	b	SE-b	Beta	t	P
Constant	70.189	9.593		7.316	0.000
Age	-1.296	.570	-.565	-2.272	0.044

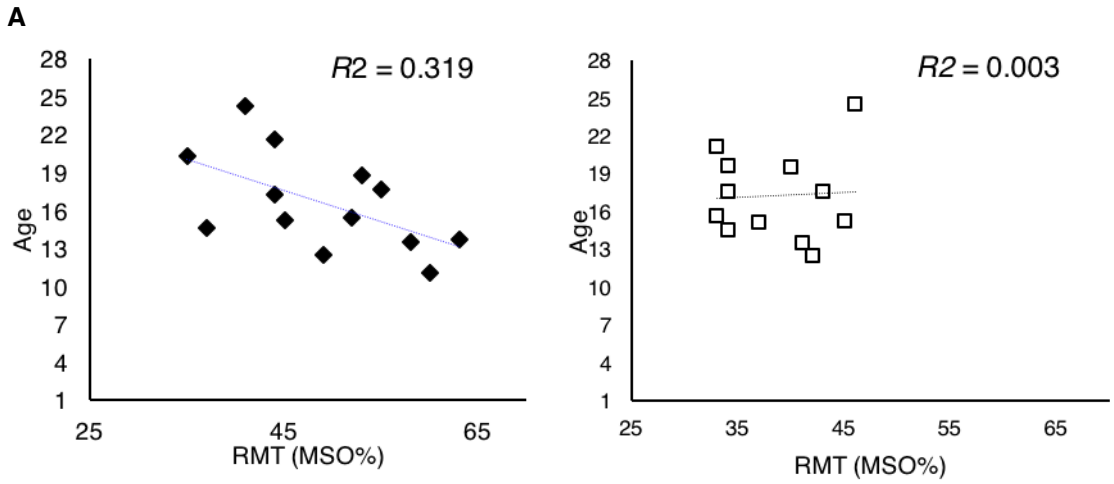


Figure 4.13: Scatterplots showing the relationship between RMT and age in **A** the TS group and **B** the CS group. One can see that whilst there is a strong negative relationship between age and RMT in the TS group there is an absence of a relationship in the CS group.

4.3.4.1 Relationship between Tic Severity and MEPs during Right-cued Trials

Multiple regression models using tic severity and MEP amplitude during right-cued trials were significant.

4.3.4.1.1 Motor tic severity predicts CSE in rhNo-Go trials

The average CSE in rhNo-Go trials was significantly predicted by motor tic severity, but not background EMG signal, age or phonic tic severity when assessed by a stepwise linear regression. The model was statistically significant ($F(2,12) = 19.324$, $p<0.001$); it predicted around 60% of the variability of rhNo-Go CSE ($R^2 = 0.637$, Adjusted $R^2 = 0.604$). The unstandardised and standardised coefficients, t

statistic and *p* value are presented in **Table 4.8**. For a visual representation of the relationship between tic severity and rhNo-Go CSE please view **Figure 4.14**. In the TS group, as motor tic severity increased, MEP amplitude in rhNo-Go trials were greater.

Table 4.8: Stepwise Regression model using motor tic severity and the covariate background EMG signal to predict rhNO-GO. This table presents: unstandardised and standardised coefficients, t-statistics and *p* value.

Model	b	SE-b	Beta	t	P
Constant	1.048	0.211		4.976	0.000
Motor Tic Score	0.061	0.014	0.798	4.396	0.001

4.3.4.1.2 Motor tic severity predicts Percentage Change in rhBin5 trials compared to Baseline (lhP1)

The average increase in MEP amplitude in rhBin5 trials (40-80% of RT) was significantly predicted by motor tic severity and background EMG noise, but not age or phonic tic severity when assessed by a stepwise linear regression. The model was statistically significant ($F(2,12) = 14.690$, $p<0.01$); it predicted around 70% of the variability of rhBin5 MEP change ($R^2 = 0.746$, Adjusted $R^2 = 0.695$). The unstandardised and standardised coefficients, t statistic and *p* value are presented in **Table 4.9.** For a visual representation of the relationship between tic severity and rhBin5 MEP Increase please view **Figure 4.14**. In the TS group, as motor tic severity increased, the MEP percentage change from baseline in rhBin5 trials was reduced (at 40-80% of movement execution).

Table 4.9: Stepwise Regression model using motor tic severity and the covariate background EMG signal to predict rhBin5. This table presents: unstandardised and standardised coefficients, t-statistics and *p* value.

Model	b	SE-b	Beta	t	P
Constant	2.246	0.190		11.812	0.000
EMG Background Signal	-0.407	0.094	-0.700	-4.312	0.002
Motor Tic Score	-0.024	0.006	-0.659	-4.057	0.002

4.3.4.1.3 Phonic tic severity predicts Percentage Change in rhBin1 and rhBin3 trials compared to Baseline (IhP1)

The average increase in MEP amplitude in rhBin1 and rhBin3 trials, collected during P1, were significantly predicted by phonic tic severity, but not background EMG noise, age or motor tic severity when assessed by a stepwise linear regression. For brevity I will only present the data for the most significant model, however, scatterplots for both are presented in **Figure 4.14**. The model (phonic tic severity predicting rhBin1) was statistically significant ($F(1,12) = 5.595$, $p<0.05$); it predicted around 30% of the variability of rhBin1 MEP Change ($R^2 = 0.337$, Adjusted $R^2 = 0.277$). The unstandardised and standardised coefficients, t statistic and *p* value are presented in **Table 4.10**. In the TS group, as phonic tic severity increased, the MEP percentage change from baseline in rhBin1 trials was reduced.

Table 4.10: Stepwise Regression model using phonic tic severity to predict rhBin1. This table presents: unstandardised and standardised coefficients, t-statistics and *p* value.

Model	b	SE-b	Beta	t	P
Constant	1.068	0.017		61.311	0.000
Phonic Tic Score	-0.004	0.001	-0.581	-2.365	0.037

No other relationships were found. Age did not relate to the above measures in the control group. CSE in any other period was not significantly predicted by handedness, age nor tic severity. Further, variability of the MEP was not related to handedness, tic severity nor age.

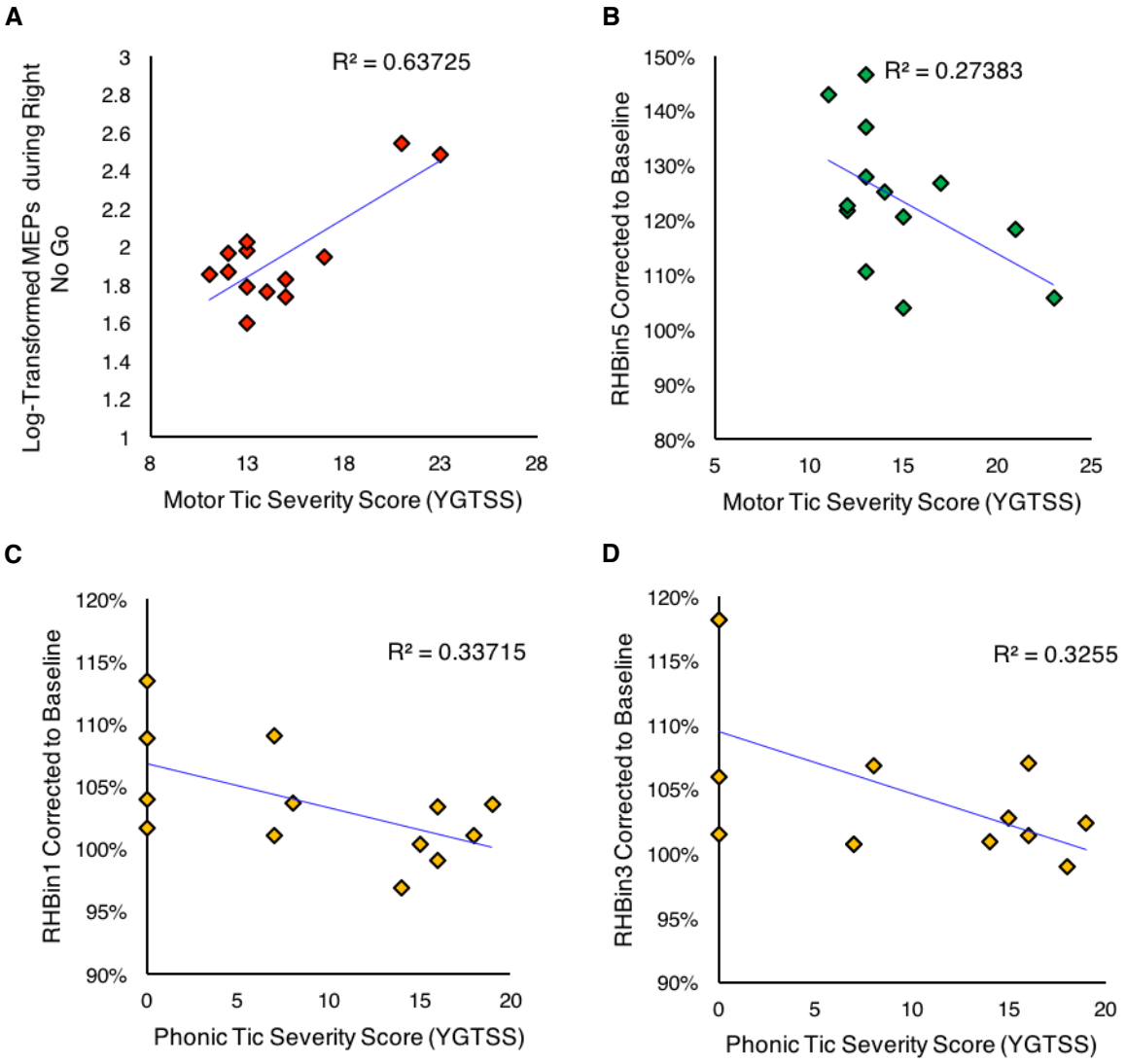


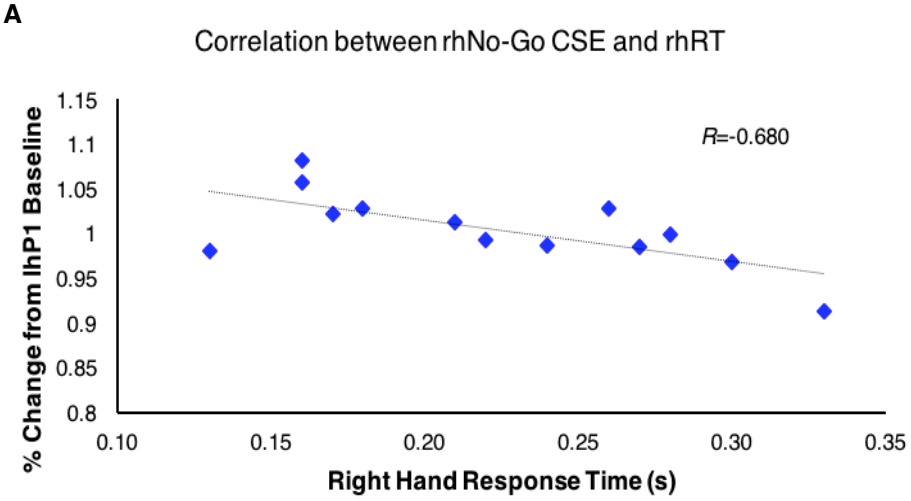
Figure 4.14: A The positive relationship found using linear regression between motor tic severity and right cued No-Go MEPs in the TS Group, greater tic severity is associated with increased MEP amplitude during RH No-Go MEPs. **B** The negative relationship found between RHBin5 (percentage change from baseline IhP1) and motor tic severity whereby greater tics are correlated with reduced ramping up of motor excitability. **C**&**D** The negative relationship between phonic tic scores and RHBin1&3 MEP percentage change from baseline IhP1 whereby reduced increase from baseline during sustained preparation for a RH response is associated with greater phonic tic scores.

4.3.4.2 Does motor cortex excitability during P1 or P2 relate to behavioural performance?

Individual Pearsons Correlations were calculated to assess the relationship between each individual's motor excitability in each trial for either rhP1, lhP1, rhP2 or lhP2 and RT for that trial. Individual correlations were varied and ranged from -0.84 to 0.74 (M = -0.09, SD = 0.28). There was no evidence of a significant relationship between MEP during P1or P2 and behavioural performance; when fitting a linear slope the mean coefficient of determination was 0.03 (lhP1 & rhP1) or 0.13 (lhP2 & rhP2, note rhP2 M = 0.20). Interestingly, when I assessed the relationship between CSE and right hand RT in each group I found differential effects. I found a negative relationship between right-cued bin 5 motor excitability (corrected to baseline) and RT in the CS group (R=-0.578, n = 12, p<0.05 [uncorrected]) and a significant negative relationship between right-cued No-Go motor excitability and RT

in the TS group (R=-0.680, n = 13, p<0.05 [uncorrected]). Neither relationship survived bonferroni correction for multiple comparison.

A



B

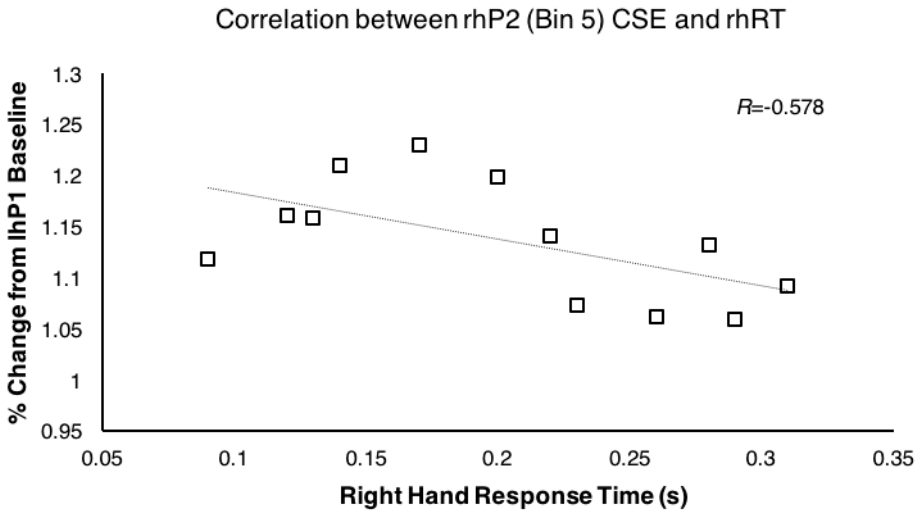


Figure 4.15: **A** shows the perhaps paradoxical relationship between right-cued No-Go motor excitability and right hand average response time in the TS group, **B** shows the relationship between motor excitability during a right hand movement and right hand average response time in the CS group.

4.4 Discussion

The results section of this chapter is extensive; I will therefore first begin the discussion of these results by providing a short summary of exactly what was found. I will then zoom in on each finding and explain or speculate as to what these results may mean.

4.4.1 Behavioural Results

Firstly, it was found that emgRT may be more sensitive that bpRT as it deduced a difference between RT between left hand and right hand trials that would have otherwise gone undetected. When assessing the RTs it was also found that there was a clear interference of TMS where participants responded faster to the imperative Go signal if TMS was delivered in P1 rather than during P2; this effect was found for both right-cued and left-cued trials. Participants were very accurate at the task and there were no differences in behavioural performance (assessed by errors made or RT) between groups; however, it is clear when looking at the table denoting RT for each group that there is a nonsignificant slowing of RT in the TS group. Importantly, this is despite there being no decision making in P2 (participants were instructed only to respond after S2 disappeared; decisions would have been made during S2 presentation unless participants were inattentive).

4.4.2 Left Hemisphere Motor Excitability at Rest

There was a clear difference in RMT between groups, replicating what I previously found in **Experiment 1**. The TS group had a significantly higher RMT suggesting a lower gain of the motor system (whereby they require a higher intensity of stimulation to induce a measurable output). However, there were no differences between the percentage of RMT required to achieve 750μV (the TP used). As in

Experiment 1, age significantly predicted RMT in the TS group (but not in the CS group). This is further support for a developmental delay in the motor system in TS; this apparent delay is not mediated by tic severity within this study.

4.4.3 Motor Excitability during an adapted Go/No-Go Task, measured during preparation of an action, withholding an action or executing an action

P1 is a condition that involves the preparation of a simple right index or left index movement; whereby all that is required (after a delay) is a fast button-press or the withholding of a response. In P1 both groups showed larger MEP amplitudes in right-cued trials vs. left-cued trials. Whilst the TS group showed no differences in MEP amplitude compared to the healthy controls in period 1, they did show increased MEP variability specifically in right-cued trials. Interestingly, whilst there was no strong evidence of modulation of motor excitability during Period 1, the percent change in right-cued MEP amplitude from left-cued trials did negatively relate to phonic tics. Those with the least severe phonic tics had the largest percent change in MEP amplitude in right-cued P1 MEPs when corrected to baseline (mean left-cued P1 MEP). This does suggest that patients with TS that engaged PMC or regions effectively connected to PMC had the most reduced tic severity.

The alternative hypothesis is of course, that the relationship with tic severity reflects that those with reduced tic severity have a greater ability to suppress motor excitability of the non-selected effector (rather than ramping up excitability for the selected effector). This would be in keeping with similar delayed-response tasks using sp-TMS in one hemisphere whilst the participant prepares for different hand responses which show evidence of MEP suppression for non-selected effectors during a task (Lewis & Bates, 2013; Mars, Bestmann, Rothwell, & Haggard, 2007; Pascual-Leone, 2000; Tandonnet, Garry, & Summers, 2011). In either scenario, this

likely necessitates the recruitment of frontal or higher order motor areas (the SMA for example).

I assessed motor excitability during P2 whilst participants withheld execution of a left-cued or right-cued response, and hence whilst they were 'stopping' a pre-potent response. Left-cued No-Go trial MEP amplitude was significantly reduced in comparison to right-cued No-Go MEP amplitude and motor excitability during all No-Go trials (irrespective of hand cued) was significantly reduced in comparison to MEPs collected during P1, which is suggestive of MEP suppression. This suppression of MEP is clearly independent of hand cued and occurs in both groups. It most probably signifies a global stop command within the motor system (Bestmann & Duque, 2015). MEP suppression in task-specific and nonspecific effectors during No-Gos has been found in similar No-Go paradigms (Hoshiyama et al., 1996; Pascual-Leone, 2000).

Paired-pulse TMS studies have demonstrated that intracortical inhibition at the level of the PMC is at least partly responsible for task-specific MEP suppression of the pyramidal tract (Coxon, 2006). Intracortical inhibition (measured by a short intracortical inhibition [SICI] TMS paradigm) not only increases after a No-Go cue in the task-specific effector, but also in a task-irrelevant effector (Stinear et al., 2009). In this experiment, it was noted that a reduction of MEP amplitude during the non-selected effector was accompanied by a marked increase in MEP amplitude variability.

Furthermore, it has been suggested that thalamocortical pathways are important for stopping of motor outputs and the regularisation of intracortical inhibition for the prevention of movement (Stinear et al., 2009). Here, one may think

of studies of intracortical inhibition in TS which tend to show (at least in adults) reduced inhibition (Moll, Heinrich, Gevensleben, & Rothenberger, 2006; Orth, Amann, et al., 2005) which is related to tic severity (Orth, Münchau, & Rothwell, 2008). This is all the more interesting given the positive relationship between motor tic severity and right cued No-Go MEPs. Greater tic severity was associated with higher MEP amplitudes during right-cued No-Gos indicative of reduced SICI and thus reduced $GABA_A$ receptor action. This is in keeping with previous literature that has consistently suggested that patients with TS have reduced SICI (Gilbert et al., 2004; K. F. Heise et al., 2010; Orth, Amann, et al., 2005; Ziemann et al., 1997).

Given that there was no group difference between MEP amplitude during 'No-Go' trials, the predictive value of phonic tic severity of motor excitability during a No-Go could be indicative of a compensatory enhancement of cortical inhibition during movement suppression. Whilst this may be due to localised change in excitability, this could also be related to mediation of the thalamocortical inputs into PMC which could require help from areas intrinsically related to stopping for example frontal regions (rIFC or pre-SMA).

As in **Experiment 2**, **Experiment 3** was designed to assess motor excitability during the execution of a movement. Unlike **Experiment 2**, the period that this was assessed was clear-cut motor-execution and the time in which TMS was delivered should not have caught any decision-making (the decision whether to 'Go' or not is assumed to have been made prior to TMS pulse delivery). Interestingly, in this experiment there was no effect of group for right-cued 'Go' trials; the characteristic rise of motor excitability as delivery of TMS pulse is closer to movement onset was found in both groups. Importantly, if 'Go' trials MEPs were corrected to a left-cued P1 baseline TS motor excitability appears to have been ramped up to a much greater

extent than in the CS group. Whilst I was unable to do this sort of analysis in **Experiment 2**, I concluded that the hypo-excitability found in the TS group in the 'Go' period was due to baseline differences that precipitated in a widespread reduction of motor excitability (rather than a specific excitability suppression related to motor preparation & execution).

The compensatory rise in activity in comparison to a preparatory baseline has not so far been reported in the literature, nor to my knowledge has it been explored. A negative relationship between motor tic severity and percentage change during the penultimate bin of data (corresponding to 41-80% of RT) was further found which replicates previous work showing that those with the most severe tics are able least to modulate motor excitability (Draper et al., 2015).

Finally, I found evidence for motor excitability as measured from left hemisphere PMC to be predictive of behavioural performance (as indicated by RT). Interestingly, the relationships found difference for the controls versus patients with TS. The extent to which participants could modulate motor excitability during movement execution appeared to predict right hand RT in controls (greater modulation was related to shorter response times). In contrast, in the patient group I found a relationship between motor excitability during right hand No-Go negatively predicted response times (i.e. those with the most reduced or suppressed MEPs had the slowest RTs).

In summary, this experiment found more evidence to support the argument that the motor system is not as mature as healthy young people of a similar age and RMT appears to normalise with age suggesting that development over adolescence results in a gain of motor output. This reduction in motor gain was not restricted to

the system at rest, the group had reduced motor excitability during the behavioural task and whilst this was a non-significant difference, this effect disappeared and reversed when the data was corrected to these baseline differences.

I found no evidence of modulation of motor excitability during a period of sustained attention where participants had to prepare for a movement with either their left or right hand and wait for the signal to tell them to move or not. Despite this, when data was corrected to baseline it appeared that the patients with TS that were able to ramp up motor excitability during Period 1, thereby engaging PMC to a greater extent than other patients, had reduced phonic tic severity.

Importantly, when correcting for baseline differences in motor output, patients with TS in fact ramped up motor excitability to a greater extent during motor execution than healthy controls. Those with more severe tics were least able to modulate their tic severity, which has been found before (Draper et al., 2015). Given the group difference (whereby TS>CS) and baseline differences (whereby CS>TS) the fact that patients with reduced tics are able to modulate motor excitability to a greater extent during a volitional movement is likely to be compensatory.

Furthermore, motor tic severity was positively related to rh No-Go amplitudes which likely relates to a pathological reduced SICI (reduced $GABA_A$ receptor action). There were no group differences between 'No-Go' MEPs and this accompanied with no performance differences in stopping supports the multitude of evidence that suggests cognitive control is not impaired in young people with TS (Baym, Corbett, Wright, & Bunge, 2008; Buse et al., 2013; Ganos et al., 2014; G. M. Jackson, Mueller, Hambleton, & Hollis, 2007). Interestingly, a negative relationship was also found between right-cued 'No-Go' MEP amplitude (not the extent of suppression)

and increased RTs which could be a consequence to increased inhibitory processes. In support of this effect being compensatory rather than pathological, this relationship did not exist in controls. In fact, in controls increased gain during motor execution predicted faster RTs – a relationship which was absent in patients.

Chapter 5: Investigation of Interhemispheric Effective Connectivity between Bilateral Primary Motor Cortices in Healthy Young Adults

5.1 Introduction

In **Chapters 1** and **2** I explore the relevance of the CC in TS and how communication between motor regions transcallosally may be altered in TS. This experiment aims to explore transcallosal communication in young adults to extend our knowledge of effective connectivity between bilateral PMC regions in the healthy population. The aim of this current experiment is to explore the differing parameters that may lead to interhemispheric facilitation (IHF) and inhibition (IHI); whilst in depth assessing whether there is any directionality to interhemispheric interactions. I aim to assess whether there is an asymmetry or symmetry to IHI/F in a healthy population of young adults and whether sex influences symmetry.

There is a body of evidence that suggests that there are both inhibitory and facilitatory interhemispheric connections and that these are distinct, from both ds-TMS studies (Bäumer et al., 2006; Hanajima et al., 2001) and TMS-EEG studies (Voineskos et al., 2010). Using ds-TMS to probe facilitatory connections between the bilateral primary motor cortices is a relatively new and less extensively studied phenomenon. To achieve this, the CP must be at a very low intensity (60-80% AMT) and facilitation of the CP has been shown that the ISI must be set at 6-8ms.

IHI is a more robust phenomenon that can be measured using a suprathreshold CP (130% RMT) and a longer ISI, ~10-12ms (Wahl et al., 2007). IHI is a more robust finding that has consistently been shown to be effective (Ferbert et al., 1992; Ni et al., 2009; Wahl et al., 2007). There has been some evidence that IHI is stronger in the left-to-right direction (Bäumer et al., 2006), however, this has not

been consistently found (Bäumer et al., 2010). Furthermore, there is some evidence for an effect of sex on IHI whereby females show greater IHI than males (De Gennaro, Bertini, et al., 2004; De Gennaro, Cristiani, et al., 2004). To my knowledge, there has been no study to date explore the effect of sex on IHF.

The aim of this current experiment is to explore the differing parameters that may lead to interhemispheric facilitation (IHF) and inhibition (IHI); whilst in depth assessing whether there is any directionality to interhemispheric interactions and importantly assessing whether there is an asymmetry or symmetry to IHI/F in a healthy population of young adults. In a subgroup of participants I explore whether there is any relationship between transcallosal interactions and other measures of left hemisphere cortical excitability – namely pp-TMS measures: short interval intracortical inhibition (SICI) (3ms, $SICI_{3,}$ and 1ms, $SICI_1$), intracortical facilitation (ICF: 10ms, ICF_{10}, and 12ms, ICF_{12}), recruitment curve slope and proton magnetic spectroscopy measures: GABA:tCr, Glu:tCr and Gln:tCr. This will help better characterize the interhemispheric interactions and the individual differences that result in the amount of inhibition or facilitation that arises from the dual site protocol in a left hemisphere-to-right hemisphere (LtoR) and right hemisphere-to-left hemisphere (RtoL) direction. I will further explore the contributions of handedness and sex to individual differences in IHI/F.

5.2 Pilot Study

5.2.1 Method

Two biphasic Magstim Rapid machines and two 40mm diameter figure-of-eight coils were used to deliver ds-TMS to the motor hotspot of the FDI of both hemispheres. First, RMTs were measured (5/10 trials that elicited at least a 50μV MEP on the EMG recording) for the left and right side of the brain. The intensity

needed to elicit an MEP between 500-750 μV was also calculated for the right hemisphere (that was needed for a TP). The hand area in the brain was anatomically located using T1 anatomical brain scans and the participant's head was co-registered the brain scan using the same method discussed in the sp-TMS experiment protocol. The location of both hotspots were continuously tracked throughout the experiments using BrainSight 2, three trackers (one of the participant's forehead, and one on each of the TMS coils) and the coils were stabilized using two manfrotto arms. One experimenter observed the coil on the left and another observed the coil on the right in order to reduce the possibility of movement between trials. Disposable EMG electrodes with a diameter of 5mm were placed on the FDI muscle in both hands in a standard belly-tendon configuration in order to record both muscles online using BrainVision software. A sample rate of 5000 Hz and interval of 200μV and two channels (the left and right hand) were recorded.

CPs tested were 60%, 80%, 120% and 130% RMT with ISIs of 4,6,8,10 and 12 ms. There were 15 trials of each above combination (conditioned trials) and 20 unconditioned trials in total. The CP was applied to the left hemisphere and the TP applied to the right; in order to assess interhemispheric connectivity in the L-to-R direction. The participants watched a neutral film throughout testing.

Three female participants were tested with the above protocol.

5.2.2 Results

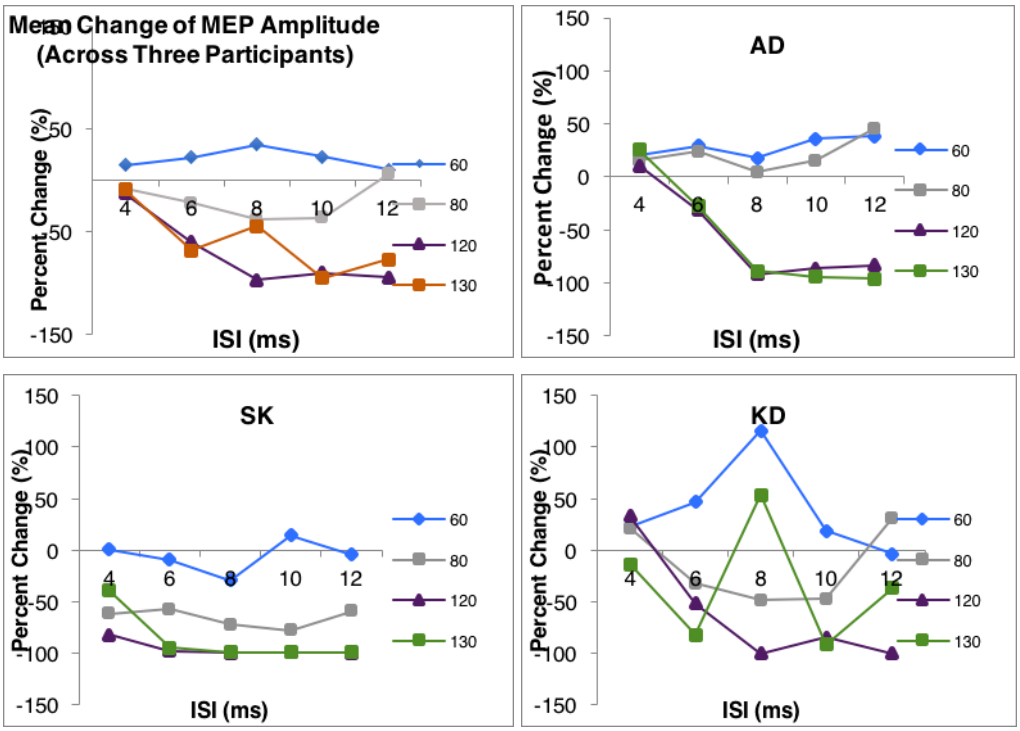


Figure 5.1: Graphs showing the mean percentage change from baseline MEP from a protocol looking at left to right interhemispheric connectivity. A conditioning pulse (CP) was given to the left hemisphere either 4,6,8,10,12 ms before a TP, at an intensity of 60,80,120,130% RMT. The CP was given over the left hemisphere and the TP was given over the right hemisphere. Measures are indices of interhemispheric facilitation (IHF) and interhemispheric inhibition (IHI).

5.2.3 Summary

Initial pilot testing raised some important considerations. Firstly, it appears that the 60% RMT CP may not be low enough for all participants to get an excitatory effect. This may be because RMT was used and not active (AMT) as suggested in Bäumer and colleague's 2006 interhemispheric facilitation ds-TMS study. Secondly the suprathreshold CPs (used to measure IHI) seemed to be too high; this did not seem necessary and meant that the participant was less comfortable (an important consideration for children and adolescents). Further testing will use either 100 or 110% of RMT (or higher of AMT) in order to look at IHI that does not reach floor. It is evident that 4ms ISI is not a useful measure and therefore only 6-12ms will be used as ISIs.

5.3 Main Experiment: Method

5.3.1 Participants

Eighteen healthy adults (females n = 7) aged between 17 and 24 years old (M = 22.07, SD = 2.30) were recruited with no history of neurological disorder and no contraindications to TMS including no medications which are known to affect the brain or central nervous system. All participants had T1-weighted MRI scans of their brain and head that were used with neuro-navigation software. 15 participants completed the Edinburgh Handedness Inventory and one participant was left-handed. 10 participants had also taken part in the experiment detailed in **Chapter 6** and ^{1}H-MRS spectroscopy measures of the left hemisphere PMC and pp-TMS measures also collected in the left hemisphere were available.

5.3.2 Transcranial Magnetic Stimulation

Ds-TMS was delivered using custom-made 40mm diameter figure-of-eight coils and two Magstim Rapid machines (biphasic pulse). Neuro-navigational software BrainSight was used where participants were registered to their 3D-curviliear reconstruction of head and brain from their T1 weighted scan and a camera and three trackers (attached to the participant and two TMS coils) were used to monitor where the participant and coils were at all times throughout the experiment. The hand area of the left and right hemisphere PMC were located anatomically on each T1-weighted brain image and a grid of possible targets were superimposed to the surface of the cerebral cortex over their 'hand area' within the PMC. BrainSight and online EMG recordings were then used to locate and fine-tune the location of the motor hotspot for the participant's left and right FDI; these were then targeted on BrainSight and used throughout the experiment. Great care was taken to ensure a

good registration of the participants actual and virtual head and to ensure the distance between their actual and virtual skin was within 3 mm of error. (Please read the methods in **Chapter 4** for a full description of how BrainSight was used).

Two experimenters held the TMS coils and each coil was held tangentially against the scalp at a 45° angle with the current travelling in a posterior-anterior/ anterior-posterior direction. Firstly, RMT was defined as the stimulator intensity that induced an MEP with the criteria of 5 out of 10 with a peak-to-peak amplitude $\geq$ 50μV. A TP was then found with the criteria of an MEP that could consistently be produced between 750 - 1000μV; else if the maximum MEP was lower than 750 μV this stimulator intensity was recorded. The motor hotspot, RMT and TP was found systematically first for the left hemisphere and then the right hemisphere. Prior to each direction of interhemispheric interaction being tested RMT for the conditioning hemisphere and TP for the conditioned hemisphere was tested and altered if necessary.

The parameters that were tested were a conditioning stimulus (CP) of 50%, 70%, 100% and 110% of RMT (CP_{50}, CP_{70}, CP_{100}, CP_{110}) and an ISI of 6, 8, 10 and 12ms (ISI_6, ISI_8, ISI_{10}, ISI_{12}). Thus including unconditioned trials, 17 different trials were tested in each direction. The CP was delivered to the left and right hemisphere to test the left-to-right and right-to-left hemisphere interhemispheric interaction. The direction tested first was counterbalanced between participants. Each CP was tested in blocks; the order was randomised within each direction tested. For consistency, one experimenter would hold the conditioning coil for the entirety of the experiment, however, for different participants experimenters altered holding either the conditioning or test coil. For each direction 20 unconditioned trials were tested and 12 trials for each combination of parameters were tested. In between each trial there

was a variable ISI between 4-6 seconds. Every 20 trials, participants were offered a break and checked for fatigue.

5.3.2.1 Electromyography

Ag-AgCl surface electrodes were used with conductive gel and were placed in a belly-tendon montage over the FDI muscle on both the right and left hand. A ground electrode was only placed on the tested hand (over the ulnar styloid). Brainamp ExG and BrainVision Recorder software (Brain Products GmbH) were used to amplify, digitize, record, preprocess and visualise the EMG signal with a sampling rate of 5000 Hz with a sampling interval of 200 μS.

5.3.3 Results

5.3.3.1 Baseline Participant Demographics

A visualisation of the spread of the participants' age, handedness scores and RMT from each hemisphere can be viewed in **Figure 5.2**. Handedness scores (M = 82.33, SD = 35.04) varied between -40 (left handed) and 100 (considered entirely right handed). Left hemisphere RMT ranged between 36 and 71 (M = 54.06, SD = 8.42) and right hemisphere RMT ranged between 41 and 73 (M = 55.29, SD = 8.12). A paired samples T-Test indicated that there was a marginal between the right and left hemisphere RMT t(15)=-1.920, $p = 0.074$; the left hemisphere RMT was marginally lower. The TP when applied to the left hemisphere was a mean of 116% of RMT (SD = 6.4%) and in the right hemisphere 114% of RMT (SD = 6.7%); these were not significantly different to each other (t(15) = 0.855, $p = 0.406$). I tested whether there was a difference between the unconditioned MEPs between both hemispheres. Grubb's outlier test removed one outlier from the unconditioned left hemisphere MEP data and a paired samples t-test revealed a marginally significant difference in unconditioned MEP amplitude from each hemisphere (t(14)=-2.156, $p =$

0.049; left hemisphere unconditioned MEP: M = 1121.32, median = 993.50, SD = 465.94, right hemisphere unconditioned MEP: M = 858.4, median = 956.75, SD = 409.38). There was a mean total difference of 262.92μV with the left hemisphere tending to have higher amplitudes. This was likely driven by a few participants that could not get the achieved TP amplitude in the right hemisphere.

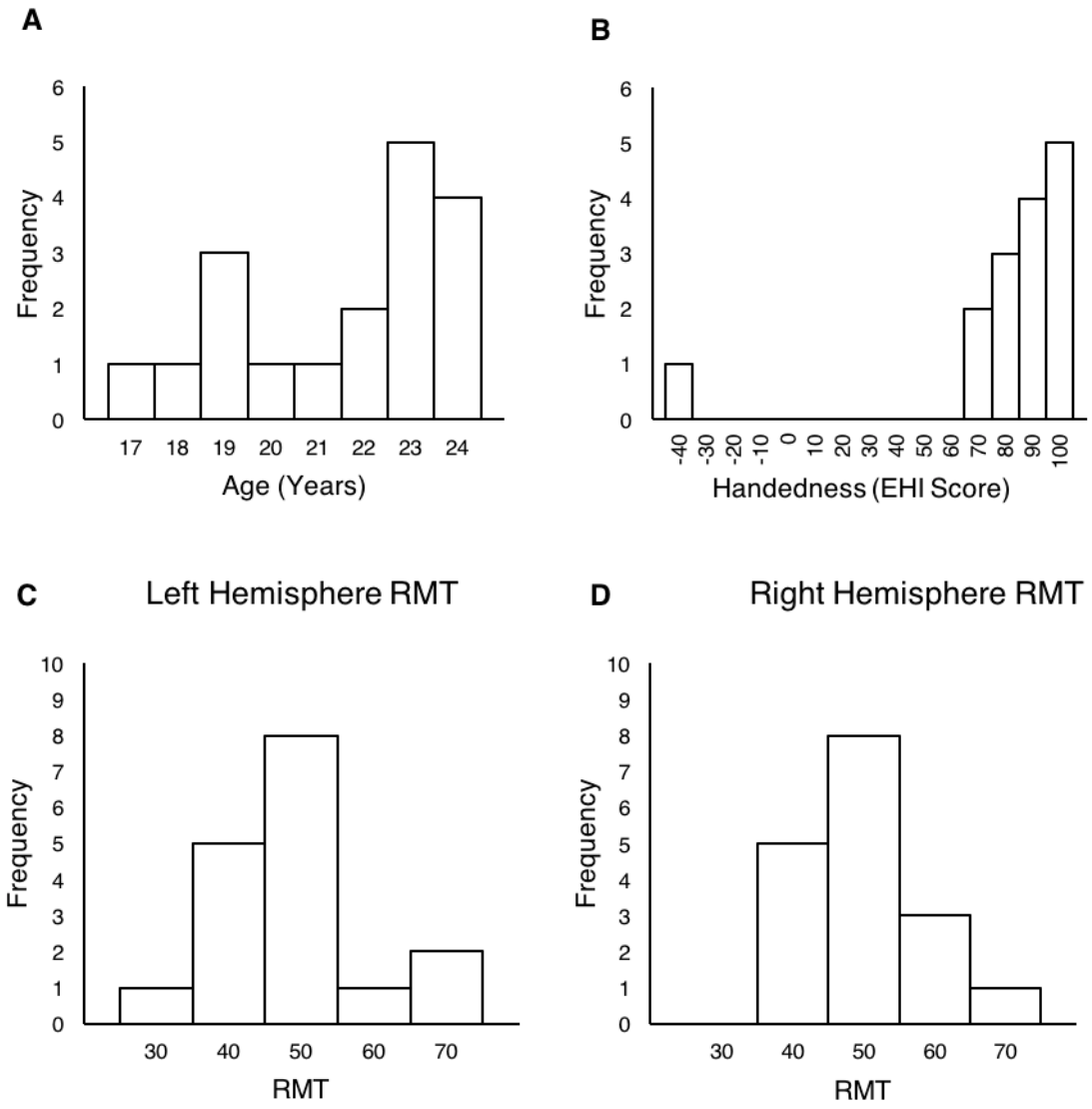


Figure 5.2: 5.2A depicts the distribution of ages of participants that took part in the ds-TMS experiment. **5.2B** shows the Edinburgh Handedness scores for the 15 participants that completed the inventory. **5.2C** and **5.2D** show the distribution of RMT of the left hemisphere (C) and the right hemisphere (D).

5.3.3.2 Data Analysis

Median of MEPs from each trial were calculated for each participant, conditioned trial medians were corrected to the unconditioned trial baseline which resulted in ratios where 1 equals no change, greater than 1 indicates facilitation and less than 1 indicates inhibition. Trials with a baseline EMG signal greater than 75μV in either hand 150ms prior to sp-TMS delivery were discarded. 2 participants only had one direction collected, all other participants were tested in the left-to-right and right-to-left direction. A grubbs outlier test was used in each condition to examine and remove outliers – this was applied to ratio data.

5.3.3.3 Left to Right Direction Effects

A dataset of 17 participants was included for analysis. The grand mean of unconditioned MEPs (taken for the left hand FDI) for all participants was 911.35μV ± 443.66 (SD).

5.3.3.3.1 Interhemispheric Interaction

One-way repeated measures ANOVAs were employed to investigate the effect of ISI for each CP and effect of CP for each ISI (**Table 5.1**). There were no effects of ISI for any of the conditioning pulses (minimum $p = 0.074$).

There was an effect of CP for ISI 6 ms ($F(1.83, 23.73) = 3.536$, $p<0.05$, G-G corrected); ISI 8ms ($F(3, 42) = 4.038$, $p<0.05$); ISI 10ms ($F(2.01, 30.21) = 10.154$, $p<0.001$) and ISI 12 ms, $F(2.02, 30.24) = 8.015$, $p<0.01$. Pairwise comparisons revealed that for an ISI of 6ms, there was only an effect of CP of 50% where it was significantly increased compared to 70%, 100% and 110% ($p <0.05$, $p<0.01$ and $p< 0.05$ respectively). 8ms a CP of 110% was more inhibitory than a CP of 70% ($p = 0.054$). For an ISI of 10ms a CP of 50% and 70% were more facilitatory than a CP of 110% ($p<0.01$; 0.001). For an ISI of 12ms a CP of 50% was more facilitatory than

100% or 110% (p<0.01); a CP of 70% was more facilitatory than 100% and 110%(p<0.05; p = 0.057). In this condition (ISI 12ms) there was no difference between 50% and 70% or 100 and 110%.

Whilst there is a clear effect of CP in this ds-TMS paradigm, it is unclear whether there is an effect of ISI. It is apparent that some ISIs may be more effective for certain CPs than others.

Table 5.1: Group mean of median and standard error of the means displayed for MEP ratios for left hemisphere conditioned, right hemisphere tested trials.

Left to Right Direction
MEP amplitude as a Ratio of Conditioned/Unconditioned Trials

	ISI of 6ms	ISI of 8ms	ISI of 10ms	ISI of 12ms
CP: 50%	1.46 ± 0.22	1.05 ± 0.13	1.32 ± 0.20	1.06 ± 0.09
CP: 70%	0.96± 0.06	0.99 ± 0.11	1.15 ± 0.10	1.24 ± 0.17
CP: 100%	0.90 ± 0.10	0.86 ± 0.13	0.96 ± 0.17	0.67 ± 0.08
CP: 110%	0.78 ± 0.09	0.64 ± 0.07	0.51 ± 0.06	0.66 ± 0.09

Further to the above tests, I assessed whether any of the conditions were different from an unconditioned MEP baseline. It was clear that there was a strong inhibitory effect of $CP_{100}ISI_{12}$, $CP_{110}ISI_{8,}$ $_{CP110}ISI_{10}$ and $_{CP110}ISI_{12}$. However, no other conditions appeared to be effective conditioning paradigms. Please view **Table 5.2** for all t and p values for the comparisons tested (p values adjusted for multiple comparisons).

Table 5.2: t values and *p* values for one sample t-tests that tested whether conditioned trials were significantly different to an unconditioned baseline

		CP: 50%		CP: 70%		CP: 100%		CP: 110%	
ISI (ms)	df	t Value	P Value	t Value	P Value	t Value	P Value	t Value	P Value
6	15-6	2.13	0.130	-0.72	0.596	-0.98	0.502	-2.61	0.0672
8	15-6	0.43	0.769	-0.12	0.905	-1.08	0.477	**-5.62**	**0.0003**
10	15-6	1.68	0.258	1.52	0.263	-0.27	0.847	**-8.65**	**0.00001**
12	14-6	0.73	0.639	1.53	0.292	**-4.15**	**0.004**	**-4.02**	**0.00533**

5.3.3.4 Right to Left Direction Effects

A dataset of 17 participants was included for analysis. The grand mean of unconditioned MEPs (taken for the left hand FDI) for all participants was 1263.35μV ± 924.08 (SD).

5.3.3.4.1 Interhemispheric Interaction

One-way repeated measures ANOVAs were used to investigate the effect of ISI for each CP and CP for each ISI. Only one CP showed evidence for an effect of ISI; this was CP 100% ($F(3,48) = 3.9$, $p<0.05$). Pairwise comparisons showed that for CP 100% an ISI of 12ms was more inhibitory than an ISI of 6ms ($p<0.05$). ISI 8 showed a marginal effect of CP ($F(3,45) = 2.819$, $p = 0.068$), ISI 10 & 12 showed a significant effect of CP (10ms: $F(3,45) = 2.848$, $p<0.05$; 12ms: $F(2.09,29.23) = 6.622$, $p<0.01$, G-G corrected). View **Table 5.3** to see the ratio of conditioned to unconditioned trials. All other comparisons were not significant.

Table 5.3: Group mean of median and standard error of the means displayed for MEP ratios for right hemisphere conditioned, left hemisphere tested trials.

	Right to Left Direction MEP amplitude as a Ratio of Conditioned/Unconditioned Trials			
	ISI of 6ms	ISI of 8ms	ISI of 10ms	ISI of 12ms
CP: 50%	1.11 ± 0.13	1.21 ± 0.12	1.33 ± 0.16	1.25 ± 0.16
CP: 70%	0.95 ± 0.09	1.17 ± 0.19	1.03 ± 0.13	1.16 ± 0.14
CP: 100%	1.13 ± 0.15	0.85 ± 0.09	0.97 ± 0.10	0.83 ± 0.09
CP: 110%	1.13 ± 0.19	0.81 ± 0.13	0.89 ± 0.13	0.70 ± 0.11

I assessed whether any of the conditions were different from no change by using multiple one-sample t-tests. This showed a different pattern of results from the

investigations in the left to right direction – no significant differences from baseline were found; no table is presented.

5.3.3.5 Differences between Conditioned Hemisphere

A two-way repeated measures ANOVA was used for each different CP to determine whether the effects were different for each hemisphere. For each CP, I entered ISI (6,8,10,12) and hemisphere (right or left) as factors into the ANOVA. In all the analyses there were no main effects of hemisphere found. An interaction between hemisphere and ISI was found for CP 50% ($F(3, 39) = 2.989$, $p<0.05$, G-G corrected). An effect of ISI was found for CP 100% ($F(3,39) = 5.586$, $p = 0.01$, G-G corrected). No other effects were found. One can view the similarities and differences between the left-to-right and right-to-left direction for each CP in **Figure 5.3** and in a different view with each CP shown separately in **Figure 5.4** for clarity.

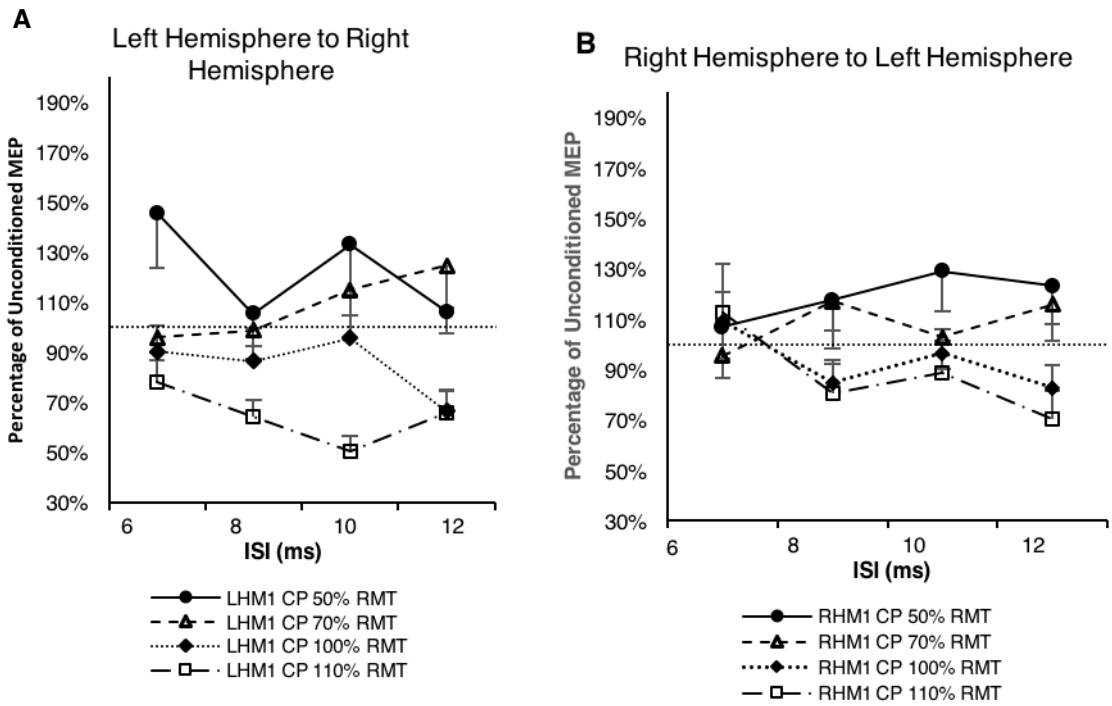


Figure 5.3: CP_{50}, CP_{70}, CP_{100} and CP_{110} plotted by ISI (6, 8, 10 and 12ms) for left hemisphere conditioned (A) and right hemisphere conditioned (B). Errors bars are standard error of the mean.

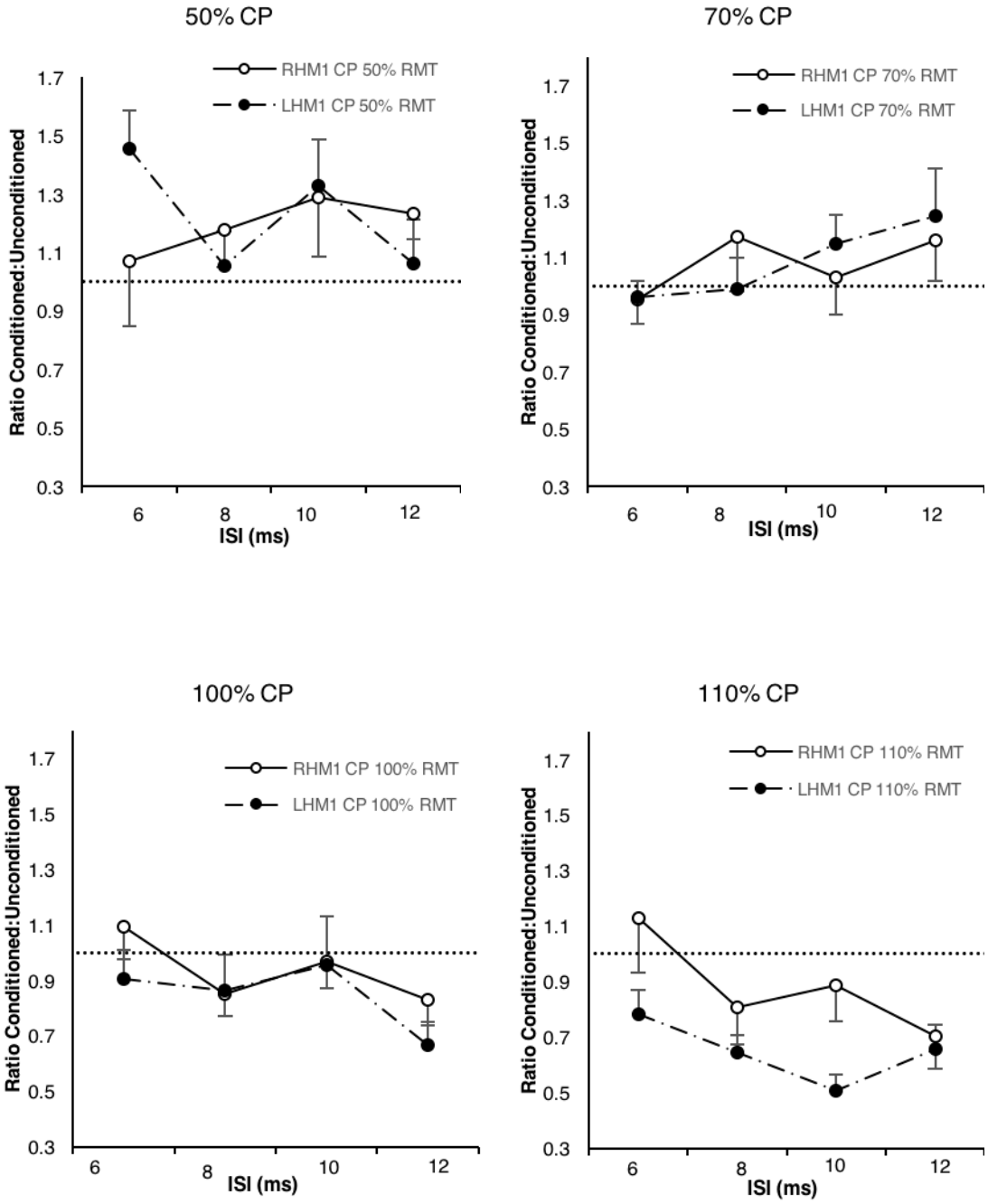


Figure 5.4: Each CP plotted by ISI in separate charts (A,B,C and D) to illustrate directional differences. Left hemisphere to right hemisphere (denoted LHM1) as a dashed line with filled circles and right hemisphere to left hemisphere (denoted RHM1) as a full line with unfilled circles. Errors bars are standard error of the mean.

5.3.3.6 Individual Trends

Individual ISI x CP curves were created (one curve for each ISI) and linear slopes were fitted to test whether there was a trend for more facilitation or inhibition depending on the CP and to determine whether this was a sensitive measure for

individuals thereby giving a holistic characterisation of directional interhemispheric connectivity. Please view **Figure 5.5** for a depiction of individual trends for each ISI plotted as a function of CP. One sample t-tests (false discovery rate [FDR]-corrected) were conducted in order to detect whether the individual slopes were any different to zero, in order to test the hypothesis that as we increase the CP intensity more inhibition prevails. The t-tests revealed that for every ISI except ISI_6 in the RtoL direction (M = 0.084, p = 0.303 uncorrected), there was a significant negative linear relationship between CP and MEP ratio. Average slopes and t-test values are reported first in the LtoR direction: ISI_6 M=-0.178, t(15)=-3.030; ISI_8 M=-0.186, t(15)=-3.482; ISI_{10} M=-0.253, t(15)=-5.885; ISI_{12} M=-0.232, t(16)=-5.331. In the RtoL direction: ISI_8 M=-0.171, t(16)=-3.136; ISI_{10} M=-0.144, t(16)=-2.767; ISI_{12} M=-0.177, t(16)=-3.132. A paired samples t-test confirmed that there was a marginal difference between directional slope value for ISI_6, between the LtoR direction and the RtoL direction, (t(14)=-2.747, p = 0.064 (FDR-adjusted)). The slopes were more negative in the LtoR direction than in the RtoL direction. There were no differences between the slopes in either direction for any of the other ISIs. Finally, Pearson's correlations were run in order to detect whether the relationships between CP intensity and MEP ratio for each ISI was similar across hemisphere conditioned. A correlation was only found between slopes in both directions in the ISI_{12} condition (R = 0.509, n = 16), however, even this did not survive an FDR-correction (p = 0.176 corrected).

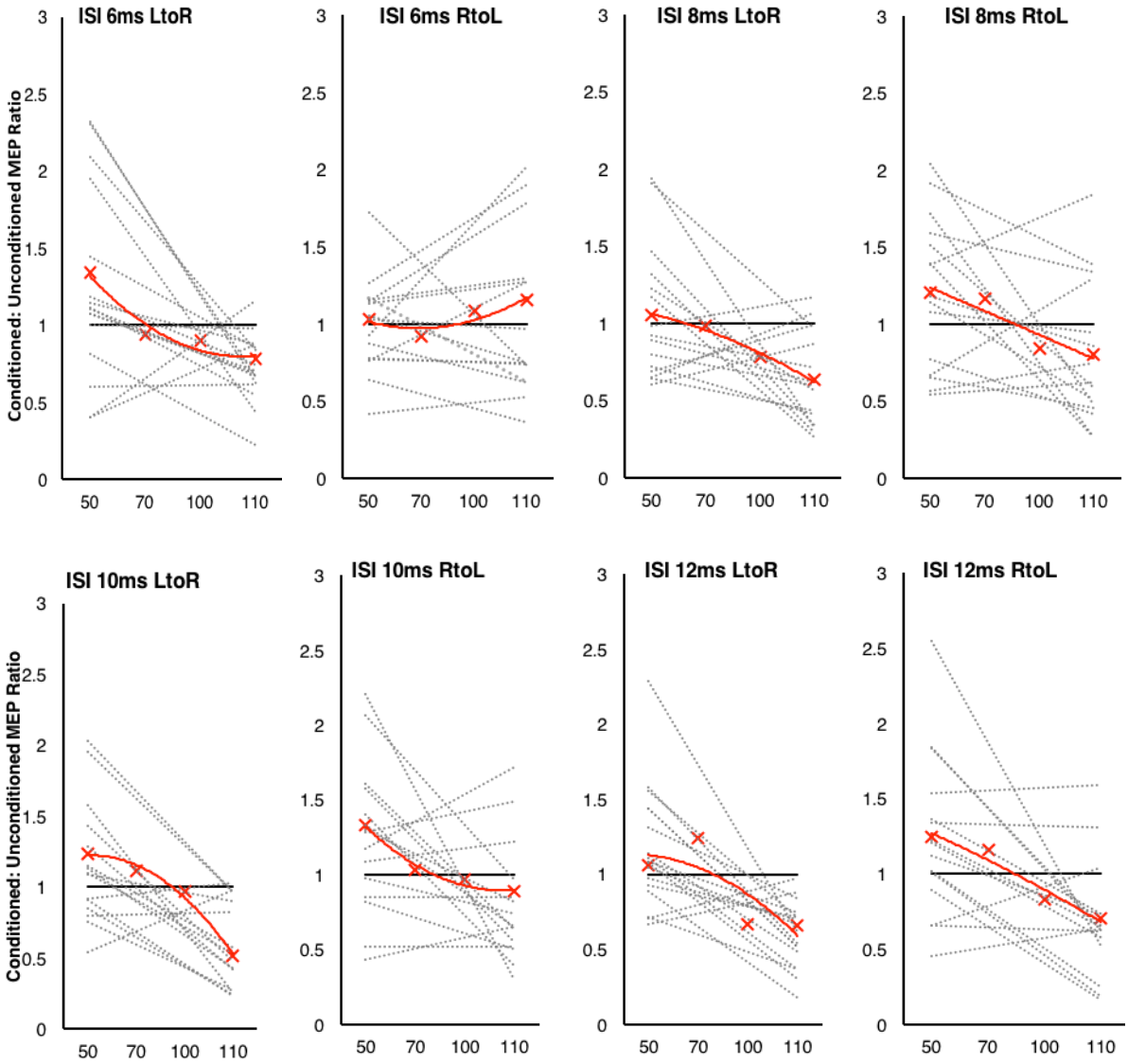


Figure 5.5: Linear slopes were fitted for each ISI and direction as a function of CP%. Grey lines show individual trends for each participants, red crosses show the mean conditioned ratio for each CP.

5.3.3.7 Relationships between Measures

I assessed the relationships between all the parameters tested (each combination of CP and ISI). The outliers identified in above analyses were removed from the analysis and N was adjusted for each correlation. Pearson's correlations were used to assess the relationships between each CP and ISI combination and a FDR correction for the multiple comparisons was adopted to control for type 1 error rates with a threshold of $p<0.05$. 23 out of 524 comparisons remained as significant correlations with this approach. All relationships that survived were within parameters

collected in one direction (i.e. no relationships were found between left-to-right measures and right-to-left measures) and were all positive. For ease, I have listed the correlations found and have presented them pictorially as correlation matrices (**Figure 5.6** and **Figure 5.7**) and a grid showing each *R* value for all pairs (**Table 5.4** and **Table 5.5**).

Relationships found in the left-to-right direction were within CP_{50}: ISI_6 and ISI_8 (R = 0.816, n = 16), ISI_6 and ISI_{10} (R = 0.827, n = 17), and ISI_8 and ISI_{10} (R = 0.831, n = 16); CP_{70}: ISI_8 and ISI_{10} (R = 0.768, n = 16), ISI_8 and ISI_{12} (R = 0.754, n = 16), ISI_{10} and ISI_{12} (R = 0.823, n = 17); CP_{100}: ISI_8 and ISI_{10} (R = *0.*893, n = 16), ISI_{10} and ISI_{12} (R = *0.*808, n = 16); CP_{110} ISI_8 and ISI_{10} (R = 0.890, n = 16), ISI_8 and ISI_{12} (R = 0.713, n = 17). Positive correlations were also found between $CP_{50}ISI_6$ and $CP_{70}ISI_{12}$ (R = 0.698, n = 17); $CP_{50}ISI_6$ and $CP_{100}ISI_6$ (R = 0.729, n = 16).

Relationships found in the right-to-left direction were with CP_{50}: ISI_6 and ISI_8 (R = 0.750, n = 15), ISI_6 and ISI_{10} (R = 0.756, n = 15), ISI_6 and ISI_{12} (R = 0.707, n = 15), ISI_8 and ISI_{10} (R = 0.736, n = 16); CP_{70}: ISI_8 and ISI_{10} (R = 0.808, n = 16), ISI_8 and ISI_{12}(R = 0.735, n = 16), ISI_{10} and ISI_{12} (R = 0.784, n = 16); CP_{110}: ISI_8 and ISI_{12} (R = 0.814, n = 15), ISI_{10} and ISI_{12} (R = 0.761, n = 15). Positive relationships were also found between different conditioning stimuli with the following flagging up as significant: $CP_{100}ISI_{12}$ and $CP_{110}ISI_{10}$ (R = 0.709, n = 17), $CP_{100}ISI_{12}$ and $CP_{110}ISI_{12}$ (R = 0.761, n = 15). It is clear that there is a blurring between the different ISIs where close and far in time ISIs are related and different conditioning stimuli ratios are related, albeit to not such a great extent. This suggests that particularly the different ISIs are probably indexing the same neurobiological construct. It is interesting to find that measures in one direction do not appear to be related to measures taken in the

other direction. This does suggest that information transfer between left and right primary motor cortices are somewhat independent.

Correlation Matrix

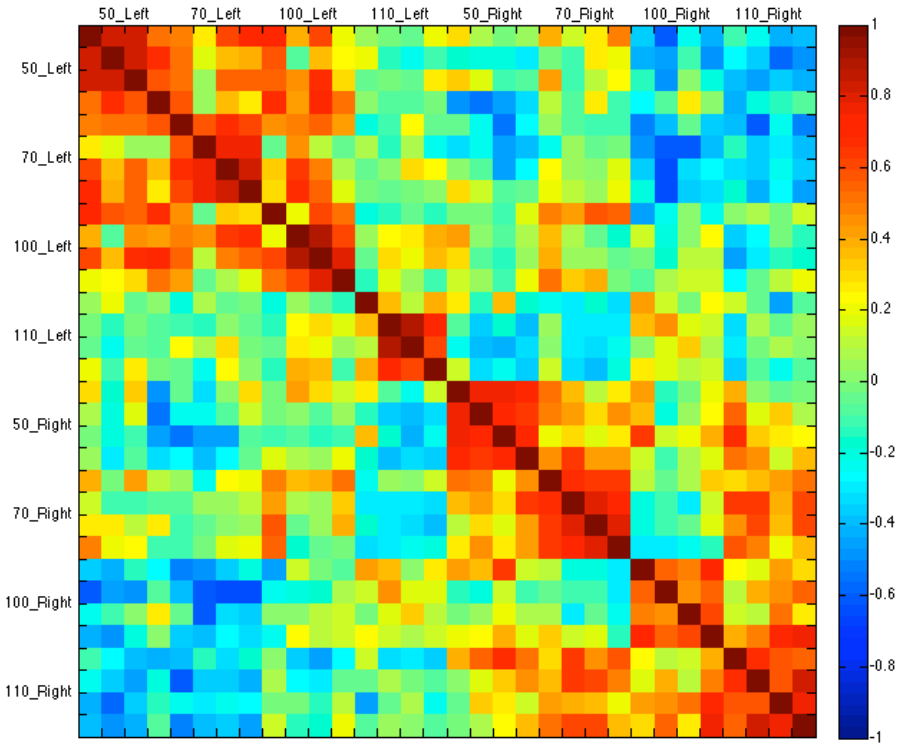


Figure 5.6: Correlation matrix depicting the correlation strength between all possible pairs between Left-to-Right and Right-to-Left TMS measures. The colour of each square denotes the correlation strength between paramaters. Each CP and direction are grouped together (e.g. CP_{50} left-to-right is at the top left, with each ISI for that CP shown as a different square in ascending order: 6,8,10,12ms). Intra and inter-hemisphere correlations are shown. The direction denoted in the axis labels is the hemisphere conditioned.

Correlation Matrix Left to Right

Correlation Matrix Right to Left

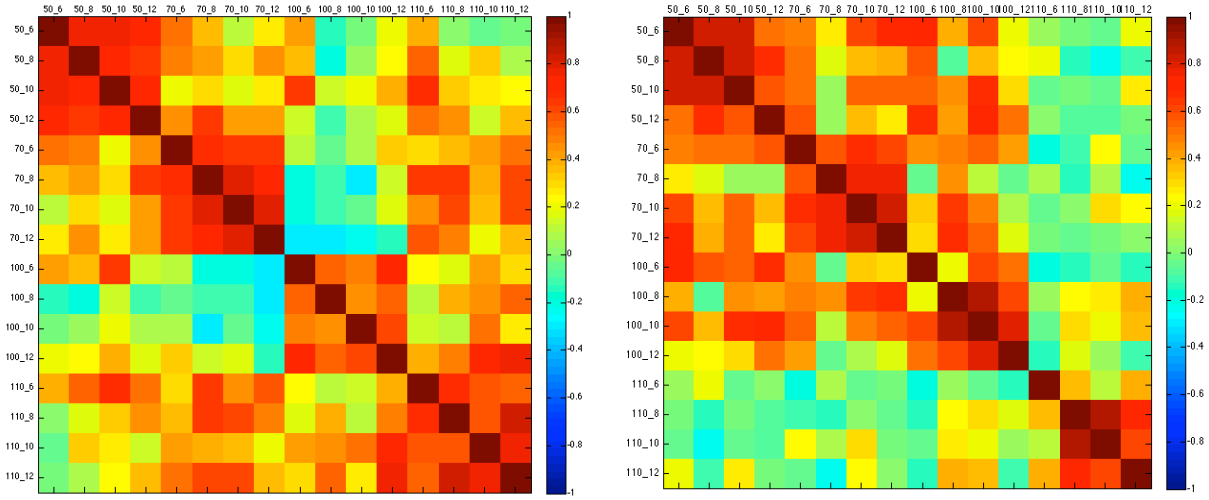


Figure 5.7: A correlation matrix for all parameters tested in the Left-to-Right direction (on the left) and the Right-to-Left direction (on the right). Each axis is labelled but each square denotes the correlation strength between each parameter within that direction. Only intra-hemisphere correlations are shown.

Table 5.4: Pearson's Correlations shown in tables, numbers are R values. The first table shows correlations in the left-to-right direction and the table below shows the right-to-left direction relationships (between median conditioned/unconditioned MEP ratio values). Dark red shows positive correlations that have survived FDR correction, medium red shows positive correlations that do not survive correction (p<0.01 uncorrected) and light red show marginal positive correlations that do not survive correction (p<0.05 uncorrected). There are no negative correlations.

Correlations

			Left Hemisphere Conditioned															
			CP 50%				**CP 70%**				**CP 100%**				**CP 110%**			
			6	8	10	12	6	8	10	12	6	8	10	12	6	8	10	12
Left Hemisphere Conditioned	**CP 50%**	6	1															
		8	**.816**	1														
		1	**.827**	**.831**	1													
		1	.517	.686	.586	1												
	CP 70%	6	.489	.531*	.518	.566*	1											
		8	.272	.159	.060	.042	.577*	1										
		1	.604*	.345	.541	.369	.664	**.768**	1									
		1	**.698**	.382	.558	.273	.595*	**.754**	**.823**	1								
	CP 100%	6	**.729**	.592*	.541	.667	.460	-.053	.340	.285	1							
		8	.403	-.064	.468	.437	.499	.468	.653	.671	.206	1						
		1	.621	.364	.669	.693	.557*	.098	.469	.550	.600	**.893**	1					
		1	.204	.224	.290	.517*	.415	-.053	.079	.176	.525	.603	**.808**	1				
	CP 110%	6	.040	.199	-.034	.017	-.203	.074	-.054	-.023	-.216	.054	-.049	-.138	1			
		8	-.008	-.149	-.028	-.093	-.102	-.128	.001	-.053	-.148	.231	.286	.162	.361	1		
		1	-.037	-.242	-.045	-.076	.237	.073	.295	-.002	-.044	.272	.204	.041	.104	**.890**	1	
		1	.193	-.101	.275	-.028	-.052	-.236	.249	.027	-.129	.393	.347	-.123	.393	**.713**	.623*	1
			Right Hemisphere Conditioned															
			CP 50%				**CP 70%**				**CP 100%**				**CP 110%**			
			6	8	10	12	6	8	10	12	6	8	10	12	6	8	10	12
Right Hemisphere Conditioned	**CP 50%**	6	1															
		8	**.750**	1														
		1	**.756**	**.736**	1													
		1	**.707**	.649	.696	1												
	CP 70%	6	.514	.499	.212	.463	1											
		8	.344	.436	.285	.644	.685	1										
		1	.098	.312	.176	.416	.655	**.808**	1									
		1	.271	.449	.253	.421	.634	**.735**	**.784**	1								
	CP 100%	6	.432	.354	.626	.144	.105	-.210	-.213	-.296	1							
		8	-.134	-.214	.130	-.097	-.035	-.117	-.110	-.301	.558	1						
		1	-.006	.042	.215	.063	.087	-.295	-.045	-.238	.490	.439	1					
		1	.189	.229	.381	.183	.333	.147	.168	-.154	.727**	.557	.597	1				
	CP 110%	6	.396	.533	.684	.504	.297	.653	.467	.592	.241	.102	.140	.385	1			
		8	.006	.175	.335	.466	.371	.631	.603	.469	.160	.381	.108	.476	.660	1		
		1	-.042	.344	.253	.132	.431	.395	.345	.217	.412	.444	.503	**.709**	.572	.572	1	
		1	-.020	.092	.235	.357	.510	.603	.603	.372	.309	.546	.253	**.761**	.538	**.814**	**.761**	1

Table 5.5: Pearson's Correlations shown in tables, numbers are R values. This table shows correlations between the left-to-right and right-to-left interhemispheric interactions (between median conditioned/unconditioned MEP ratio values). Light red show marginal positive correlations that do not survive correction (p<0.05 uncorrected), light blue show marginal negative correlations that do not survive correction (p<0.05) and medium blue show negative correlations that do not survive correction (p<0.01). There are no negative correlations.

Correlations

			Left Hemisphere Conditioned															
			CP 50%				**CP 70%**				**CP 100%**				**CP 110%**			
			6	8	10	12	6	8	10	12	6	8	10	12	6	8	10	12
Right Hemisphere Conditioned	**CP 50%**	6	.292	-.177	.320	-.487	-.052	-.341	.012	.299	-.017	.409	.288	.143	.257	-.078	-.233	.142
		8	.072	-.193	.169	-.544	-.220	-.235	-.086	.134	-.030	.026	-.078	.042	-.138	-.348	-.389	-.318
		10	-.026	-.200	-.095	-.446	-.534	-.450	-.439	-.066	-.102	-.092	-.141	-.118	.345	-.170	-.419	-.224
		12	.032	-.311	-.074	-.316	-.251	-.385	-.272	-.037	.159	.092	.036	.201	-.172	-.391	-.326	-.345
	CP 70%	6	.381	-.011	.412	.119	.062	-.232	.243	.196	.491	.361	.397	.503	-.227	.044	.027	.136
		8	.156	-.122	-.105	-.069	-.078	.058	.053	.102	.416	.083	.035	.327	-.292	-.294	-.293	-.319
		10	.270	.272	.118	.274	-.103	-.051	.028	.056	.585	-.061	.057	.382	-.179	-.288	-.323	-.381
		12	.479	.204	.236	-.095	-.098	-.016	.157	.192	.544	-.160	-.036	-.014	-.326	-.290	-.246	-.212
	CP 100%	6	-.353	-.429	-.134	-.264	-.505	-.473	-.355	-.192	-.462	.143	-.018	-.072	.432	.346	.001	.265
		8	-.604	-.446	-.488	-.091	-.376	-.597	-.641	-.655	-.245	-.213	-.157	.063	.135	.444	.179	.169
		10	-.221	-.097	.025	.253	-.058	-.619	-.330	-.357	.007	.026	.102	.125	-.020	.157	.342	.109
		12	-.415	-.492	-.208	.024	-.373	-.401	-.275	-.335	-.221	.236	.097	.149	.242	.149	.075	.126
	CP 110%	6	-.094	-.274	-.384	-.426	-.397	-.155	-.322	-.183	.022	-.365	-.447	-.277	.137	-.334	-.293	-.358
		8	-.247	-.373	-.461	-.192	-.613	-.354	-.363	-.415	.073	-.263	-.282	-.059	-.039	.080	-.014	-.089
		10	-.430	-.586	-.366	-.130	-.221	-.286	-.227	-.265	-.017	-.024	-.126	.119	-.438	-.038	.075	-.230
		12	-.385	-.471	-.419	-.083	-.531	-.405	-.364	-.462	.131	-.127	-.169	.197	-.088	.060	.002	-.067

5.3.3.8 Relationships between unconditioned MEP and conditioned ratios

As well as assessing the relationship between conditioned interhemispheric measures, I assessed the relationship between the unconditioned MEP and the conditioned MEP. This may be informative of whether the resulting conditioned MEP is related to the current state of excitability of the conditioned motor cortex or whether it is more related to the state of excitability of the connected motor cortex. Pearson's correlations were conducted between the unconditioned MEP from each hemisphere and the ratio of conditioned:unconditioned MEP from each combination of CP and ISI.

65 pairs were tested and only 1 pair was significantly associated when adopting FDR correction. Right hemisphere unconditioned MEPs were negatively correlated with LtoR $CP_{50}ISI_6$ MEP ratio (R=-0.782, n = 16), i.e. smaller unconditioned MEPs in the right hemisphere tended to exhibit more facilitation when conditioned by a CP_{50} pulse 6ms earlier to the left hemisphere. Several trends were also found and depicted below in **Figure 5.8,** however, none of these were strong enough associations to survive multiple comparison correction.

Bayesian correlations were conducted to assess the strength of the evidence for the relationships shown in **Figure 5.8**. Bayes Factors (BF_{10}) were calculated using JASP (JASP-Team, 2016). JASP uses the Bayesian correlation test proposed by Jeffreys (1961). Bayes Factors above 1 show support for H_1 whilst below 1 show support for the H_0. The magnitude of the BF_{10} shows the strength of the evidence in support of either the H_1 or H_0. For cut-off values for the different strengths of evidence please see **Table 5.6**.

Table 5.6: Category values for BF_{10}, table adapted from Wetzels & Wagenmakers (2012)

Bayes factor BF_{10}			Interpretation
> 100	-	∞	Decisive evidence for H_1
> 30	-	< 100	Very strong evidence for H_1
> 10	-	< 30	Strong evidence for H_1
> 3	-	< 10	Substantial evidence for H_1
> 1	-	< 3	Anecdotal evidence for H_1
	1		No evidence
> 1/3	-	< 1	Anecdotal evidence for H_0
> 1/10	-	< 1/3	Substantial evidence for H_0
> 1/30	-	< 1/10	Strong evidence for H_0
> 1/100	-	< 1/30	Very Strong evidence for H_0
0	-	< 1/100	Decisive evidence for H_0

Extreme evidence was shown for the relationship between $CP_{50}ISI_6$ and the unconditioned LtoR MEP (BF_{10} = 163.025) and strong evidence was shown for the relationship between RtoL $CP_{50}ISI_8$ and RtoL unconditioned MEP (BF_{10} = 10.586). Moderate evidence was found for the relationship between unconditioned LtoR and RtoL MEP (BF_{10} = 6.074); LtoR $CP_{50}ISI_{10,}$ $CP_{70}ISI_{12}$, $CP_{100}ISI_6$, $CP_{100}ISI_{10}$ and unconditioned LtoR MEP (BF_{10} = 3.828, 4.671,5.293 and 3.687 respectively) and RtoL $CP_{50}ISI_{10}$ and $CP_{70}ISI_6$ and unconditioned RtoL MEP (BF_{10} = 3.685 and 2.887). Anecdotal evidence was found for the remaining trends (LtoR $CP_{50}ISI_8$ and $CP_{100}ISI_8$ with unconditioned LtoR MEP, BF_{10}<1.306; RtoL $CP_{50}ISI_6$, $CP_{50}ISI_{12}$ and unconditioned RtoLMEP, BF_{10}<1.773). Of note, in general trends were not found between unconditioned MEPs of the conditioning hemisphere and conditioned MEPs – only with unconditioned MEPs of the test hemisphere and conditioned MEPs. However, a Bayesian correlation matrix did find anecdotal evidence for LtoR unconditioned MEP being related to both RtoL $CP_{50}ISI_6$ and $CP_{70}ISI_{12}$ (BF_{10}<1.583).

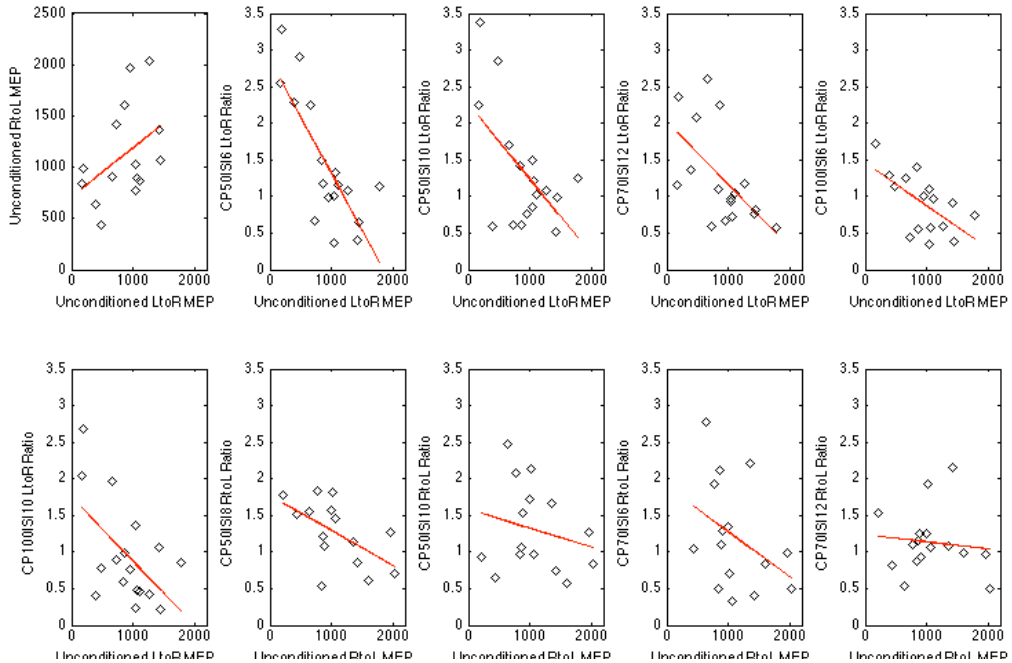


Figure 5.8: Correlations between unconditioned MEPs and conditioned ratios for relationships that are moderately associated when testing with Bayesian statistics. Left-to-right denoted LtoR and right-to-left denoted RtoL

5.3.3.9 Relationships ds-TMS measures and pp-TMS and ^{1}H-MRS spectroscopy measures of excitatory and inhibitory neurochemicals (measured on different days)

pp-TMS and ^{1}H-MRS spectroscopy measures were collected on a different day to ds-TMS as a part of the experiment detailed in **Chapter 6**. For full methods that were followed to collect pp-TMS and ^{1}H-MRS quantifications of neurochemicals please read **Chapter 6.** 10 participants in this experiment have measures of 1ms SICI curve slope ($SICI_1$), 3ms SICI curve slope ($SICI_3$), ICF median (median composite of 10ms and 12ms) and recruitment curves as well as GABA, glutamate and glutamine (as a ratio to total Creatine) for the left hemisphere PMC. Pearson's correlations were conducted to determine whether left hemisphere measures of excitation and inhibition related to either LtoR or RtoL interhemispheric inhibitory or excitatory interactions. Correlations were conducted between $ISI_{6\text{-}12}$ LtoR and RtoL slopes and $SICI_1$ slope, $SICI_3$ slope, median ICF, recruitment curve slope, GABA:tCr, Glu:tCr and Gln:tCr. One significant relationship survived FDR, 3 in total had a

p<0.05. This may be partly due to multiple comparisons and an underpowered sample. The relationship found was between ISI_8 RtoL slope and GABA:tCr (R = 0.951, n = 8, p<0.05 (adjusted)). Interestingly, there was a correlation (although not significant when corrected) found also between ISI_8 RtoL slope and $SICI_1$ (R=-0.746, n = 9, p = 0.370 (adjusted)). A marginal correlation was also found between ISI_6 RtoL and ICF (R=-0.934, n = 7, p = 0.056 (adjusted)).

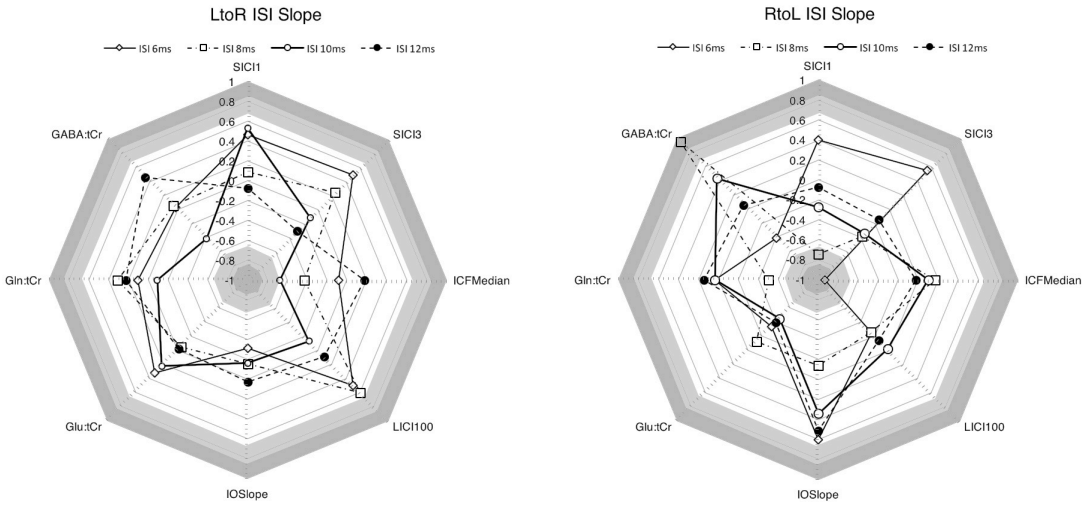


Figure 5.9: Radar plots showing correlations with ISI slope and pp-TMS & ^{1}H-MRS measures of physiological inhibition and facilitation. 9A shows correlations with ds-TMS measures collected in the LtoR direction, 9B shows correlations with ds-TMS measures collected in the RtoL direction. The darker grey shading denotes the critical R that is required when corrected for the pp-TMS/MRS measures for an n = 9 (R=±0.82) and the lighter grey denotes critical R for n = 9 uncorrected (when alpha = 0.05), R=±0.66).

Bayesian correlations were utilised in order to help clarify whether there was any evidence for some of the marginal correlations found. These revealed 'anecdotal evidence' for relationships between some of the RtoL interhemispheric interactions and pp-TMS and ^{1}H-MRS measures. Namely, anecdotal evidence supported a positive relationship between ISI_6 RtoL slope and $SICI_3$ slope (BF_{10} = 1.087). Moderate evidence was found to support that ISI_8 was also negatively related to $SICI_1$ (BF_{10} = 4.084). Strong evidence was shown to support a negative relationship

between ISI_6 RtoL and ICF (BF_{10} = 19.597) and ISI_8 RtoL and GABA:tCr (BF_{10} = 89.283). Importantly, only strong relationships were found between the RtoL direction and pp-TMS/ ^{1}H-MRS measures.

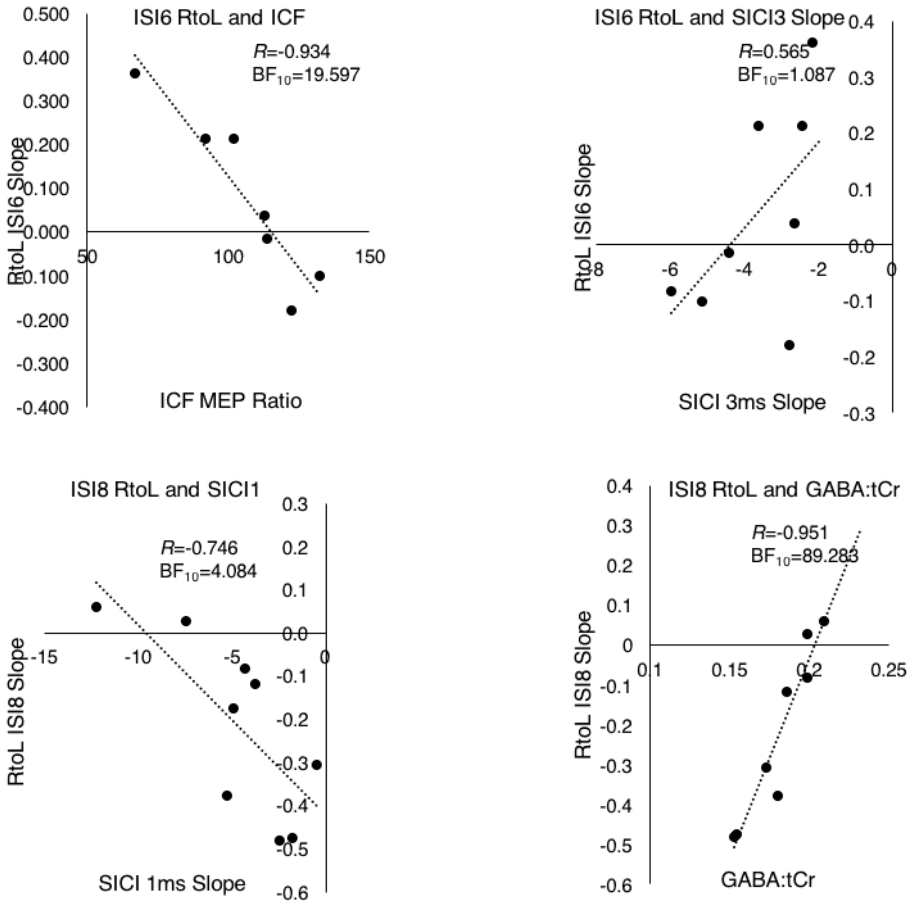


Figure 5.10: Images of correlations between ds-TMS and pp-TMS/ ^{1}H-MRS measures that were shown to have some evidence supporting their relationships.

3.5.1.2 Sex Effects

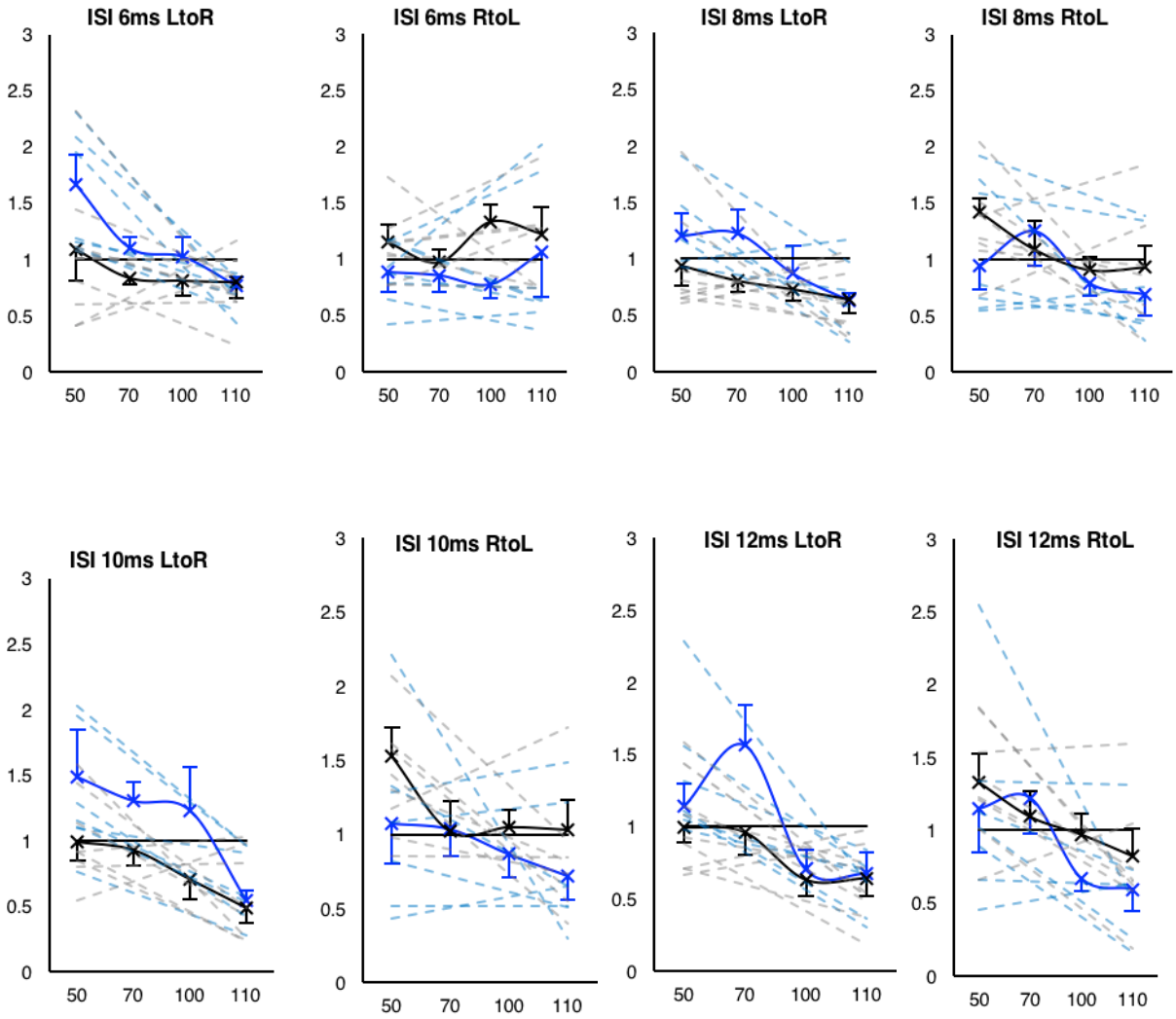


Figure 5.11: Graphs to show differences between sex in interhemispheric interactions in the LtoR direction and RtoL direction. For comparison for each ISI a graph was formed (CP% along x axis and MEP ratio along the y axis) and for each ISIs both directions were placed side to side. LtoR on the left and RtoL on the right. Pale dashed lines are individual ISI trends across CP, light blue represents females and light grey represent males. The bold crosses and lines on top represent the grand mean for females (blue) and males (black) with error bars representing standard error of the means.

A two-way mixed model ANOVA was conducted to look at the effect of Sex. Within-subject independent variables 'ISI slope' and 'Direction' and between-subject independent variable 'Sex' were entered into the ANOVA (n = 7 for males and females). A main effect of ISI Slope ($F(3,36) = 3.655$ $p<0.05$) and an interaction effect between Direction and Sex ($F(1,12) = 13.832$, $p<0.01$) were found. There were

no main effects of Sex (p = 0.138) or Direction (p = 0.066) and no other interaction effects found (minimum p = 0.214). Importantly, handedness scores did not differ between sexes (U(14) = 16.00, p = 0.189).

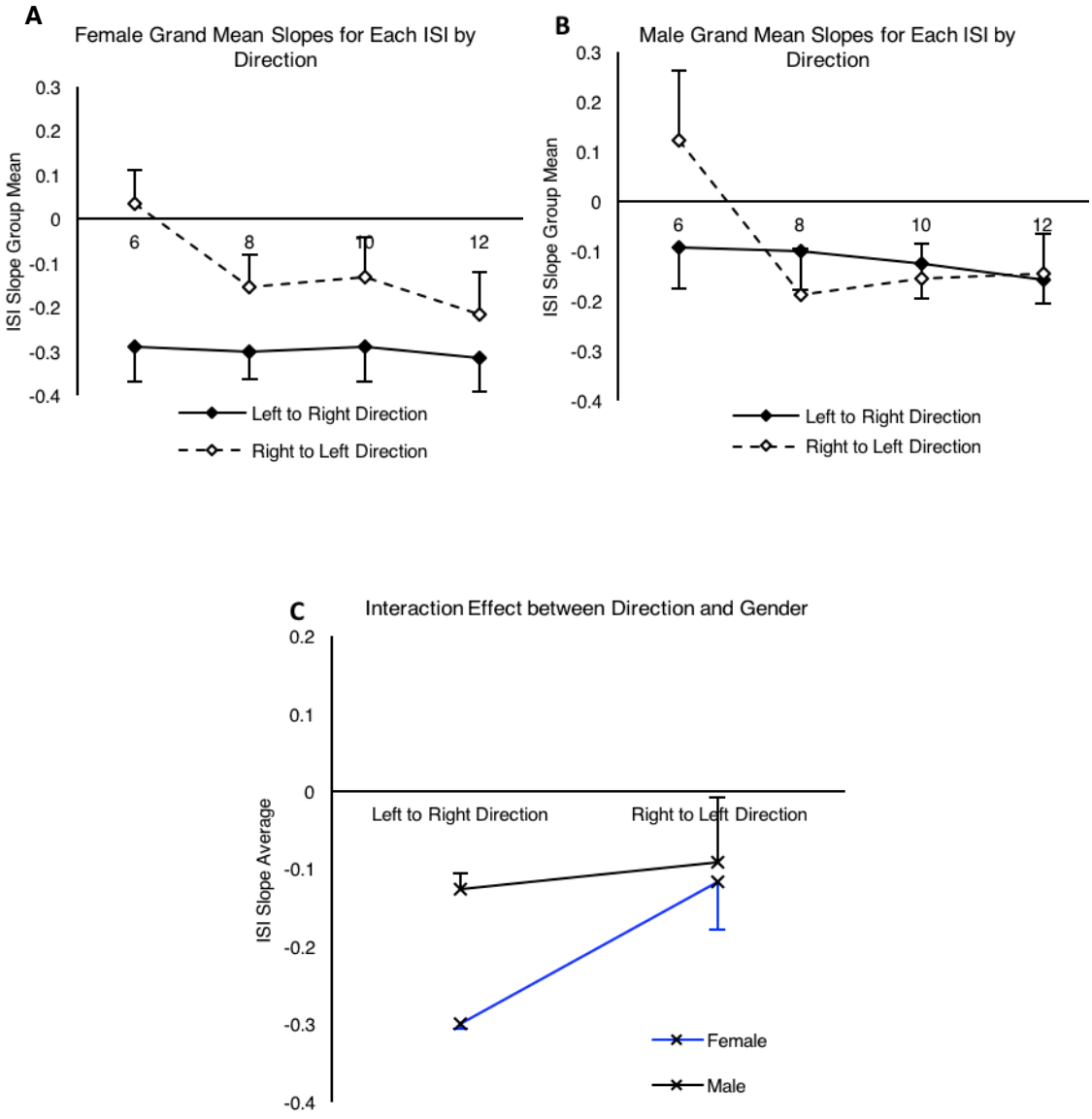


Figure 5.12: Graphs A & B show grand mean of individually fitted slopes (to ISIxMEP Ratio for each ISI) in the left to right and right direction. Graph A represents data from the female participants (n = 7) whilst graph B shows the data for male participants (n = 7). Graph C demonstrates the interaction effect in the two-way mixed model ANOVA with sex as a between group variable, ISI and direction as within factors and slope as the dependent variable.

It is evident from **Figure 5.11** that the interaction between direction and sex is primarily driven by a steeper slope in female ISI whereby the females appear more sensitive to change in CP. When one looks at the individual trends although there

does not appear to be a great difference in overall facilitation or inhibition at the lower or higher CPs, stronger negative trends are evident in the female group (blue dashed lines). Handedness did not appear to modulate the results in any way – these measurements did not relate to handedness.

5.4 Discussion

This experiment was designed to probe transcallosal effective connectivity between both primary motor cortices using dual site TMS and explore the differing effects of parameters on IHI and IHF. I specifically wanted to explore the directionality of transcallosal connections in light of evidence that demonstrated that adults with TS had asymmetrical IHI whereas healthy controls has symmetrical (Bäumer et al., 2010). I will first summarise the results described above and will then discuss them in relevance to the literature on transcallosal effective connectivity in the healthy population and in patients with TS.

I tested a range of parameters varying both conditioning stimuli (CP) intensity, ranging from 50%, 70%, 100% and 110% of RMT and ISI (6ms, 8ms, 10ms and 12ms). Whilst there was no effect of ISI in each CP in the left-to-right (LtoR) direction there was an effect of ISI for CP_{100} in the right-to-left (RtoL) direction; this interestingly was the only effect of ISI found in the data showing that ISI did not modulate interhemispheric interactions in a strong manner. The effect of CP was more robust whereby there was a clear effect of CP in each ISI tested for both directions; except for ISI 6ms in the RtoL direction. The general pattern was as expected, the higher the CP the more inhibition preceded. When assessing whether the individual parameters were different from a baseline it appeared only a few combinations were robust enough to be considered different from baseline; interestingly whilst there was a clear inhibitory effect for CP_{100} (ISI_{12}) and CP_{110} (ISI_{8},

ISI_{10} and ISI_{12}) in the LtoR direction there were no parameters that tested as significantly different from baseline in the RtoL direction. This may suggest that the left hemisphere is somewhat better at modulating, or at least inhibiting, the excitability of the connected right hemisphere.

A weak but nonsignificant facilitation is in keeping with the previous literature. Previous reports have shown facilitation of a response sized 0.2mV (Ugawa, Hanajima, & Kanazawa, 1993) but not 0.3mV (Hanajima et al., 2001) testing ISIs 7-8ms and CP of 5-30% of maximum stimulator output; thus using a larger test response (0.75-1mV) may have contributed to weaker inhibition. One study that used similar parameters to those used in this experiment found a weak significant facilitation effect using parameters 60% AMT and an ISI 6ms in a LtoR direction which is entirely consistent with our results (Bäumer et al., 2006). Further, the significant reduction of MEP found using a suprathreshold CP and later ISIs (8-12ms), i.e. IHI, when the left PMC is conditioned is in keeping with the literature (R. Chen, Yung, & Li, 2003; Ferbert et al., 1992; Ni et al., 2009; Wahl et al., 2007).

In order to explore individual differences in the dataset I fitted linear slopes to data across the different CPs in each ISI (IH_{slope}); I hoped this would be a sensitive measure of the interactions between CP and transcallosal interaction whilst reducing the amount of comparisons needed in analysis. Again, there was a clear effect of CP where linear slopes were significantly different from an expected slope of 0 (confirming the null that CP did not have an effect) in each ISI for both directions except for ISI_6 in the RtoL direction. This provides further evidence for directionality for ds-TMS PMC-to-PMC with ISI_6. When testing this explicitly, there was a marginal difference between LtoR and RtoL ISI_6 slopes. IH_{slope} measured an interaction between hemispheres that was not related to the current state of left or right PMC,

when assessed by sp-TMS MEP peak-to-peak amplitude. In a small sub-group I was able to explore this further and assess whether the IH_{slope} were related to other pp-TMS measures of physiological inhibition/ facilitation or ^{1}H-MRS measures of neurometabolites measured at the left PMC on a different day.

Two strong relationships were apparent where the RtoL ISI_8 slope was positively related to GABA:tCr and ICF was negatively related to RtoL ISI_6. There were also marginal relationships also found between RtoL ISI_6 and $SICI_3$, LtoR ISI_{10} and GABA:tCr, and lastly RtoL ISI_8 and $SICI_1$. However, due to the small sample size and relative weakness of the correlations these results should be treated with caution.

These results are interesting in two ways. Firstly the stronger relationships were between left PMC excitability and the RtoL direction suggesting that the state of excitability of the test hemisphere may be important for the amount of inhibition or excitation the conditioned hemisphere produces; secondly neurometabolite GABA:tCr and TMS-assessed ICF were able to be related to specific ISI interhemispheric interactions in the LtoR direction suggesting that the different ISIs may be tapping into subtly different mechanisms despite the overall effect being similar.

Whilst GABA:tCr was positively related with RtoL ISI_8 (suggesting that more positive slopes were related to higher GABA); ICF was negatively related to RtoL ISI_6 (suggesting that higher intracortical facilitation was related to steeper negative slopes). Greater negative IH_{slopes} indicate a greater interhemispheric interaction, whereby an individual with a more negative IH_{slope} shows greater facilitation at $CP_{50/70}$ and greater inhibition at $CP_{100/110}$. These results can therefore be interpreted as

follows: greater ICF at the left PMC relates with greater facilitation/ inhibition at the left PMC induced by a conditioning stimulus at the right PMC 6ms prior to the test stimulus. Further, greater GABA concentrations as measured by ^{1}H-MRS at the left PMC relates with less facilitation/ inhibition at the left PMC induced by a conditioning stimulus at the right PMC 8ms prior to the test stimulus. These results are consistent with the idea that the CP delivered at the conditioned hemisphere engages with the excitatory callosal fibers and it is only at the tested hemisphere that different intracortical circuits are engaged (Daskalakis, Christensen, Fitzgerald, Roshan, & Chen, 2002).

The relationship found between with ICF and interhemispheric interactions, to my knowledge has not been tested before, and is most probably driven by the more increased facilitation at subthreshold CP that results in a more negative IH_{slope} for ISI_6; it therefore may follow that a subthreshold CP coupled with the short ISI of 6ms may tap into similar intracortical circuits to ICF. It has been previously found that IHI does not interact with ICF (Daskalakis et al., 2002), however, this has only been explored in IHI; i.e. only for suprathreshold CPs with longer ISIs.

The relationship found between GABA:tCr and ISI_8 RtoL slope resonates with the concept that GABA:tCr as measured by 7T ^{1}H-MRS may be measuring extracellular pools of GABA that may have a tonic effect of inhibition on the tissue that is measured (Draper et al., 2014; Stagg, Bachtiar, & Johansen-Berg, 2011). This would be consistent with a reduced slope (reflecting reduced facilitation at the lower CPs and reduced inhibition at the higher CPs) coinciding with increased GABA:tCr concentration at the left PMC.

In order to unpack further whether the parameters tested were independent of each other or whether they were tapping into the same physiological mechanism, I ran multiple correlations looking at how the different parameters related to each other within one direction and across both directions. The results of this demonstrated that there were most certainly similarities across ISIs within each CP, however, there were fewer relationships across different CPs. This was only the case within each transcallosal direction and in fact, across directions there appeared to be no relationships whatsoever; again, supporting the case that there is a directionality to IHI/F as measured by TMS, where the observed effect in each direction are somewhat independent.

Finally, there was an interaction found between sex and ds-TMS interhemispheric interaction whereby the slopes fitted to each ISI interacted with sex whereby females appeared to have steeper negative slopes (and therefore greater facilitation with subthreshold CP and greater inhibition with super/ra-threshold CP), only in the LtoR, direction than males. This finding mirrors previous studies that have demonstrated that there is greater inhibition in female IHI than male IHI (De Gennaro, Bertini, et al., 2004; De Gennaro, Cristiani, et al., 2004). This is likely related to the influence of sex hormones during the menstrual cycle where whilst there have not been any direct ds-TMS studies exploring this issue; there have been a multitude of varying methodologies looking at interhemispheric crosstalk and the effect of menstural cycle in females.

One TMS study found a great effect of menstrual cycle phase and amount of transcallosal inhibition as measured by ipsilateral silent period (iSP) (Hausmann et al., 2006). iSP is measured by the subject contracting the hand ipsilateral to the hemisphere being tested whilst a sp-TMS is delivered to cortex contralateral to

resting hand; and the silent period is measured. Both iSP duration and transcallosal conduction time (i.e. the difference between the onset of the silent period in the ipsilateral hand and the onset of the MEP recorded at the contralateral hand) were related to levels of hormones in the blood and menstrual phase. There was a clear reduction of iSP duration in the follicular phase compared to the luteal phase; iSP was further negatively correlated with estradiol in the follicular phase and not to progesterone and was negatively correlated with progesterone in the luteal phase but not estradiol. Transcallosal conduction time was not related to hormones or menstrual phase. Given that iSP is thought to reflect interhemispheric inhibitory processes the authors suggest that this result may be related to the menstrual cycles effects on neuromodulators such as GABA and glutamate. Progesterone has been shown to increase GABAergic inhibitory function and reduced glutamatergic excitatory function, as a synthetic injection (S. S. Smith, Waterhouse, Chapin, & Woodward, 1987) and across menstrual phases (M. J. Smith et al., 1999). It is worth keeping in mind that dynamic changes in interhemispheric interactions may be partially modulated transcallosally but also via influencing intracortical circuits.

Chapter 6: Examining the Relationship between Physiological Measures of Inhibition and Neurochemicals γ-ammino butyric acid, Glutamate and Glutamine as Measured by Magnetic Resonance Spectroscopy

6.1 Introduction

6.1.1 Background: Altered Neurotransmission Tourette Syndrome

One highly influential theory regarding the occurrence of tics suggests that an imbalance of excitatory and inhibitory neurotransmission leads to disinhibition in the CSTC pathways, resulting in the inappropriate reinforcement of stereotyped behaviour, i.e. tics (Albin & Mink, 2006). In support of this, dysfunction in neurotransmission has been well documented in Tourette Syndrome with much of the evidence focused on alterations in GABA. Alterations in GABAergic signalling appear to occur throughout the cortico-striatal cortico-thalamic (CSTC) network.

Animal studies of tic-like behaviour have shown that when selective $GABA_A$ antagonists are microinjected or infused into various loci in the striatum different tic-like behaviours proceed. For example, abnormal tic-like movements have been observed in primate models following bicuculline microinjections in the posterior putamen (Bronfeld, Belelovsky, & Bar-Gad, 2011; McCairn et al., 2009; Worbe et al., 2013) and dorsal part of the anterior striatum (Worbe et al., 2013). This has also been shown in the rat brain using bicuculline (Bronfeld et al., 2013) and with another more selective $GABA_A$ antagonist, picrotoxin (Pogorelov, Xu, Smith, Buchanan, & Pittenger, 2015).

Other modalities have been used in TS and support a theory of disrupted GABAergic signalling in TS. Imaging studies have repeatedly established that

caudate volume is reduced in Tourette Syndrome (M. H. Bloch et al., 2005; Peterson et al., 2003), the volume of which inversely relates to tic severity. The reduction of volume is likely explained by a reduction in parvalbumin positive (PV+) GABAergic interneurons (Kalanithi et al., 2005; Kataoka et al., 2010) and cholinergic interneurons (Kataoka et al., 2010) in the same area, found in post-mortem studies. In addition to aberrant GABAergic architecture, disrupted function has also been implicated in TS. Reduced $GABA_A$ receptor binding in the Cd, Pt, nucleus accumbens and globus pallidus has been shown using PET Imaging (Lerner et al., 2012). Outside of the basal ganglia, cortical sites have been implicated. Two important tools that have been used to neurotransmission in the brain, particularly in cortex, are Transcranial Magnetic Stimulation (TMS) and Magnetic Resonance Spectroscopy (^{1}H-MRS).

TMS has been used time after time in TS to investigate synaptic function at the PMC, the greatest output of the CSTC. Paired pulse TMS (pp-TMS) takes various measures that when taken together can give us a broad picture of PMC excitability; two important measures used in TS are termed short interval intracortical inhibition (SICI) and intracortical facilitation (ICF). SICI is an index of $GABA_A$ receptor action that has been consistently found to be reduced in TS (Gilbert et al., 2004; K. F. Heise et al., 2010; Orth, Amann, et al., 2005; Ziemann et al., 1997). The evidence concerning ICF is inconsistent (Orth & Rothwell, 2009); in one study patients with TS had increased ICF whereas others reported no effects (Gilbert et al., 2005; Ziemann et al., 1997). The effect of ICF results from a net facilitation that partly comprises the an inhibitory post-synaptic potential mediated by $GABA_A$ receptors (Ziemann, 2013). NMDA receptor antagonists and $GABA_A$ agonists reduce ICF; whilst glutamate agonists/ Noradrenaline agonists increase it (Ziemann et al., 2015). ICF is a notoriously variable measure to acquire (Orth et al., 2003), possibly due to the varied

neurotransmitters it is dependent on. Given the heterogeneous nature of TS and difficulty obtaining reliable ICF measures, it is unsurprising that the evidence surrounding ICF in TS is inconsistent.

^{1}H-MRS is an MR imaging technique which allows for the quantification of neurometabolites within pre-specified volumes of interest (VOIs). ^{1}H-MRS is an important tool in measuring alterations in various neurometabolites in neurodevelopmental disorders; it is likely that these biochemical changes may occur before structural changes and may provide important and sensitive information about changes within tissue structures that are associated with TS and other disorders. Of note, ^{1}H-MRS studies in TS report no differences were found in the PMC between patients and controls despite well documented differences in GABAergic synaptic neurotransmission (Draper et al., 2014; Tinaz et al., 2014). In other brain regions, reduced GABA concentration has been shown in a sensorimotor voxel (Puts et al., 2015) and increased GABA concentration has been found in the SMA (Draper et al., 2014).

6.1.2 Magnetic Resonance Spectroscopy as a Useful Tool in Neuropsychiatric Disorders

MRS is a popular tool used to probe GABAergic and glutamatergic signalling in neurological and psychiatric disorders. For example, in addition to the evidence discussed surrounding TS, reductions in GABA have been reported in sensorimotor areas in ASD (Puts et al., 2015, 2017) and ADHD (Edden, Crocetti, Zhu, Gilbert, & Mostofsky, 2012). In schizophrenia, reduced PFC GABA levels (Marsman et al., 2014) and decreased glutamate in medial frontal areas (Marsman et al., 2013) have been implicated in cognitive functioning and positive symptoms.

The right balance of glutamatergic and GABA levels in the brain are clearly vital for healthy brain function and a disturbance in signalling of either of these neurotransmitters is associated with various neuropsychiatric disorders. There is therefore a great necessity to use non-invasive tools such as ^{1}H-MRS to gain a greater understanding of neurotransmission in health and pathology. However, the specifics of what exactly ^{1}H-MRS is measuring and what it signifies behaviourally is unclear.

Behaviourally, increased GABA concentration in motor cortex has been related to slower response times and reduced BOLD signal change in functional MRI (Stagg, Bachtiar, & Johansen-Berg, 2011a). Increased GABA and decreased glutamate in the ventromedial PFC has been associated with superior performance in reward-based decision tasks (Jocham, Hunt, Near, & Behrens, 2012); whilst increased glutamate and GABA in the dorsal anterior cingulate cortex (dACC) has been related to increased use of learnt information (Casey et al., 1997). It is therefore unsurprising that the GABA and glutamate that is visible to ^{1}H-MRS does not simply reflect stable trait states, but is to some extent dynamic. Functional ^{1}H-MRS demonstrates that 7T ^{1}H-MRS measured GABA in the PMC is changeable over a short period and reduces during a simple motor task (C. Chen et al., 2017). Brain stimulation technique anodal transcranial direct current stimulation has been shown to reduce ^{1}H-MRS -measured GABA concentration at 3T (Bachtiar, Near, Johansen-Berg, & Stagg, 2015) and 7T MRI (Kim, Stephenson, Morris, & Jackson, 2014).

6.1.3 How do Magnetic Resonance Spectroscopy and Transcranial Magnetic Stimulation Measures of Neurotransmission Relate?

In order to explore what pools of neurochemicals ^{1}H-MRS is measuring it seems sensible to use it alongside TMS. The physiological inhibition and facilitation

that TMS have been well-studied in pharmacological studies and there is a good basis of understanding for the mechanisms that contribute to them. There have already been several studies exploring the link between ^{1}H-MRS -measured GABA and glutamate to TMS-measured inhibition and facilitation. Stagg and colleagues (2011) and Tremblay and colleagues (2013) both published highly influential papers on the matter; both studies of which were conducted using 3 Tesla MR scanners. Stagg et al. (2011) found that GABA concentrations were negatively associated with 1ms SICI ($SICI_1$), which has been replicated since (Mooney, Cirillo, & Byblow, 2017), and a positive relationship between IO slope and Glutamate concentrations. Tremblay et al. (2013) found a positive relationship between cortical silent period (CSP), a measure of $GABA_B$ receptor activity, and the concentration of a composite of Glutamate+Glutamine (Glx). Whilst these studies are interesting and important first steps there are inherent flaws with using ^{1}H-MRS at the field strength 3T in which using an ultra-high field strength 7T scanner solves.

Using 7T to acquire ^{1}H-MRS greatly improves the SNR and increases chemical shift dispersion. The SNR increase means that 7T ^{1}H-MRS is highly sensitive to detecting metabolites, this greatly advantages the detection of metabolites that have intrinsically low concentrations such as GABA. Due to spectral overlapping, detection of GABA, Glu and Gln is challenging in ^{1}H-MRS at the typical field strengths used (3T or below). 7T thus has the advantage of producing a visually discernible GABA-peak without the need for an edited protocol, therefore being susceptible to motion artefacts (Wittfoth et al., 2012). Furthermore, the Glutamate and Glutamine peak can be distinguished (rather than acquiring the composite Glx). These advantages are important advancements that allow us to acquire more sensitive measures of neurometabolite concentrations - an imperative for relating findings to behaviour or pathology.

The principle aim of this study is to explore further the relationship between physiological inhibition and excitation and ^{1}H-MRS measured GABA, Glu and Gln. It achieves this, adding to previous literature by using high-field 7T ^{1}H-MRS and various TMS measures of physiological inhibition and excitation: RMT, 1ms SICI ($SICI_1$), 3ms SICI ($SICI_3$), 10ms ICF (ICF_{10}), 12ms ICF (ICF_{12}), 100ms LICI ($LICI_{100}$) and recruitment curves (IO). A large sample of 29 healthy adults were recruited to have sufficient power to explore relationships between the two complementing measures.

6.2 Methods

6.2.1 Participants

29 healthy adults were recruited to take part in this experiment (females n = 14). The age of the participants ranged between 18 and 29 (M = 23.11 ± 2.49). Inclusion criteria were that participants had no contraindications to MRI or TMS including no history of neurological or psychiatric illness.

6.2.2 MR acquisition

All MR data were acquired using an ultra-high field 7T Philips Achieva system (Philips Healthcare, Best, Netherlands) with a 32-channel radio frequency head coil at the Sir Peter Mansfield Imaging Centre (SPMIC), University of Nottingham. Participants were placed supine and head-first into the scanner. Foam pads were inserted between the participant's head and the coil to minimise and control head movement; a pair of prism glasses were provided to allow participants to view a screen outside the magnet bore. The bed was then positioned so that the participant's head was in the isocenter of the scanner.

At the start of each imaging session ^{1}H image localiser and B0 maps were acquired, followed by BOLD-fMRI T_2^*-weighted images, which were acquired to guide placement of the left primary motor cortex (PMC) spectroscopy voxel in the following ^{1}H-MRS scans. The BOLD-fMRI used a single shot EPI sequence (TR/TE = 1999/25 ms, FOV = 208×120×192 mm^3, matrix = 112×112, 30 slices, 160 dynamics). During the fMRI scan, eight blocks of bimanual finger-to-thumb opposition tapping were performed in a blocked-trial paradigm as follows. The words 'TAP' and 'REST' were alternately displayed for 8s and 32s, respectively. Participants were asked to tap their thumbs to each finger with both hands simultaneously and continuously during the 'TAP' phase and to rest (withhold movement) during the 'REST' phase. Maximum activation of the left M1 was found by analysing the BOLD response on-line using the Philips IViewBOLD software.

T_1-weighted anatomical images were then acquired with a magnetization prepared rapid acquisition gradient echo (MP-RAGE) sequence (TR/TE/TI = 7.3/3.4/998 ms, FA = 8^o, FOV = 224×224×120 mm^3, isotropic resolution = 1mm^3) for tissue segmentation. Anatomical landmarks from these images were also used to assist in the placement of the left PMC voxel for ^{1}H-MRS.

In vivo ^{1}H-MRS data were acquired from a voxel of interest (VOI = 20×20×20mm^3) placed over the hand area in the left M1 (**Figure 6.1A**) using a STEAM sequence (TE/TM/TR = 17/17/2000ms, sample size = 4096, spectral bandwidth = 4000Hz, phase cycling = 8, 288 averages, 9.6mins). Water suppression was performed using multiply optimised insensitive suppression train (MOIST) (Murdoch, 1993). Prior to this, a non-suppressed water reference spectrum (16 averages) from the same VOI was acquired for eddy current correction and quantification. B0 shimming of the VOI was performed automatically by the Philips pencil beam (PB) algorithm (Gruetter,

1993) in order to increase B0 field homogeneity. For VOI placement and an example of the spectrum acquired please view **Figure 6.1**.

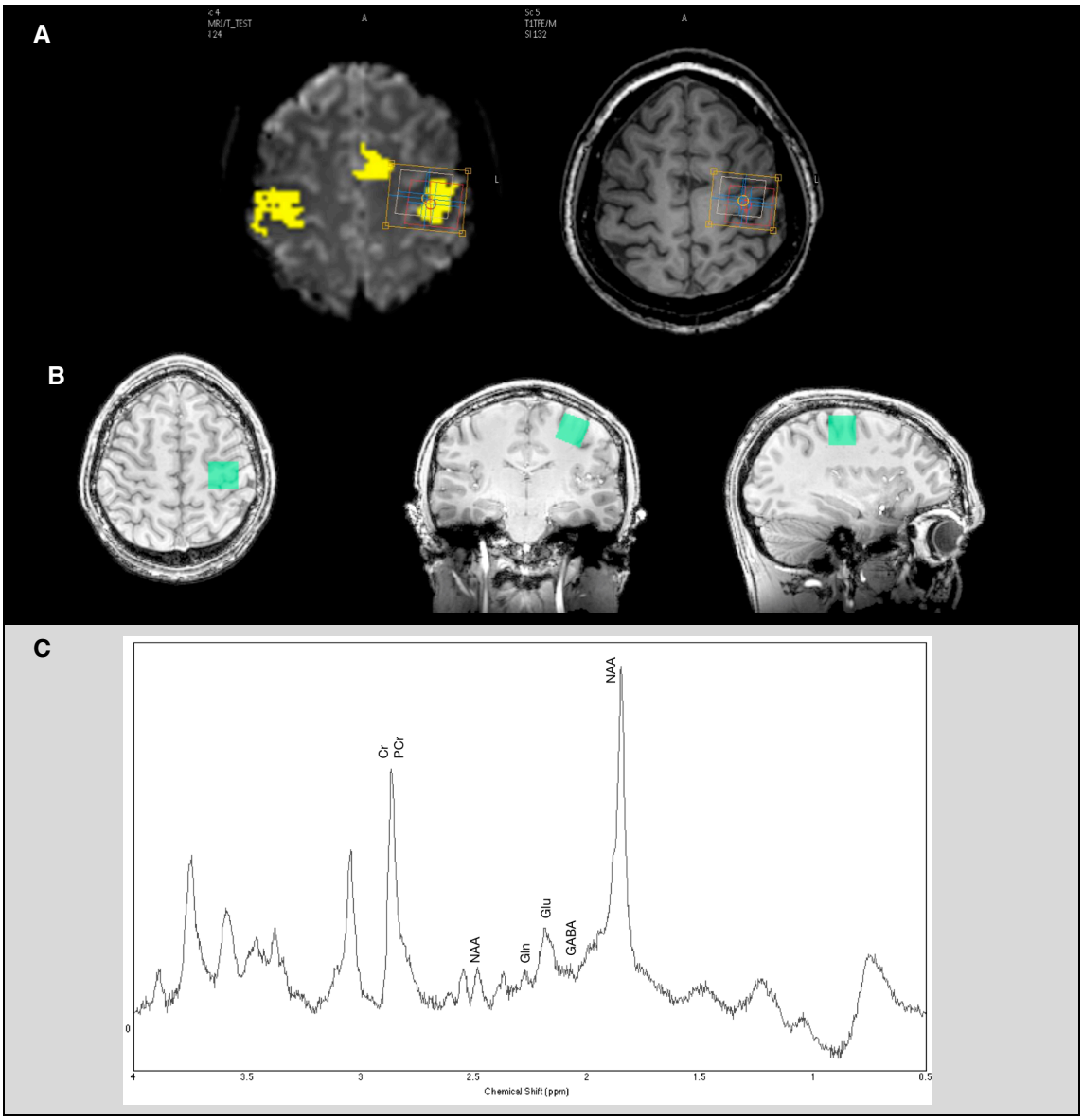


Figure 6.1: A) On the left, BOLD activity in bilateral PMC and SMA from finger tapping task in scanner, and on the right an MP-RAGE anatomical scan. Placement of VOI can be viewed (overlaying peak BOLD activity and anatomical landmark for hand area). B) VOI placement from one participant in three views: an axial, coronal and sagittal slice. Care was taken to ensure the VOI did not go outside cortex. C) An example of the spectrum acquired with relevant peaks labelled: Gln Glu and GABA are all in close proximity between 2-2.5 ppm.

6.2.3 Transcranial Magnetic Stimulation

The MP-RAGE images that were acquired at the imaging centre were used in combination with neuro-navigation software BrainSight (Rogue Resolutions). This allowed the experimenter to firstly anatomically locate the left PMC and target it

ready for brain stimulation and secondly, to co-register the participant's head with the software's reconstruction of their virtual head. The targeted location was then used as a guide to deliver the initial sp-TMS and use online EMG recording to adjust the target and find the motor hotspot of the FDI of the right hand; the hotspot was chosen to be the most reliable location on the scalp to induce MEPs with consistently the largest MEPs for a given stimulator output intensity.

The experimenter acquired paired pulse TMS (pp-TMS) using a Magstim Bistim^2 machine with a 70mm wing diameter figure-of-eight coil in a branding-iron configuration. Magstim Bistim^2 is a TMS machine that comprises of two monophasic stimulators and a connecting module; this arrangement permits the delivery of two TMS pulses in rapid succession at different intensities. A Manfrotto arm was used to hold the coil in position throughout the experiment, whilst the participant relaxed with their chin resting on a chin-rest, sitting in a fully adaptable chair. The location of the coil was monitored continuously and the coil position was adjusted when needed. All trials were programmed into and triggered by Matlab software.

6.2.3.1 Electromyography

Disposable surface EMG silver-silver chloride (Ag/AgCl) electrodes with a 24mm diameter were adhered to the skin in a belly-tendon montage around the right FDI muscle with the ground placed on the wrist. The signal from the FDI was amplified using Brainamp ExG and recorded using Brain Vision Recorder (sampling rate: 5000Hz, sampling interval: 200 μs).

6.2.3.2 Protocol

TMS measurements were acquired immediately after the scanning procedure in the School of Psychology, Nottingham; the average time in between the end of the

final scan and starting the initial TMS protocol was circa 30 minutes. Once the FDI hotspot was found, as described above, resting motor threshold (RMT) was estimated as the minimum intensity required to produce an MEP peak-to-peak amplitude of ≥50μV in 5/10 trials at rest. A TP was then defined as the intensity required to consistently produce an MEP peak-to-peak amplitude of 1mV.

An array of physiological data was collected: recruitment curves (10 trials of sp-TMS at 100-150% in increments of 10% of RMT) and various pp-TMS measures. The pp-TMS paradigms that were applied were 1 and 3ms short interval intracortical inhibition ($SICI_1$ and $SICI_3$), 100ms long interval intracortical inhibition ($LICI_{100}$) and 10 and 12ms intracortical facilitation (ICF_{10} and ICF_{12}). 30 unconditioned trials (at TP) were collected as a baseline to compare the pp-TMS. Refer to **Table 6.1** to view parameters tested.

Table 6.1: All parameters tested in pp-TMS.

pp-TMS Paradigm	CS (%RMT)	ISI (ms)	Number of Trials
$SICI_1$	45 50 55 60	1	40 (10 each CS)
$SICI_3$	60 65 70 75	3	40 (10 each CS)
$LICI_{100}$	110	100	20
ICF_{10}	75	10	20
ICF_{12}	75	12	20

Recruitment curves were assessed before pp-TMS responses were collected. There was an ITI of 5s in between each trial. Participants were instructed to relax the upper effectors and to adjust their position to stay comfortable throughout the study.

6.3 Results

6.3.1 Data analysis

6.3.1.1 STEAM Data

For the STEAM spectroscopic data, cerebral metabolites were quantified using a LCModel software package. In LCModel, the water scaling was performed using the non-suppressed water reference. The LCModel analysis was performed within the chemical shift range 0.2 to 4.2 ppm, assuming water concentration is 55.51 mol/g. The absolute concentration for each cerebral metabolite was reported in institutional units with its estimated standard deviation (Cramer-Rao lower bound (CRLB)) expressed in percentage. The GABA:tCr ([GABA]/[tCr]) ratio was calculated by dividing the absolute concentration of GABA by the absolute concentration of total Cr (i.e. [PCr + Cr=tCr]). This same procedure was used to calculate ratios for Glu:tCr, Gln:tCr and Naa:tCr.

A model was fitted to the spectra acquired and neurometabolite concentrations were automatically quantified using LCmodel software package (Provencher, 2001). A basis set included a previously acquired macromolecule spectrum and model spectra for 20 metabolites. Analysis was restricted to the chemical shift range 0.5 – 4.2 ppm. Control parameters were used within LCmodel, which were taken from (Menon, Adleman, White, Glover, & Reiss, 2001).

Total Cr (tCr, i.e., PCr+Cr) was used as the internal reference for quantification due to its relatively high and stable concentration in the human brain (Danielsen & Ross, 1999; Roshan et al., 2003).

I assessed spectral quality in the data in two ways in order to identify whether any datasets should be excluded. The two markers I used to quantify quality were

FWHM and CRLB – I then assessed whether there were any outliers within these measures. A Grubbs outlier test was used to detect whether there were any outliers in FWHM and CRLB measures. Resulting spectra had the following mean CRLBs: Glu = 2% ± 0%, Gln: 6.71% ± 2.80% and GABA: 8.63% ± 1.95%. The mean FWHM was 9.69 ± 1.03 Hz. Tests of normality were conducted (shapiro wilk) and all measures were considered normal. Please refer to **Figure 6.2** for a graph depicting spectra that were retained for analysis and spectra that were identified as outliers.

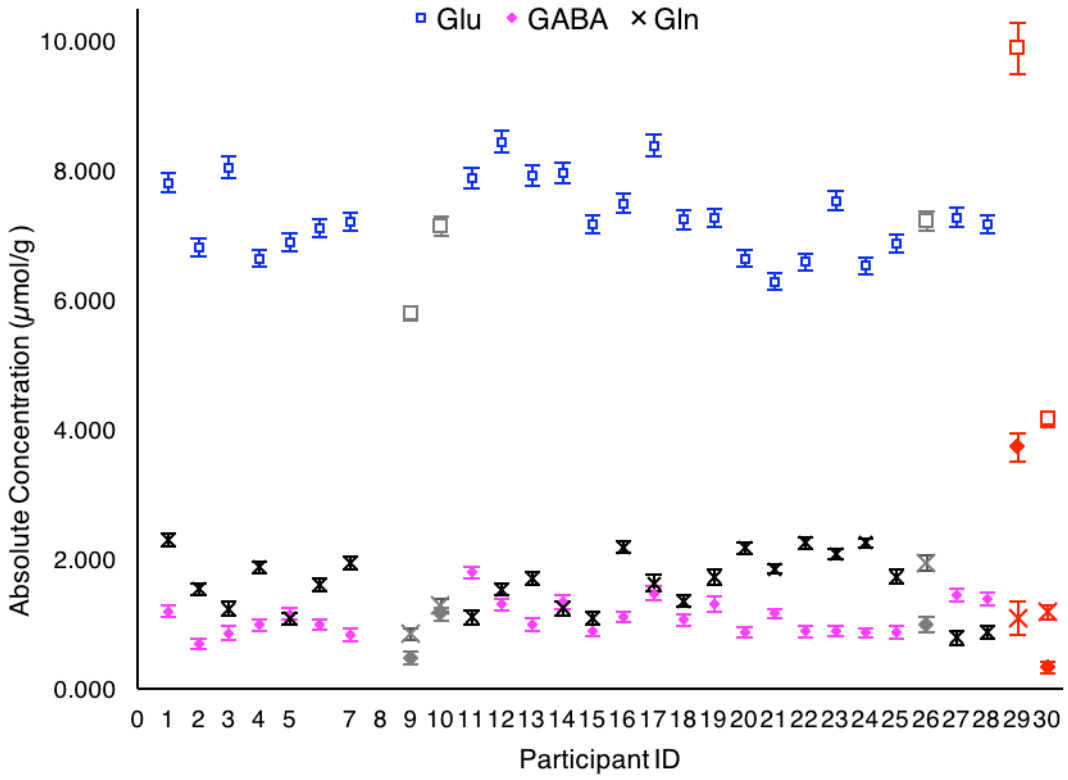


Figure 6.2: Absolute concentrations of glutamate (Glu), GABA and glutamine (Gln) for each participant. Error bars are Craemer-Rao Lower Bound (CRLB). Data points in grey had ^{1}H-MRS spectral acquisitions that exceeded 2 SDs outside FWHM. Data points in red exceeded 2 SDs outside CRLB. Both suggest that the spectroscopy for these subjects were not optimal (9,10,26,29,30)

6.3.2 Data Analysis of TMS

EMG signals were assessed and analysed using an in-house built Matlab programme; care was taken to exclude any trial that was contaminated by contraction of the muscle prior to sp-TMS delivery and peak-to-peak amplitude of the

test stimulus was calculated. For each individual, a median was calculated for each trial type; significant outliers were identified from the group and removed using Grubbs test. Tests of normality were conducted and several measures were indicated as not being normally distributed. A Shapiro Wilk test highlighted that RMT Median MEP, ICF_{12}, $LICI_{100}$, Sigmoid Slope and Sigmoid Slope Variable were not normally distributed. Non-normal data were log-transformed in order to allow the application of conventional parametric statistics. Only $LICI_{100}$ still had an element of non-normality after being transformed; although this was largely corrected, any statistics using $LICI_{100}$ should be treated with caution. Please view **Table 6.2** for details about the distribution (mean, median, standard deviation, skewness and kurtosis) and Shapiro Wilk Test Statistic for all variables and **Figure 6.3**.

Table 6.2: All measures were subject to normality tests; the mean median, standard deviation, skewness, kurtosis and statistics from the Shapiro-Wilk normality test are listed. Measures highlighted in blue significantly deviate from normality according to the Shapiro-Wilk test.

Tests of Normality

	Distribution					Shapiro-Wilk		
	Mean	Median	Std Dev.	Skewness	Kurtosis	Statistic	df	Sig.
Age	23.11	23.33	2.49	0.073	-0.117	0.975	29	0.696
RMT	46.24	46.00	6.13	-0.364	-0.374	0.969	29	0.521
RMT MedMEP	42.08	34.75	30.22	0.96	0.191	0.893	27	0.009
TP	55.17	55.00	7.10	-0.168	-0.702	0.966	29	0.453
CoV_100_IO	1.37	1.30	0.53	1.27	1.975	0.893	29	0.007
CoV_150_IO	0.32	0.31	0.13	1.02	0.844	0.92	29	0.031
IO Slope	13671.22	10981.43	11082.67	2.279	7.73	0.805	29	0.000105
IO Slope Plateau	3285.17	2897.33	1645.67	0.844	0.868	0.946	27	0.17
SICI 1ms Median	57.53	61.96	25.68	-0.498	-0.435	0.953	28	0.232
SICI 3ms Median	50.84	48.98	24.78	0.445	-0.119	0.975	28	0.71
SICI 1ms Slope	-5.27	-4.89	2.86	-0.678	0.719	0.955	28	0.269
SICI 3ms Slope	-3.60	-3.58	2.02	-0.011	-0.037	0.984	28	0.931
ICF 10ms	130.80	127.46	45.82	0.229	0.263	0.979	27	0.846
ICF 12ms	143.00	126.88	65.23	1.359	1.766	0.88	28	0.004
LICI 100ms	55.40	28.30	76.95	2.575	8.517	0.709	28	0.000004
Glu:tCr	1.30	1.31	0.10	0.395	0.633	0.961	24	0.451
Gln:tCr	0.29	0.30	0.09	0.129	-0.746	0.968	24	0.627
GABA:tCr	0.20	0.19	0.04	0.935	0.294	0.917	24	0.051
Naa:tCR	1.27	1.29	0.08	-0.34	-0.51	0.97	24	0.645

Tests of Normality (Log-transformed Data)

	Distribution					Shapiro-Wilk		
	Mean	Median	Std Dev	Skewness	Kurtosis	Statistic	df	Sig.
RMT MedMEP (Logged)	3.4628	3.5482	0.80145	-0.288	-0.887	0.948	27	0.191
IO Slope (Logged)	9.2246	9.304	0.84627	-0.651	0.787	0.967	29	0.473
CoV_150_IO (Logged)	-1.2013	-1.1648	0.392	0.207	-0.638	0.976	29	0.716
ICF 12ms (Logged)	4.8751	4.8429	0.41841	0.357	-0.152	0.979	28	0.817
LICI 100ms (Logged)	2.7078	3.3108	2.07831	-0.485	-1.072	0.919	28	0.034

6.3.3 Reproducibility of 7T ^{1}H-MRS Acquisition

The ^{1}H-MRS measurements were acquired at two points on the same day for a sub-set of 12 participants. Several aspects can affect reliability of spectroscopy

data: the size of the volume one is acquiring the spectroscopy from, the region of the brain it is acquired from, field strength and the sequence used. There has been previous literature suggesting that a STEAM sequence at 7T has excellent reproducibility (Stephenson et al., 2011; Wijtenburg, Rowland, Edden, & Barker, 2013), however they also demonstrated that the reproducibility differed between brain regions. The authors did a test-retest study looking at STEAM sequence in the ACC and DLPFC and reported that GABA reproducibility was superior in the ACC (Coefficient of Variation percentage [CV%] (M) = 3.5%) compared to the DLPFC (mean CV = 13.6%). Research collected at the same scanner that this data was collected reported mean CV% for low concentration metabolites in the ACC as follows: GABA CV% = 10%, Glu CV% = 4%, Gln CV% = 10%. They compared reproducibility values to data collected at 3T and found a critical difference (21%, 8% and 29% mean CV% respectively). Since neither group tested reproducibility values for low concentration metabolites in the PMC with a comparable sized VOI, I have analysed the data in this sub-set to determine whether the quality of this dataset is equivalent to that used within these two articles.

In order to be directly comparable to the above papers CV% was calculated for the three metabolites GABA, Glu and Gln. The mean and median CV values for the group of 12 participants are as follows: GABA CV% = 11.91% (μ = 5.56%), Glu CV% = 3.52% (μ = 3.46%) and Gln CV% = 18.05% (μ = 12.66%).

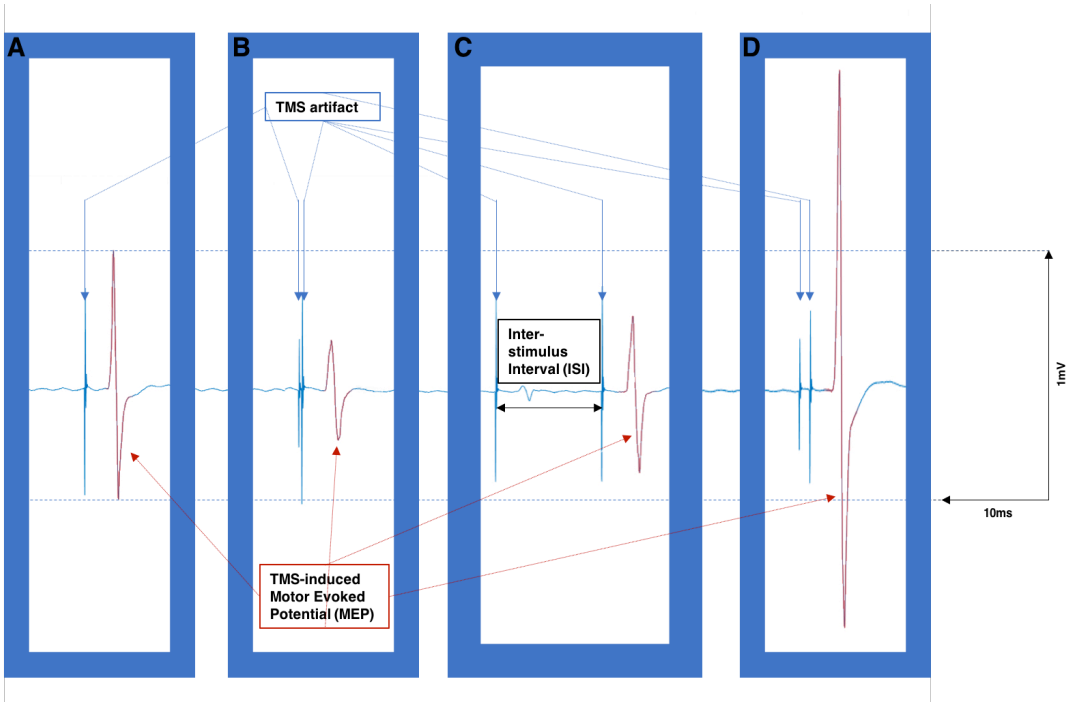


Figure 6.3: An image depicting the effect of a conditioning stimulus on TMS-induced motor evoked potentials (MEP). The MEP is in red. Figure **6.3**A is the unconditioned MEP (test MEP); Figure **6.3**B is an example of what short intracortical inhibition (SICI), in this case the TMS pulses are separated by an ISI of 3ms; Figure **6.3**C is an example of long intracortical inhibition (LICI) where the conditioning stimulus precedes the test stimulus by 100ms; Figure **6.3**D is intracortical facilitation where the test stimulus occurs 10ms after the conditioning stimulus. One can clearly see a reduction in MEP amplitude in comparison to the test MEP of 1mV in Figure **6.3**B and Figure **6.3**C (SICI and LICI) and a large facilitation of MEP in Figure **6.3**D (ICF).

6.3.4 Sex Effects

First, I assessed whether there were any sex differences of neurometabolites as assessed by 7T STEAM ^{1}H-MRS (Naa:tCr, Glu:tCr, Gln:tCr and GABA:tCr) and TMS physiological measures (RMT, RMT MedMEP (Logged), IO Slope (Logged), IO Plateau, CoV_100_IO, CoV_150_IO (Logged), $SICI_1$ Median, $SICI_1$ Slope, $SICI_3$ Median, $SICI_3$ Slope, ICF_{10}, ICF_{12}, $LICI_{100}$ (Logged)). Multiple t-tests confirmed that there were no differences between sexes in any of the measures collected; the only comparison that approached significance was a marginal difference between $SICI_3$ Slope – however, this had a $p = 0.078$ uncorrected and thus is unlikely to be of any importance.

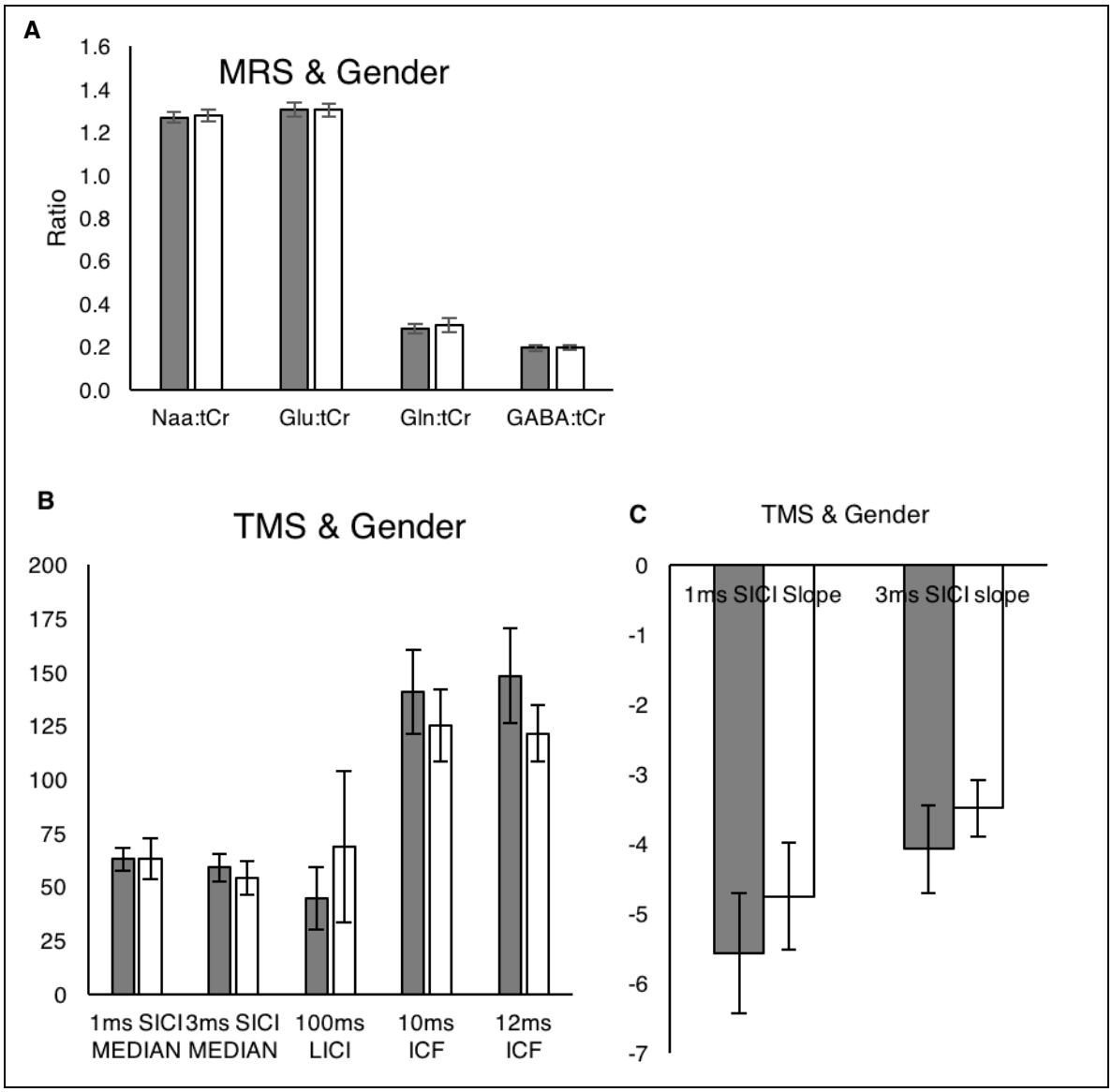


Figure 6.4: Concentrations of Naa, Glutamate, Glutamine and GABA in reference to total Creatine were collected in the PMC, in **6.4A** the ratios are depicted for males and females, grey= females. **6.4B** displays the percentage of inhibition and facilitation for all pp-TMS measures (<100% is inhibition), grey=female. **6.4C** shows the slopes calculated for $SICI_1$ and $SICI_3$ (grey= female). In each of the measures collected, both ^{1}H-MRS and TMS, there are no differences in the concentrations for any of the neurochemicals between sexes.

6.3.5 Relationships between TMS and ^{1}H-MRS Measures

Exploratory Pearsons' correlations and supporting Bayesian correlations were conducted to explore whether there were any relationships between physiological measures of excitation and inhibition of the PMC as assessed by TMS and neurometabolite concentrations quantified by 7T proton-MRS. The important question I explored is whether neurometabolites commonly considered as measuring

inhibitory or excitatory neurotransmitters reflect the physiological inhibition or excitation that we measure with TMS. Radar plots are used to display the array of correlation coefficients found for each neurometabolite. Multiple comparison corrections were applied for each TMS measure tested (i.e. 13). The findings were fairly unremarkable, however, Glutamate (Glu:tCr) appeared to be positively related to ICF_{10} (R = 0.580, n = 22) , $LICI_{100}$ (Logged) (R = 0.693, n = 23) and negatively correlated to IO Plateau (R=-0.727, n = 22). Namely, more glutamate was measured in the PMC of those that showed higher excitation in ICF_{10} and less inhibition in $LICI_{100}$ measures. Further participants with higher PMC glutamate had a lower amplitude of maximum MEP (i.e. the max output of the corticospinal system as measured by MEPs was lower in those that showed higher Glu:tCr).

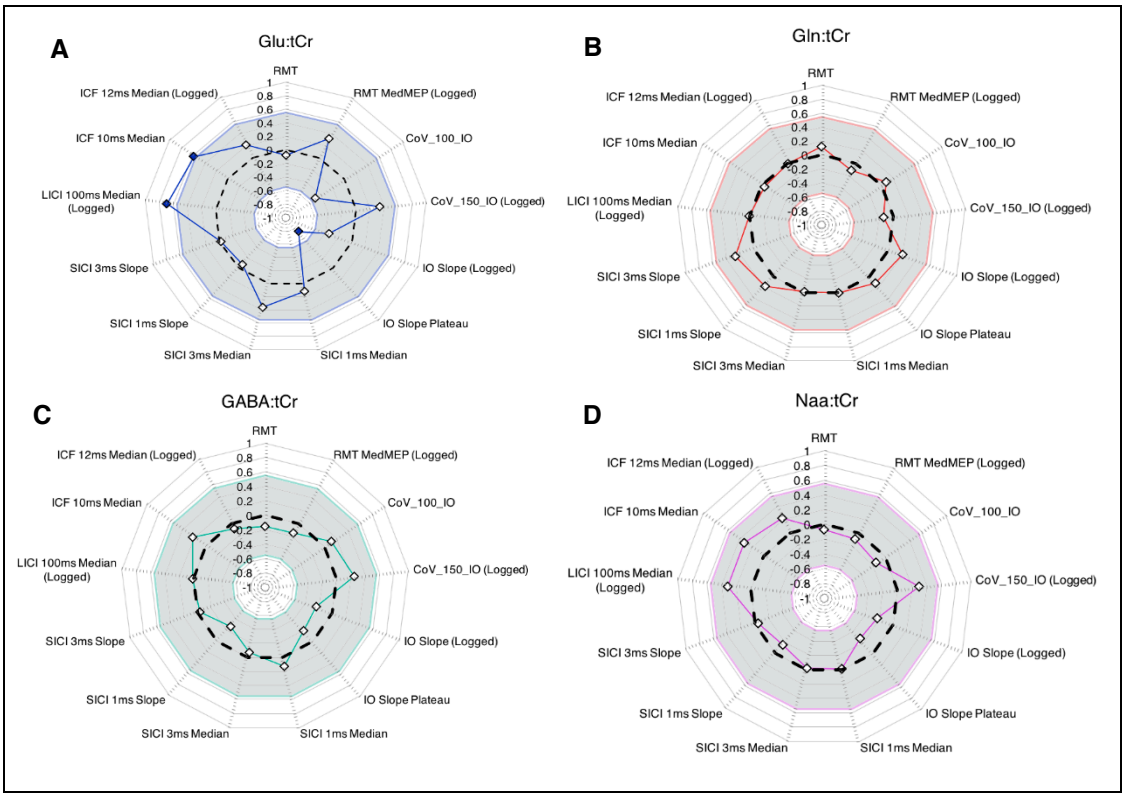


Figure 6.5: Spider's web plots are used to illustrate the Pearson correlation coefficients for individual values for each TMS measure relative to ^{1}H-MRS measurements of Glutamate (5A), Glutamine (5B), GABA (5C) and Naa (5D). Pearson correlation coefficients are plotted from -1 to 1, the dashed line in the middle represents a correlation of 0 (perfectly uncorrelated) and the grey shaded region represents the area of the graph that is not significantly correlated. Any significant correlations are signified by a filled diamond. The only significant correlations relate the following TMS measures to Glutamate:tCr: IO Slope Plateau (R=-0.727), $LICI_{100}$ (R = 0.693) and ICF_{10} (R = 0.580).

6.3.5.1 Bayesian Statistics

Bayesian statistics were employed primarily to evaluate whether there was evidence in favour of the null hypothesis (H_0), i.e. that a metabolite bore no relationship with neurotransmitter function as assessed by TMS. It further allowed us to quantify the strength of the relationship of any correlations found (evidence in support of the alternative hypothesis, H_1). Please refer back to **Table 5.6** (**Chapter 5**) for cut-off values.

The Bayesian Hypothesis Test confirmed the findings of conventional statistics. Decisive evidence was found that Glu:tCr is related to IO plateau (BF_{10} = 259.056) and $LICI_{100}$ (BF_{10} = 140.911) and strong evidence that ICF 10ms is related to Glu/tCr (BF_{10} = 11.309). Substantial evidence was also found for Glu:tCr being related to CoV of 100% RMT. Further, there is substantial evidence that the majority of TMS measures do not relate to ^{1}H-MRS measures of Glu:tCr, Gln:tCr, GABA:tCr or Naa:tCr. Most notably there is substantial evidence for no relationship between SICI 3ms and SICI 3ms slope and GABA:tCr (BF_{10} = 0.272 and 0.261 respectively). The results of the Bayesian Hypothesis Test support that the absence of relationships are not likely to be the consequence of an underpowered sample, but simply a true null effect. Please refer to for a depiction of the significant correlations between Glu:tCr and TMS measures and **Table 6.3** for a full list of all relationships tested with their corresponding Bayes Factor.

Table 6.3: Bayesian hypothesis test for correlations. Data presented are each BF_{10} calculated to test the relationship between ^{1}H-MRS metabolites and TMS measures.

	Glu:tCr	Gln:tCr	GABA:tCr	Naa:tCr
RMT	0.270†	0.280†	0.372	0.265†
RMT MedMEP (Logged)	0.686	1.670	0.427	0.289
CoV 100% IO	4.307*	0.240†	0.262†	0.321†
CoV 150% IO	1.406	0.314†	0.508	0.595
IO Slope (Logged)	0.903	0.381	0.466	0.444
IO Plateau	259.056***	0.299†	0.372	0.530
SICI 1ms	0.293†	0.259†	0.301†	0.259†
SICI 3ms	1.003	0.259†	0.272†	0.260†
SICI 1ms Slope	0.275†	0.345	0.547	0.327†
SICI 3ms Slope	0.259†	0.542	0.261†	0.262†
LICI 100ms (Logged)	140.911***	0.259†	0.259†	0.734
ICF 10ms	11.309**	0.269†	0.426	0.727
ICF 12ms (Logged)	0.411	0.259†	0.278†	0.421

*substantial evidence for H1, **strong evidence for H1, ***decisive evidence for H1, †substantial evidence for H0. $BF_{10} > 1$ supports H1, $BF_{10}<1$ supports H0, $BF_{10} = 1$ suggests no evidence.

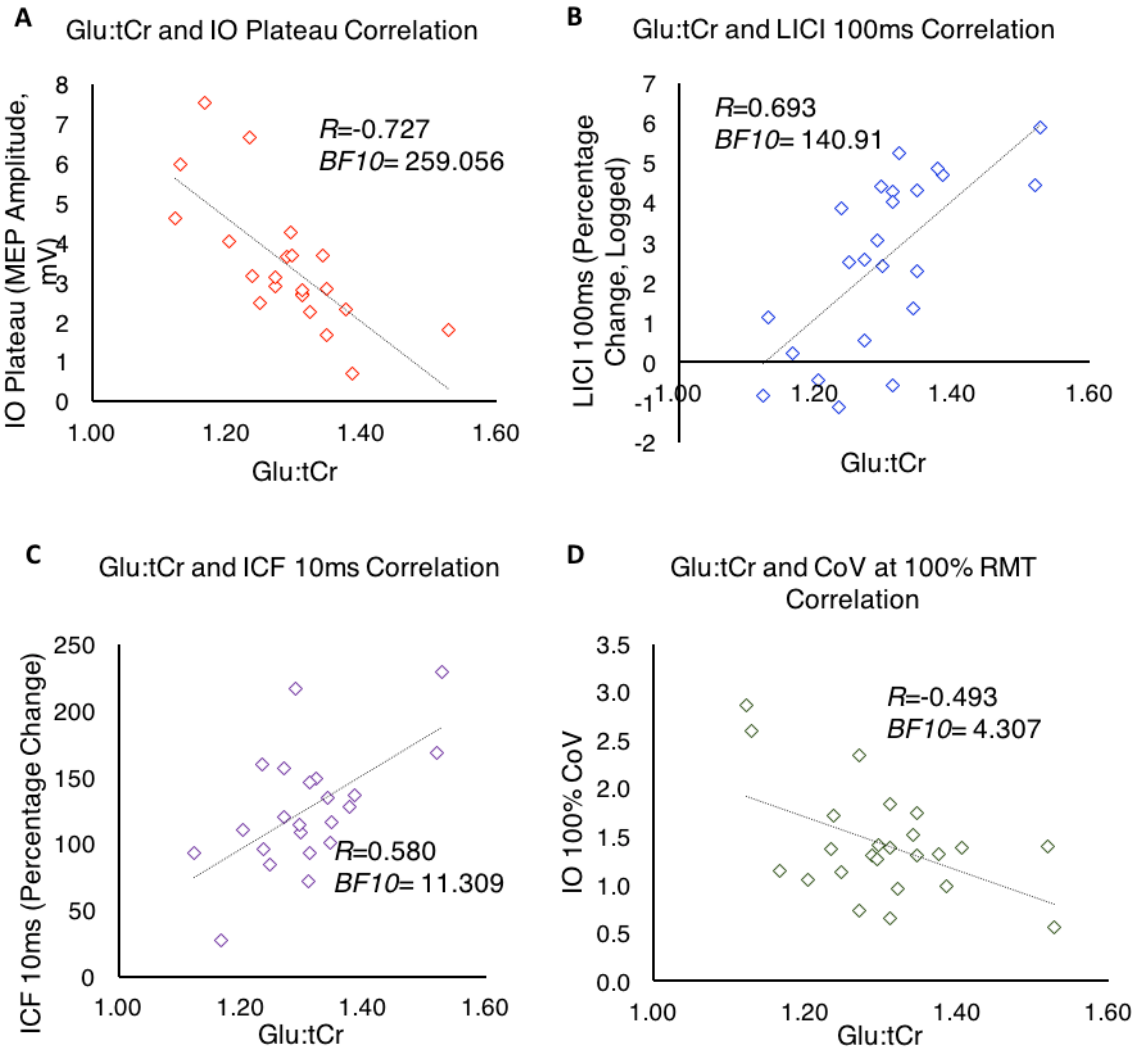


Figure 6.6: Scatterplots illustrating the relationship between IO plateau, $LICI_{100}$, ICF_{10} and CoV for 100% RMT and Glutamate:tCr.

6.3.6.1 Glu:tCr – Regression

Given that several TMS measures related to Glu:tCr a stepwise regression was adopted in order to explore the relationship between the variables IO plateau, $LICI_{100}$, ICF_{10} and CoV at 100% RMT with Glu:tCr. When all TMS measures were entered into a forward stepwise regression, only $LICI_{100}$ (logged) ($t = 3.576$, $p<0.01$) and ICF_{10} ($t = 2.124$, $p = 0.05$) were significant predictors ($F(2, 18) = 13.382$, $p<0.001$). The significant linear model explained circa 58% of the variance (R^2=.598, Adjusted R^2= .553). $LICI_{100}$ was weighted as the primary predictor. Please see **Table**

6.4 for details of unstandardized, standardised coefficients, Pearson's correlation, squared semi-partial correlations and structure coefficients.

Table 6.4: A Stepwise Regression model using $LICI_{100}$ and ICF_{10} to predict Glu:tCr. This table presents for each factors: unstandardised and standardised coefficients, Pearson's correlation, squared semipartial correlations, and their structure coefficients.

Model	b	*SE-b*	Beta	Pearson *r*	sr^2	Structure Coefficient
Constant	1.159	.037				
$LICI_{100}$	.022	.006	.577	.705	.534	.746
ICF_{10}	.001	.000	.343	.559	.317	.444

6.3.7 TMS Correlations

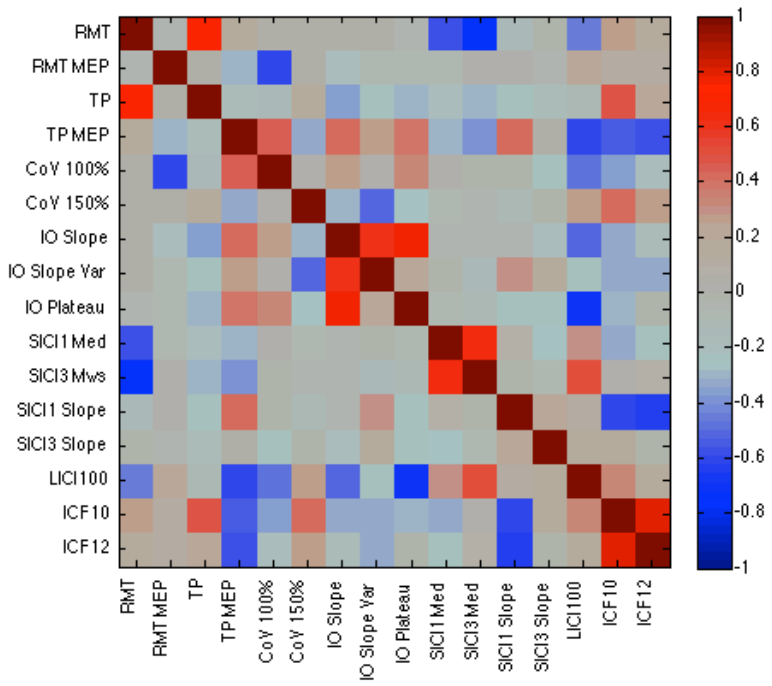


Figure 6.7: A correlation matrix depicting the strength of multiple Pearson correlation coefficients for all TMS measures utilised within this study: RMT (% of maximum stimulator output to evoke an MEP>50μV in 5/10 trials), MEP amplitude at threshold, TP (% of maximum stimulator output required to evoke an MEP = 1mV), MEP amplitude at TP, CoV of MEP amplitude at 100% RMT, CoV of MEP amplitude at 150% RMT, IO Slope (the maximum linear slope), IO Slope (variable slope required to produce the sigmoid), IO plateau, $SICI_1$ median % inhibition, $SICI_3$ median % inhibition, $SICI_1$ slope, $SICI_3$ slope, $LICI_{100}$ median % inhibition, ICF_{10} median % facilitation, ICF_{12} median % facilitation. Log-transformed data was used for RMT median MEP, CoV 150% RMT, IO Slope, ICF_{12} and $LICI_{100}$. The coloured bar to the right indicates what colour corresponds to what strength correlation, whilst grey indicates no correlation, deep blue indicates a negative correlation and deep red indicates a positive correlation.

Exploratory multiple correlations were used to assess the patterns of relationships between physiological measures of inhibition and excitation as assessed by TMS. A correlation matrix is shown in **Figure 6.7** showing the strength and direction of the relationships between each of the TMS measures. Out of 120 relationships tested 17 relationships were significant when FDR-corrected. Firstly, unsurprisingly the MSO%s used for RMT and TP were highly positively correlated (R = 0.700 p<0.01) and CoV for MEPs collected at 100% RMT were negatively correlated with the log-transformed median MEP collected at 100% RMT (R=-0.621, p<0.01). The slope parameters calculated for individual recruitment curves were also, predictably, correlated with each other: IO plateau and IO maximum slope (R = 0.762, p<0.001), IO variable slope and CoV of MEPs collected at 150% RMT (R=-0.521, p<0.01) and finally IO maximum slope and IO variable slope (R = 0.612, p<0.01).

Whilst the above correlations were strongly expected some other interesting relationships precipitated. Several relationships were found between both RMT values (MSO% required to produce MEPs at threshold criterion) and TP MEP values (unconditioned MEPs collected as test) with pp-TMS measures. RMT values negatively correlated with both $SICI_1$ and $SICI_3$ median inhibition (R=-0.570 and -0.724 respectively, p<0.01). TP MEP values inversely correlated with ICF_{10}, ICF_{12} median facilitation and $LICI_{100}$ median inhibition (R=-0.597, -0.533 and -0.583 respectively, p<0.01).

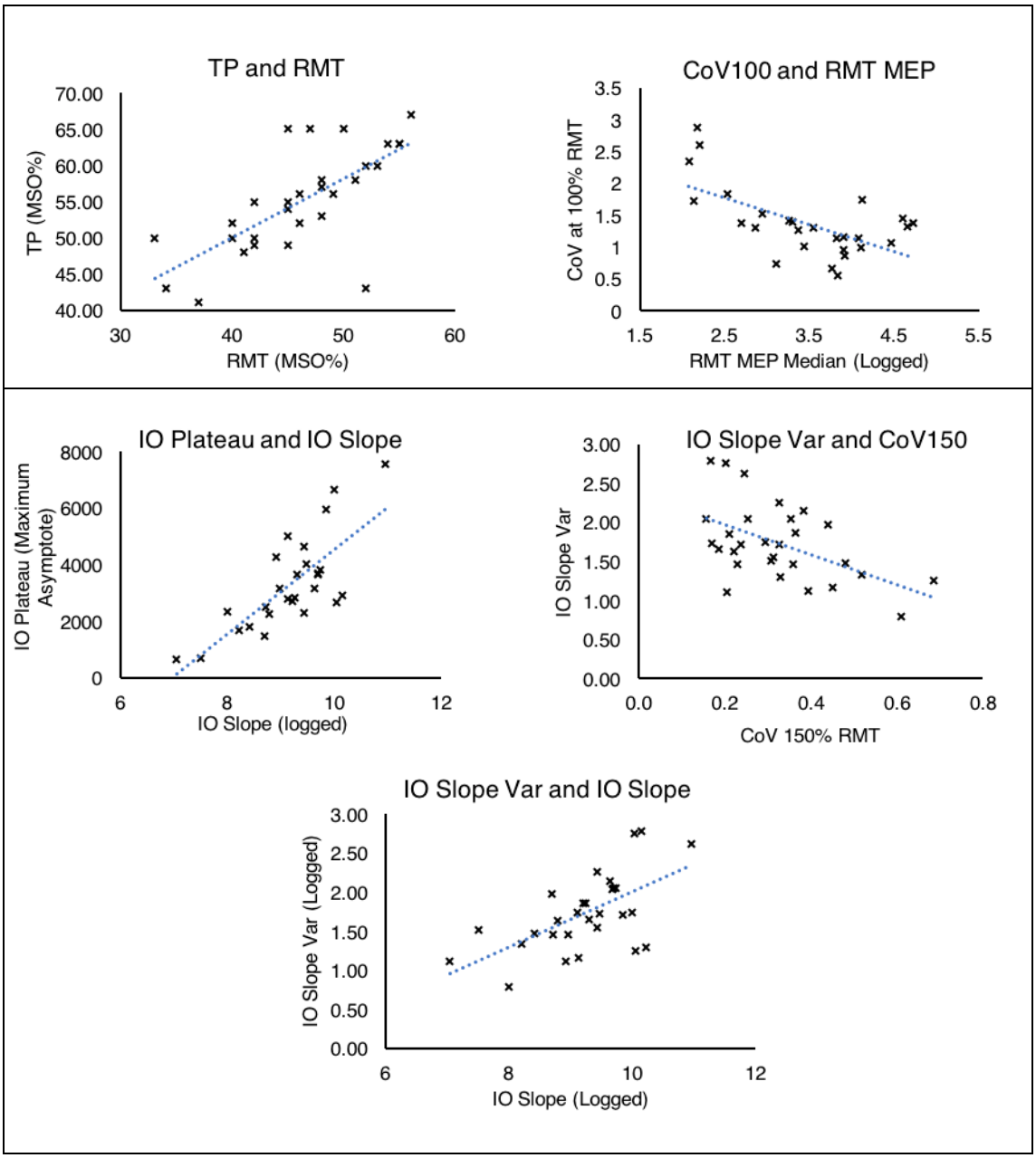


Figure 6.8: Scatterplots indication the significant relationships between sp-TMS measures (threshold, TP, IO Slope).

Furthermore, there appeared to be relationships between the amount of inhibition and excitation measured from independent pp-TMS measures. $SICI_1$ and $SICI_3$ median inhibition are positively related ($R = 0.649$, $p<0.01$); $SICI_3$ and $LICI_{100}$ median inhibition are positively related ($R = 0.513$, $p<0.01$); $SICI_1$ slope is related to ICF_{10} and ICF_{12} median excitation (R=-0.596 and -0.649, $p<0.01$) and somewhat unsurprisingly ICF_{10} and ICF_{12} median excitation are tightly positively related ($R =$

0.807, $p<0.001$). Importantly, the $SICI_1$ and $SICI_3$ relationship remains even after controlling for RMT (of which both are significantly related to); a partial correlation showed a positive relationship between $SICI_1$ and $SICI_3$ whilst controlling for RMT ($r(25) = 0.417$, n = 28, $p<0.05$, two-tailed). The relationship between ICF_{10} and ICF_{12} still stands when controlling for TP_{MEP} ($r(23) = 0.726$, n = 6, $p<0.0001$).

Given the results of the partial correlations, I conducted further partial correlations to determine whether both variables ($SICI_1$ and $SICI_3$) were related to RMT whilst controlling for the other. The partial correlations suggest that $SICI_3$ was negatively correlated with RMT ($r(25) = -0.567$, n = 28, $p<0.05$), whilst $SICI_1$ was not ($r(25) = -0.190$, n = 28, $p = 0.342$). Finally, when I looked in detail at the relationship between ICF_{10}/ ICF_{12} and TP_{MEP} whilst controlling for one another the effect disappeared ($p = 0.447$ and $p = 0.198$ respectively).

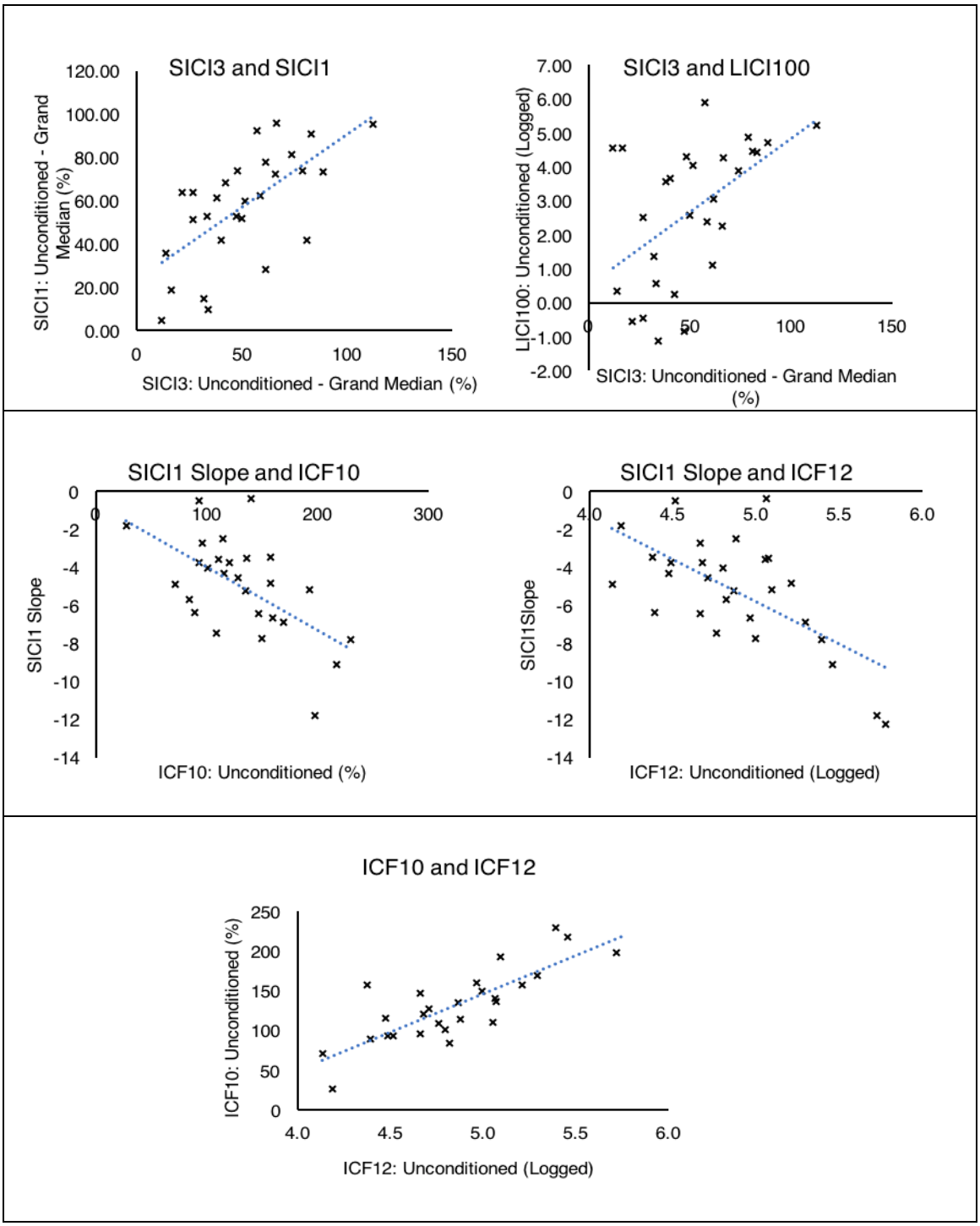


Figure 6.9: Scatterplots illustrating the significant relationships between pp-TMS measures.

Interestingly, there also appeared to be a relationship between different recruitment curve slope parameters (IO plateau and IO maximum slope) with $LICI_{100}$ (R=-0.703 and R=-0.510 respectively, p<0.01).

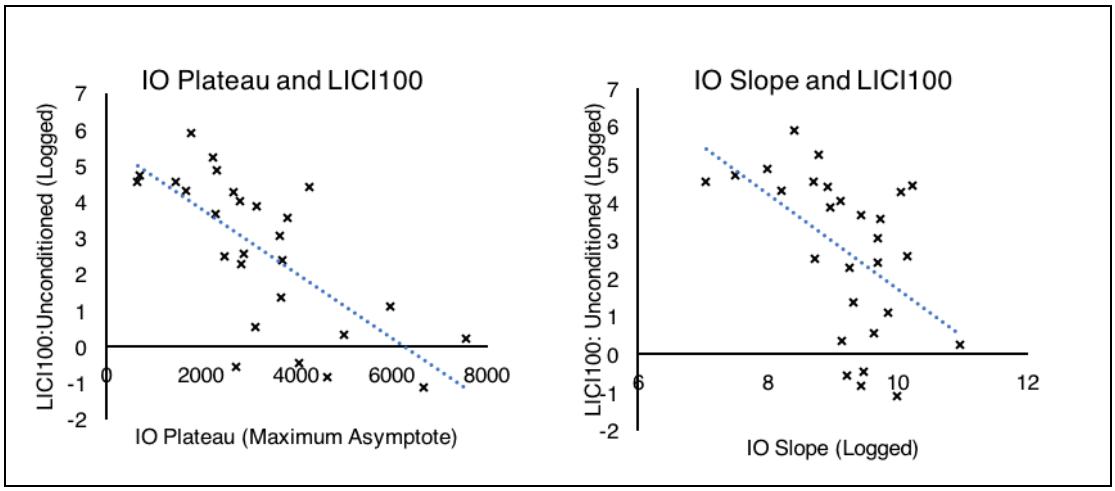


Figure 6.10: Scatterplots illustrating the relationship between IO plateau and IO slope with $LICI_{100}$.

6.3.8 MRS Correlations

Two significant relationships were found between the metabolites Glu:tCr, GABA:tCr, Gln:tCr and Naa:tCr as measured by 7T ^{1}H-MRS and quantified by LCModel (minimum p = 0.126, FDR-corrected). Gln:tCr correlated negatively with GABA:tCr (R = -0.537, n = 24, p<0.01) and Glu:tCr correlated positively with Naa:tCr (R = 0.492, n = 24, p<0.05). However, the Bayes Factors (BF_{10}) were only 7.952 and 4.247 suggesting only substantial evidence against the null hypothesis. Further there was only anecdotal or substantial evidence for the null hypothesis in the unrelated pairs.

6.4 Discussion

Physiological measures of inhibition and excitation of the PMC were collected using single and paired-pulse TMS and were related and compared to neurometabolite concentrations collected from the PMC, quantified by 7T ^{1}H-MRS. The ^{1}H-MRS acquisitions were highly reproducible, in keeping with prior literature for

7T ^{1}H-MRS (Stephenson et al., 2011; Wijtenburg et al., 2013), despite using a smaller 20x20x20mm^{3} VOI in the PMC. This dataset is not only reproducible but the small VOI means that the measures are specific, capturing little extraneous tissue. This is highly important when relating neurometabolite quantifications from this VOI to the TMS measures acquired.

Before exploring these relationships, I first explored the effect of sex in this large dataset. Sex specific hormones are powerful neuromodulators and there is evidence that there is a greater variability when measuring neurometabolites such as GABA in the brain and excitatory/inhibitory mechanisms using TMS in females. It has been shown that the ^{1}H-MRS -measured GABA fluctuates in specific brain regions across the menstrual cycle in female individuals. Specifically, higher GABA levels are typically found in the mid/late follicular phase compared to luteal phase of the menstrual cycle (De Bondt, De Belder, Vanhevel, Jacquemyn, & Parizel, 2015; Epperson et al., 2002, 2005; Harada, Kubo, Nose, Nishitani, & Matsuda, 2011). These are region-specific fluctuations in GABA, namely frontal, PFC and occipital cortices, lentiform nucleus but not the anterior cingulate. There have been no studies to date, to my knowledge, that have investigated GABA fluctuations using ^{1}H-MRS in the PMC.

Although there have been no reports of fluctuations in ^{1}H-MRS measured GABA in the PMC there have been reports of sex differences in CSE. For instance, specific features of recruitment curves have been shown to differ between male and females (Pitcher et al 2003). However, other studies have found no baseline differences in PMC CSE (Cahn, Herzog, & Pascual-Leone, 2003; Livingston, Goodkin, & Ingersoll, 2010). Akin to the ^{1}H-MRS studies, fluctuations throughout the menstrual cycle in CSE have been recorded where greater inhibition has been found

in the luteal phase (the same phase where there is a reduction in GABA) (M. J. Smith et al., 1999).

This evidence at the very least suggests that there may be greater variability in data collected from female subjects. One other study that compared ^{1}H-MRS measurements of GABA, glutamate and glutamine to TMS measurements of physiological inhibition and excitation chose only to study male subjects in light of the above evidence (Roessner et al., 2012). I felt it important, therefore, to assess whether there was any such sex effect in this dataset and can confirm that there was no evidence to support differences between sex in spectroscopy or TMS measures.

Secondly, I explored the relationship of the 7T ^{1}H-MRS quantifications of GABA, Glutamate, Glutamine and Naa (as ratio to total Creatine) to TMS measurements of corticospinal excitability. Despite the range of TMS measures taken (RMT, MEP at RMT, coefficient of variation for 100 and 100% of RMT, recruitment curve maximal slope and plateau, $SICI_1$ and $SICI_3$ (median and slope), $LICI_{100}$ median, ICF_{10} and ICF_{12} median), there were no relationships found with GABA:tCr, Gln:tCr or Naa:tCr.

Glu:tCr, however, appeared to be related to ICF_{10}, $LICI_{100}$ and IO Slope Plateau where higher PMC glutamate corresponded to lower inhibition induced by $LICI_{100}$, higher facilitation induced by ICF_{10} and a lower maximum MEP (i.e. a lower plateau estimated by the sigmoidal slope model for IO curves). Substantial evidence was also found using Bayesian correlations for a relationship between Glu:tCr and variability of MEP at threshold (CoV 100% IO). When explored further a forward stepwise regression revealed that $LICI_{100}$ was the greatest predictor of Glu:tCr

followed by ICF_{10}. IO Slope Plateau and the CoV of 100% RMT did not add any predictive value to the model.

$LICI_{100}$ is known to be mediated by $GABA_B$ receptors and it has been suggested that $GABA_B$ receptors modulate and are also modulated by glutamate receptors (Kantamneni, 2015). Whilst Tremblay et al. (2013) did not find a relationship between $LICI_{100}$ and Glu:tCr they did find a relationship between CSP and Glu:tCr which is also mediated by $GABA_B$ receptors.

The finding that ICF_{10} is positively related to Glu:tCr in the PMC is perfectly compatible with our understanding of ICF, given that it is partly mediated by glutamatergic neurotransmission (Ziemann et al., 2015). It is perplexing why ICF_{12} is not related to Glu:tCr. One possibility is that the differing ISI makes it less sensitive in determining the glutamatergic contribution of the ICF effect. ICF is evident using ISIs ranging from 7-20ms and there has been no literature investigating the distinction between these ISIs to my knowledge.

It is clear, however, that none of the metabolites captured by 7T ^{1}H-MRS map on perfectly to the TMS measures acquired in this study. This is no surprise given that higher concentrations of specific neurochemicals are unlikely to simply give rise to net excitation or inhibition. Glutamate, GABA and glutamine interact in a balanced and finely tuned manner to influence neurotransmission. Furthermore, whilst TMS assesses reactive, phasic, neurotransmitter function in response to stimulation, ^{1}H-MRS assesses ambient levels of neurochemicals. Whilst it is likely that TMS may capture ambient levels of neurochemicals (particularly if they give rise to tonic inhibition/ disinhibition) it certainly does not exclusively measure these levels. Function of a specific neurotransmitter probably does not alter the total concentration

of that neurochemical to such an extent that ^{1}H-MRS measured concentrations reflect neurotransmitter function sensitively across humans. Despite this it has been demonstrated that ^{1}H-MRS-quantified neurochemicals such as GABA are likely to be physiologically relevant given the way it varies across brain regions and has been related to human behaviour such as reaction times (Stagg, 2014; Stagg, Bachtiar, et al., 2011a).

This study has related Glu to various TMS measures. It is probable that these TMS measures either depict glutamatergic signalling cohesively or some measure of excitatory/inhibitory balance that ^{1}H-MRS-quantified Glu concentration reflects.

Sadly, the two prior studies in the literature (Roessner et al., 2012; Tremblay et al., 2013) that look at how ^{1}H-MRS relate to TMS are not synonymous with each other nor with the results found in this experiment. Whilst the results are not consistent, the methods differ in a manner that could explain these differences. Both Stagg and Tremblay acquired spectroscopy from 3T Siemens MR Scanners. Stagg, Bestmann, et al. (2011) used a spin-echo full-intensity acquired localised (SPECIAL) sequence. One paper that compared this sequence at 3T and 7T suggested that GABA measured using this sequence at 3T may not be reliable. In this article, concentrations of GABA using this sequence at 7T found were 1.3mmol/kg which is in keeping with the literature whilst concentrations at 3T were overestimated at 2.5mmol/kg (Mekle et al., 2009). Tremblay et al. (2013) used a MEGA-PRESS sequence; which has been shown to detect GABA and Glx with high reproducibility (O'Gorman, Michels, Edden, Murdoch, & Martin, 2011). In **Experiment 5**, all spectra was were collected at the field strength 7T which negated the need for a GABA-edited sequence and a STEAM sequence was used which has been demonstrated to

have good reliability at high field strengths (Menon et al., 2001; Wijtenburg et al., 2013).

Another likely source of method difference that contributed to the differing results between these studies are the different use of TMS measures. Tremblay et al. (2013) collected: $SICI_3$ with one CS (70%), $LICI_{100}$ (CS to evoke a 1mV response), CSP (CS: 120 and 130%). Stagg, Bestmann, et al. (2011) collected: $SICI_1$ and $SICI_{2.5}$ (CS: 60, 70, 80 and 90% aMT), ICF_{12} (60, 70, 80, 90% aMT), $LICI_{150}$ (CS to evoke a 1mV response) and recruitment curves (50-150% and 80-140% of intensity of evoke 1mV response). Given the varying parameters it is difficult to recognise exactly what caused the inconsistencies between these studies. The one thing we can take home is that it is clear that ^{1}H-MRS quantified neurochemicals such as GABA, Glu and Gln although may be physiological relevant do not neatly measure synaptic neurotransmission.

Investigations were also conducted within TMS measures alone and within ^{1}H-MRS measures alone.

6.4.1 TMS

Important findings are as follows:

1. RMT (MSO%) is inversely related to $SICI_3$
2. TP MEP amplitude is negatively related to both ICF_{10}, ICF_{12} and $LICI_{100}$
3. $SICI_1$ and $SICI_3$ are positively correlated
4. $SICI_1$ slope is negatively related to both ICF_{10} and ICF_{12}
5. ICF_{10} and ICF_{12} are positively related

6. IO plateau and $LICI_{100}$ are negatively related
7. IO maximal slope and $LICI_{100}$ are negatively related.

RMT was found to be negatively related to $SICI_3$ but not $SICI_1$ when controlling for the relationship between $SICI_1$ and $SICI_3$. The finding that TP_{MEP} amplitude is negatively related to ICF_{10}, ICF_{12} and $LICI_{100}$ has been shown before (Daskalakis et al., 2002). However, whilst increasing TP_{MEP} amplitude would lead to a decrease in LICI and ICF there was no correlation between the degree of LICI/ICF and TP_{MEP} amplitude. The authors suggested that one potential cause of this relationship is the refractoriness of the interneurons: whilst a small test stimulus may leave an interneuron partially refractory a larger test stimulus may overcome the refractoriness and induce more inhibition. This result could suggest that similar neuronal populations mediate both LICI and ICF.

I observed that IO slope (both plateau and maximal linear slope) correlated with $LICI_{100}$. This result in addition to the similar relationships found between IO slope plateau, $LICI_{100}$ and Glu:tCr suggests that they index overlapping mechanisms. Prior literature describes that the maximum IO curve plateau does not simply reflect the maximum output of the corticospinal system (Devanne, Lavoie, & Capaday, 1997; Houdayer et al., 2008; Kouchtir-Devanne, Capaday, Cassim, Derambure, & Devanne, 2012). It may instead reflect the intrinsic balance of excitation and inhibition at the PMC (Devanne et al., 1997). Few articles discuss the cortical origins that lead to the plateau of a recruitment curve. One experiment shows decreases in maximum plateau across different active muscle conditions is accompanied by reductions in SICI and LICI but not in ICF (Kouchtir-Devanne et al., 2012) and have speculated that this may relate to the functional coupling between *intra*cortical circuits within the PMC. This fits with the negative relationship that I have found

showing that reduced plateau coincides with reduced $LICI_{100}$; which is accompanied by high concentrations of Glu:tCr.

The finding that $SICI_1$ and $SICI_3$ medians are related is controversial; in this study $SICI_1$ and $SICI_3$ slopes are not found to be correlated and so the result is not in direct opposition to Stagg, Bestmann, et al. (2011) paper. However, the argument put forward by the authors that $SICI_1$ and $SICI_3$ are distinct physiologically is unlikely; there must be some overlap in the mechanisms that the differing SICI paradigms are tapping into. This is in keeping with the suggestion that $SICI_1$ inhibition is a result of synaptic inhibition as well as axonal refractoriness (Fair et al., 2009; Peurala, M. Müller-Dahlhaus, Arai, & Ziemann, 2008) and not that $SICI_1$ solely reflects axonal refractoriness or tonic inhibition. Neither $SICI_1$ nor $SICI_3$ is related to GABA:tCr in this study, which does not help clarify the matter.

There are two important differences between this study and Stagg, Bestmann, et al. (2011)'s paper: firstly, the longer phase SICI used in Stagg's paper was 2.5ms, not 3ms, and secondly, the subthreshold conditioning pulse intensity used was 60-90% of an active motor threshold whereas the intensity used in this experiment was 45-60% RMT for $SICI_1$ and 60-75% RMT for $SICI_3$. The subtle differences in the paradigms used to collect SICI measures potentially contributes to the differing results. Importantly, however, this study has greater power; the results of this study are based on data from 28 participants for $SICI_1$ and $SICI_3$ measures, as opposed to 11.

6.4.2 MRS

Finally, a positive relationship between Glu:tCr and Naa:tCr and a negative relationship between GABA:tCr and Gln:tCr was found. The relationship between

Glutamate and Naa is not novel; the relationship between Glx (a composite of glutamate and glutamine measured in ^{1}H-MRS using 3 or 1.5 Tesla MR scanners) and Naa has been shown elsewhere (Petersen, Pyndt, & Nielsen, 2003). Naa and Glu and are tightly linked in the metabolic pathways: the glutamate-glutamine cycle and tricarboxylic acid cycle. To my knowledge, there has been no published work describing a correlation between ^{1}H-MRS measured Glutamine and GABA, however, these two metabolites are known to be tightly linked. One study has at least described opposing correlations with GABA (positive) and Gln (negative) with scores on a social cognition test, in which Glutamate was not related (Cochran et al., 2015). Most ^{1}H-MRS studies cannot disassociate between Glu and Gln and instead collect the composite Glx as the field strength of the scanner used tends to be too low to distinguish between the Glu and Gln peaks in the spectra. It is therefore difficult for me to draw parallels between this study and others in regards to this finding.

Chapter 7: General Discussion

Within this thesis there were two principle aims. The first aim was to explore how neurodevelopment affects PMC excitability. I used TMS to measure motor excitability in young patients with Tourette Syndrome (TS) at rest and during the preparation and execution of volitional action. In this way, I provided support for the hypothesis that CSTC motor circuitry is dysfunctional in TS. Furthermore, findings in this thesis suggest that mechanisms for tic suppression and remission primarily come from alterations either in local changes in PMC cortical inhibition or alterations in influences from secondary motor areas. The second aim was to explore different ways of characterising PMC excitation, inhibition, neurochemical composition and interhemispheric effective connectivity and the ways these measures interact in healthy young adults. This is important for two reasons: firstly, in order to bring forward the methodology used within this thesis in future experiments investigating neural mechanisms in TS pathology, tic remission and therapy (pharmacological, behavioural and stimulation [TMS, tDCS and DBS]). Secondly, the results from these studies have implications on the interpretations of the current state of evidence and theory for neural mechanisms in TS using these methods.

7.1 Motor excitability in Tourette Syndrome

I have used TMS to explore motor excitability in young people with TS. The output of the PMC can be a useful readout in both neurodevelopmental and motor disorders; specifically, due to its ability to track various indices of motor state at rest and within a functional context. In **Chapters 3** and **4** I have utilised TMS in order to explore CSE in TS at rest where I have measured resting motor threshold (RMT) and recruitment curves (IO Curves) and have explored how motor excitability changes

during different circumstances broadly covering movement selection and preparation, motor execution and response inhibition.

Indices of motor excitability have been well established as changing over childhood into adolescence in the healthy population. It has been shown that RMT is increased in young children and decreases into mid adolescence to a comparable level to adults (Croarkin, Wall, & Lee, 2011; Garvey et al., 2003). Furthermore, MEP amplitudes (assessed using IO curves) are reduced in young children but alike RMT normalises to an adult-like level by mid adolescence (Garvey & Gilbert, 2004). It is believed that these changes in motor excitability track maturational changes that occur throughout adolescence. Structural changes including synaptogenesis, synaptic pruning and myelination (particularly of intracortical white matter and corpus callosum tracts) occur throughout childhood and adolescence and are largely responsible for changes in TMS-measured motor excitability (Garvey & Mall, 2008).

My work detailed in **Chapters 3** and **4** has allowed me to establish evidence for an apparent delay in the development of motor networks in TS which is characterised by increased RMT, increased MEP variability and reduced gain of motor excitability (shallower IO curve slopes) compared to age-matched controls. Critically, these indices were associated with age in TS patients where the youngest patients had the most increased RMT, variability and reduced gain.

Evidence for the delay in the development of the neuromotor system is in keeping with imaging studies of TS. Functional neural networks are characteristically immature in both children and adults (Church et al., 2008; Worbe et al., 2012). Structural networks further show evidence of an abnormal maturation trajectory (Wen, Liu, Rekik, Wang, Zhang, et al., 2017; Worbe et al., 2015). I have detailed

problems with neuroimaging data extensively in **Chapter 1** whereby many common findings in TS could be related to head motion artefact and so it is wise to be cautious when interpreting data, particularly that of functional connectivity. My data shows that patients with TS have TMS measures of CSE that resemble children of a younger age. In my two cross-sectional studies, it appears that in the majority of patients these measures normalise with age perhaps due to normal developmental processes such as myelination of intracortical white matter and synaptic pruning. Of note, RMT has been shown to relate to cortico-cortico white matter connectivity where increased RMT is inversely associated with FA (a DTI measure of white matter integrity) (Klöppel et al., 2008).

It is unclear whether my findings are a part of the pathology of TS which contributes to tic onset and maintenance, in which normalisation leads to tic remission. Another possibility is that normalisation of these measures of motor output have no consequence to tics. Data in adults are inconclusive regarding RMT with the majority of papers showing no differences in threshold between patients and controls (Bäumer et al., 2010; K. F. Heise et al., 2010; Orth, Amann, et al., 2005; Suppa et al., 2011) and one showing increased RMT in patients (Orth & Rothwell, 2009). Shallow IO curve slopes have been shown in adulthood and has further been shown to positively relate to tic severity (Orth et al., 2008). Reduced gain in adults suggests that a failure to normalise may contribute to severe persevering tics.

The baseline differences I found in motor excitability between patient and controls precipitated throughout motor execution assessed by a simple sp-TMS experiment in a one cue 'Go/ No-Go' task in **Chapter 3.** Motor gain during the execution of a movement was also reduced, replicating previous work in this field (Draper et al., 2015). My results from **Chapter 4**, however, demonstrated that young

people with TS show a normalisation in motor output during the preparation and execution of a movement. During normal movement execution, TMS-induced MEPs increase rapidly as TMS stimulation is closer to movement onset which is accompanied by a reduction in variability thought to reflect an gain in neuronal firing pattern which is needed to achieve action onset (Klein-Flügge et al., 2013). In fact, in my data presented in **Chapter 4**, when corrected to baseline patients with TS show an increased ramping of motor excitability during movement execution, compared to healthy controls, but not preparation. Motor tic severity predicted the extent to which a patient modulated CSE during movement execution; an increased capacity to ramp up excitability in TS was related to reduced tic severity.

In **Chapter 4** I used TMS alongside a two-cued Go/ No-Go Task, which allowed me to disassociate movement preparation from movement execution. Sp-TMS was delivered to the left hemisphere PMC after a first stimulus (S1), which instructed participants that they were to use their left hand or right hand after presentation of the second stimulus (S2) which was either a 'Go' signal (respond) or a 'No-Go' signal (inhibit response). I was able to assess motor excitability for effector specific choices (left hemisphere CSE during a right-hand trial) and non-selected effectors (left hemisphere CSE during a left-hand trial); it further allowed me to assess motor excitability during response inhibition (a 'No-Go' trial). Patients showed the same general trends as healthy controls including increased CSE for the selected effector versus non-selected effector and MEP suppression during 'No-Go' trials which was greater for the non-cued effector. In contrast, patients showed increased MEP variability during right-cued motor preparation and during inhibition of right hand (selected effector) responses. As I have already detailed, patients also showed an increased ramping up of motor excitability during right-cued motor execution.

Increased MEP variability in the contralateral PMC during motor preparation fits with the idea that altered processes within PMC or connected regions are reducing neuronal firing pattern consistency perhaps as a mechanism to gate for tics. Whilst inhibitory mechanisms may be at play throughout preparation for action in the non-selected effector, the PMC is left vulnerable when preparing for action in the selected effector. There are no consistent inputs for action execution yet motor excitability is generally increased ready for action. However, variability did not relate to age nor tic symptoms (**Chapter 4**). It is of course possible that this increased variability is related to the increased CSE variability found at rest in **Chapter 3.** I argued in **Chapter 3** that increased MEP variability at rest was supporting evidence for a delay in the development of neuromotor networks. Instead, when the brain is actively participating in movement preparation and execution it appears that increased variability may be functional.

Patients and healthy controls largely showed similar patterns of motor excitability that co-occur with motor selection/ preparation and response inhibition. Some interesting relationships were found that may hint at both pathology and compensatory mechanisms that occur in response to tics. For instance, in patients with TS the motor excitability measured during right hand No-Gos was predicted positively by motor tic severity. Increased tics resulted in increased CSE. Furthermore, percent change of rhNo-Gos from baseline motor excitability negatively related with right hand response times in patients with TS (but not controls). Given that the extent of MEP suppression in No-Go trials has been shown to be related SICI and thus $GABA_A$ receptor action (Coxon, 2006) it appears that those with the most severe tics had the most reduced inhibitory function which is indicative of more CSTC disinhibition and core pathology. Those that were able to actively suppress

MEPs in comparison to their baseline excitability had the behavioural consequence of increased, and thus slowed, response times.

To summarise, in both **Chapters 3** and **4** I found that young people with TS had increased response times in simple motor tasks. In **Chapter 4** the participants were to respond after the Go stimulus disappeared from the screen and thus slowed response times are likely to reflect a general slowing of action rather than a slowing of the selection and preparation processes involved in voluntary action. The finding of increased response times in patients with TS had been found in adults (Thomalla et al., 2014) and children (Eichele et al., 2010; Shephard et al., 2016) with TS before with similar tasks. The relationship of change in motor excitability during rhNo-Gos and rh RT was absent in healthy controls; whose RTs were more associated with modulation of motor excitability during rh Go trials. Healthy controls with the greatest increase in gain during a Go trial had the fastest response times, which was not true in patients.

7.2 Interhemispheric Interactions in Young Adults

The intention of **Chapter 5** was to characterise PMC-to-PMC interhemispheric interactions in a group of young people (aged 17-24 years). My main question was to explore whether there was a directionality to these interhemispheric interactions and whether the inhibition or excitation caused by different IHI or IHF protocols was related to measures of neurochemicals or pp-TMS measures. Finally, I was interested in whether sex affects a large range of ds-TMS measures that aimed to capture interhemispheric interactions.

I tested various CPs and ISIs in order to capture a range of effects: 50%, 70%, 100% and 110% of RMT as CP and ISIs of 6ms, 8ms, 10ms and 12ms. RMT

was found to be marginally increased in the right hemisphere compared to the left hemisphere. Firstly, I found that whilst there was evidence for IHI in my data by various suprathreshold conditioning stimuli and longer ISIs in the left-to-right direction; I did not find strong evidence for IHF effects and no significant inhibitory effects in the right-to-left direction. The left hemisphere appeared to have a stronger inhibitory effect on the right hemisphere than in the right-to-left direction. This has been found before in right handers (Bäumer et al., 2006).

I assessed whether the different parameters related to each other inter-directionally and intra-directionally. Whilst there were various positive relationships of different CP and ISIs within one direction there were no relationships between one direction and the other, i.e. left-to-right inhibition or facilitation did not correlate with right-to-left inhibition or facilitation. This is suggestive of a directionality to interhemispheric influences in which capturing effective connectivity between primary motor areas using TMS cannot simply be characterised using one direction. In addition, there were no relationships that survived correction for multiple comparisons between using suprathreshold CP_{110} and other conditioning stimuli suggesting that whilst there is some overlap of mechanisms between the other different conditioning stimuli CP_{110} (the most inhibitory parameter) is presumably tapping into different intracortical circuits than the other parameters used.

Previous work has shown that IHI interacts with intracortical inhibition. For instance IHI has been shown to reduce SICI, and LICI has been found to reduce IHI (Daskalakis et al., 2002). The effect of TP on IHI and LICI are similar, suggesting that their effects are both somewhat reliant on $GABA_B$ receptor activity (Daskalakis et al., 2002). However, research using $GABA_A$ and $GABA_B$ agonists have failed to modulate IHI (Irlbacher, Brocke, Mechow, & Brandt, 2007). Whilst I did not investigate how

IHI/IHF causally interacted with intracortical inhibition or facilitation in the same participant I had a wide range of physiological and neurochemical measures for a subgroup of participants. These measures included pp-TMS: $SICI_1$, $SICI_3$, ICF_{10}, ICF_{12}, $LICI_{100}$, IO curve, RMT and ^{1}H-MRS measures of neurochemicals: GABA, Glu and Gln. I used linear slopes to capture interhemispheric interactions for each ISI whereby greater negative IH_{slopes} indicated a greater interhemispheric interaction. An individual with a more negative IH_{slope} shows greater facilitation at $CP_{50/70}$ and greater inhibition at $CP_{100/110}$.

The relationships I found that survived multiple comparisons were between pp-TMS and neurochemical measures taken at the left hemisphere and interhemispheric interactions measured in the right to left direction. ICF was negatively related to ISI_6 and $SICI_3$ was positively related to ISI_6, $SICI_1$ was negatively related to ISI_8, and GABA was positively related to ISI_8. A marginal positive relationship also existed between ISI_{10} in the left to right direction and GABA. These relationships are indicative of two things. Firstly, interhemispheric interactions are mediated partly by the current state of excitability of the tested hemisphere rather than the hemisphere that is conditioned. Secondly, different ISIs used to test interhemispheric interactions are likely measuring differing mechanisms. The strongest relationships were with ICF and RtoL ISI_6 (negative) and with GABA and RtoL ISI_8 (positive). Greater ICF at the left PMC relates with greater facilitation/ inhibition at the left PMC induced by a conditioning stimulus at the right PMC 6ms prior to the test stimulus. Greater GABA concentrations in the left PMC relates with less facilitation/ inhibition at the left PMC induced by a conditioning stimulus at the right PMC 8ms prior to the test stimulus.

My final finding of the influence of sex on interhemispheric interactions is particularly interesting given the literature on differing CC changes in males and females in TS. I found that females had more negative fitted slopes for each ISI in the LtoR direction suggesting a greater influence of conditioning stimulus in females. This follows previous literature showing more inhibitory IHI in females compared to males (De Gennaro, Bertini, et al., 2004; De Gennaro, Cristiani, et al., 2004). This is likely to be hormonally mediated (Bayer, Kessler, Güntürkün, & Hausmann, 2008; De Gennaro, Bertini, et al., 2004; Hausmann et al., 2006) and both estradiol and progesterone have been shown to have effects on neuromodulators GABA and glutamate (M. J. Smith et al., 1999; S. S. Smith et al., 1987). I did not find any relationships between my measures and handedness. However, only one participant was left handed.

7.3 ^{1}H-MRS Measurements of Neurochemicals

^{1}H-MRS is a tool that has gained popularity in recent years for exploring abnormalities of neurochemicals within specific regions of the brain in neuropsychiatric and neurodevelopmental disorders; in TS, seven ^{1}H-MRS papers have been published in the last three years as compared to one study prior to 2014. The findings of which have been inconsistent. For instance, in the realm of PMC both reduced GABA (Puts et al., 2015) and no differences in GABA (Draper et al., 2014; Tinaz et al., 2014) in patients with TS compared to controls have been reported for sensorimotor regions. In addition, correlations between neurochemical concentrations and clinical measures have been reported in some papers but not others. The finding of reduced GABA concentration has further been described as having the consequence of reduced GABAergic inhibition (Puts et al., 2015). This relates back to the reductions in $GABA_A$ receptor-mediated inhibition that have been

found in TMS studies in TS (Gilbert et al., 2004; K. F. Heise et al., 2010; Orth, Amann, et al., 2005; Ziemann et al., 1997). Increased GABA concentrations in PMC as measured by ^{1}H-MRS certainly have been shown to have behavioural consequences that could be considered inhibitory, for instance increased RTs in behavioural paradigms (Stagg, Bachtiar, et al., 2011a). However, when tested explicitly it does not appear that GABA concentrations measured by ^{1}H-MRS related to phasic GABAergic inhibitory mechanisms as measured by TMS (Roessner et al., 2012; Tremblay et al., 2013). Whilst this explicit relationship has been tested before using 3T MR scanners, the results have been inconsistent and sample sizes are small. Furthermore, there are inherent problems measuring neurochemicals of such small concentrations such as Glu, Gln and GABA at 3T whereby their peaks overlap on the spectrum. Edited sequences are used in order to measure GABA and measurements of Glu and Gln tend to be taken as the composite Glx thus making measurements insensitive.

The aim of **Chapter 6** was thus to explore how ^{1}H-MRS measurements of important neurochemicals of interest in neurodevelopmental disorders (GABA, Glu and Gln) relate to TMS-measured inhibitory and excitatory mechanisms in ultra-high field MRI (7T) with a large sample size. Importantly measurements were taken close in time (separated by ~45 minutes), the VOI used was small (2x2x2mm) and TMS was neuro-navigated using anatomical scans taken on the day. Furthermore, measures taken of neurochemicals were highly reproducible.

The sample size that was used within this study was large (a total of 29 recruited) and there was an equal sex divide with 14 females. Importantly I showed that there were no sex differences found in the pp-TMS or ^{1}H-MRS demonstrating that increased variance from testing females was not an issue within this dataset.

Interestingly, using the STEAM sequence at 7T was sensitive enough to draw relationships between the neurochemicals measured: Glu and Naa positively related and GABA and Gln were negatively associated. This demonstrates the disassociation of Glu and Gln which is important when assessing changes in Glu/Gln levels in brain disorders. Further it highlights a tight linking of neurochemicals that are necessary for the balance of excitation and inhibition in the brain.

^{1}H-MRS measures of GABA and Gln did not relate significantly to any physiological measures of excitation or inhibition as measured by TMS. Whilst this is true it is of note that in a smaller sub-group of ten participants, GABA was positively related to RtoL interhemispheric interactions measures using an ISI of 8ms (as detailed in **Chapter 5**). Increased GABA was related to a shallower slope and thus related to a reduced influence of the right hemisphere on left hemisphere motor excitability or perhaps even a greater resistance of the left hemisphere to be modulated by the right hemisphere. In contrast, Glu was related to a variety of TMS measures, namely $LICI_{100}$, ICF_{10}, IO curve plateau and marginally to individual MEP variability at RMT. Since these TMS measures all correlated well with each other a regression confirmed that only $LICI_{100}$ and ICF_{10} predicted a significant amount of the variability of Glu; the other TMS measures did not add significantly to the model. Glu was in fact positively related to both $LICI_{100}$ and ICF_{10} indicating that higher levels of Glu predicted lower inhibition measured by $LICI_{100}$ and higher facilitation induced by ICF_{10}. IO plateau was negatively related to $LICI_{100}$ and thus increased inhibition was paradoxically related to increased maximum MEP amplitude in an individual and so Glu was related to reduced IO plateau.

These results were in contrast to the previous studies completed at 3T MRI, however, as discussed in **Chapter 6** there were a great number of methodological

differences between these studies. In previous literature, it was asserted that the ^{1}H-MRS GABA signal reflects largely ambient 'tonic' levels of GABA in the brain (Roessner et al., 2012). Theoretically, heightened GABAergic tone dampens network excitability and exerts inhibitory control over widespread neuronal firing (Bachtiar & Stagg, 2014; Belelli et al., 2009). Whilst this study does not provide evidence to support the concept that ^{1}H-MRS measured GABA at 7T reflects a specific pool of GABA more so than others, it does show that the ^{1}H-MRS GABA signal does not relate to phasic synaptic signalling of GABA measures. There is marginal evidence for the GABA signal relating to tonic levels of inhibition in a small subgroup (the positive relationship between GABA and RtoL ISI_8 slope); however, more work is needed.

7.3.1 Relationships between Single-Pulse and Paired-Pulse TMS Measures

Given the large sample of participants and large variation of measures taken using pp-TMS I was able to use multiple correlations in order to explore how different TMS measures relate to each other. RMT was inversely related to $SICI_3$ and $SICI_1$ suggesting that those with increased thresholds had greater inhibition. TP MEP was negatively related to ICF_{10}, ICF_{12} and $LICI_{100}$ suggesting that those with higher MEP amplitudes for test pulses had increased inhibition during ICF and LICI protocols. $SICI_1$ and $SICI_3$ were positively correlated suggesting that the mechanisms that mediate both forms of SICI are overlapping. $SICI_1$ slope was negatively related to ICF_{10} and ICF_{12} suggesting that those with greater inhibition at higher CP stimuli and greater facilitation and lower CP stimuli were related to increased intracortical facilitation. Furthermore, both plateau and IO slope were negatively related to $LICI_{100}$ suggesting that those with steeper slopes and higher maximal MEP amplitudes also had increased inhibition.

These correlations show that TMS measures of intracortical inhibition and facilitation do not vary entirely independently from each other, a view that is precedent (Wassermann, 2002). The mechanisms for ICF, LICI and IO Curves overlap with a likely glutamatergic element. $SICI_1$ and $SICI_3$ are likely to have overlapping mechanisms suggesting that $SICI_1$ does not solely reflect axonal refractoriness and is somewhat dependent on synaptic inhibition (Fair et al., 2009; Peurala et al., 2008) – a view which is still of some controversy (Fisher et al., 2002; Roessner et al., 2012). The relationship with RMT and SICI is difficult to resolve from this data alone. Given that there is no relationship between RMT MEP and RMT or SICI and RMT MEP this is unlikely due to experimental error. The inverse relationships between TP MEP and ICF/LICI are likely to be glutamatergic in nature given the positive relationships between ICF_{10} and $LICI_{100}$ and Glu.

7.3.2 The relationships between GABA, Glu, Gln and Measures from Chapter 3 and 4 that were found to be Abnormal in a Sample of Young People with Tourette Syndrome

In **Chapters 3** and **4** I found a strong negative association between age and RMT, MEP variability and IO curve slopes in a group of young people with TS that was absent in healthy controls. This resulted in a group difference in RMT and IO curve slopes where patients with TS' PMC was characterised as having an increased motor threshold and reduced gain. Whilst I looked at these measures in my final study using ^{1}H-MRS I failed to find a relationship between the majority of these measures and concentrations of GABA, Glu and Gln in the brain. Sadly, there were no relationships between RMT or MEP variability with the neurochemicals measured in **Chapter 6**. There was, however, a hub of related measures surrounding IO slope curves (IO slope, IO plateau, ICF_{10}, $LICI_{100}$ and MEP variability at 100% RMT) that seemingly has some basis in glutamatergic neurotransmission. Interestingly in this

chapter (**Chapter 6**) I found a negative relationship between IO plateau and Glu and whilst there was no found relationship between IO Slope and Glu, IO plateau and slope did strongly correlate. This indicated that rather paradoxically increased glutamate related to reduced IO plateaus. This is certainly curious when thinking about reduced gain in TS, a disorder in which there is a clear imbalance of glutamatergic to GABAergic activity (Leckman, Bloch, Smith, Larabi, & Hampson, 2010). There have been no similar findings in the literature and only one publication showing the opposite finding using 3T MRI, i.e. Glx positively related to IO curve slope (Roessner et al., 2012). I would, therefore, not want to speculate on the glutamatergic mechanisms that may underlie my findings in **Chapter 3**.

7.4 Limitations and Considerations for Future Work

7.4.1 Research in TS

I have touched upon the topic of heterogeneity of TS in **Chapter 1**. TS is complex and can be viewed as a spectrum (Cavanna & Rickards, 2013). Without sub-classifications of TS, or better still different core components of symptom spectrums, it is difficult to identify core pathophysiological and compensatory mechanisms in TS. It has been suggested that there is more than one phenotype of TS, however, these different phenotypes have not been agreed to date. Broadly speaking it appears that there are clusters of symptoms including tics (sometimes clustered as simple and complex separately), compulsions, ADHD, aggression, anxiety/depression (Alsobrook & Pauls, 2002; Grados et al., 2008; Mathews et al., 2007). A large limitation of my work is thus the small sample sizes used, which is clearly not optimal when studying heterogeneous disorders. However, it is of a comparable size (or larger) to most studies in the same field (e.g. Draper et al., 2015; Heise et al., 2010; Orth et al., 2005, 2008). Some patients within **Chapter 3** and **4**

had diagnoses of co-occurring comorbidities, which is another source of variability. However, previous studies have included comorbidities and it has been shown that measures of motor excitability such as RMT does not differ between patients presenting with TS alone or TS plus comorbidities (Orth & Rothwell, 2009). In addition, some patients were taking medication in the experiments detailed in **Chapter 3**, however, all patients were unmedicated in **Chapter 4**. Of note the finding of increased RMT in patients with TS compared to controls which was inversely related with age is the same across both studies. Within my work, I have focused on the motor system and thus concentrated on motor symptoms and motor paradigms This focus certainly has benefits with working with a heterogeneous disorder that has core motor symptoms. However, I cannot make claims about whether the finding of an immature motor system in young people with TS is distinct from or translational to other networks within the brain.

Furthermore, given that I have used a small cross-sectional sample of young people I am unable to distinguish whose tics will ameliorate and whose will remain into adulthood. It is therefore very difficult to explore adaptive compensatory mechanisms that may be helpful in tic suppression in TS. Whilst research in **Chapter 3** and **4** do suggest that patients' motor systems may catch up in adulthood from an initial immature or developmentally delayed state; the relationships between motor excitability and tic severity showed that there is the possibility of an interplay between normal development and compensatory mechanisms brought about by tic suppression. Whilst compensatory mechanisms may be at play these may not necessarily bring about tic remission in adulthood; an alternative hypothesis would be that those with milder tics are able to compensate in a way that those with severe tics (due to more severe pathology) cannot. In addition, in my sample the older

patients that still have moderate tics are likely to continue to have them into adulthood whereas the younger patients may not.

In order to explore the interplay of pathology, normal development, compensatory mechanisms and tic remission it really is necessary to explore multi-modal core reproducible measures in a large longitudinal study. The development of a biomarker in TS is necessary in order to explore how the brain responds to pathology. However, as I have explained in **Chapter 1** this is challenging given that the aetiology of TS is polygenic and the likelihood of TS comprising of multiple phenotypes. This would therefore a large feat. Employing multi-modal techniques is an important step. However, the current state of neuroimaging in TS needs to rapidly move forward and address concerns related to the fact that head motion can cause systematic bias in neuroimaging data. Whilst there have been developments of prospective motion correction devices these do not appear to work well for tics given that tics tend to have high velocity; the latency for such systems to detect motion is currently not sensitive enough (Callaghan et al., 2015). Despite this, using such a device accompanied by scanner practice and video monitoring of tics in the scanner may help to alleviate these problems.

In addition to requiring multiple test visits of a patient, one of the major restrictions in being able to recruit larger samples and testing various core measures across time is financial. Furthermore, adolescents are particularly difficult to follow given the various commitments they have over such a critical time period, for example: exams, university applications, beginning work for the first time. One other possibility would be to test adults with either continuing or remitted tics and explore the differences between these groups. Of course, recruitment within adults with

remitted tics may be difficult as people are unlikely to return to clinic and have less to gain from research.

7.4.2 Transcranial Magnetic Stimulation

7.4.2.1 Chapter 4

In **Chapter 4** I chose to deliver TMS stimuli in the time period between the end of stimulus presentation and beginning of the hand movement in order to not interrupt processing of the stimulus. This means that during the period that required movement preparation but not execution I was unable to explore how motor excitability dynamically changed from the beginning of processing the stimulus. This may have been useful temporally in order to determine whether there was any rapid gain of motor excitability following stimulus processing. In addition, if I were to develop this experiment for future use I would add a condition in which a TMS pulse would be delivered during a fixation cross where whilst there is an element of task engagement and continual attention there is not any motor preparation.

7.4.2.2 Chapter 5

The most obvious limitation of **Chapter 5** is the fact that most of the dataset was unimodal. It may have been interesting to explore how TMS measures of interhemispheric interactions rely on the white matter connectivity in the motor region of the CC and future work could explore the interaction between DTI, ^{1}H-MRS and pp-TMS measures from bilateral PMC and IHI/IHF. This could help characterise the source of facilitation and inhibition during IHI and IHF. Furthermore, whilst all ds-TMS measures were collected in the same session the pp-TMS and ^{1}H-MRS data was collected on a different day with varying lengths of time in between. Pp-TMS measures have been shown to be fairly reliable such as RMT, IO Curves and SICI

and ICF (Du, Summerfelt, Chiappelli, Holcomb, & Hong, 2014; H. Liu & Au-Yeung, 2014; Ngomo, Leonard, Moffet, & Mercier, 2012). Some, however, have suggested that ICF is fairly variable (Orth et al., 2003). It is worth then keeping in mind the relationship between RtoL interhemispheric interaction (ISI_6 slope) and ICF when interpreting the results.

Furthermore, I would have liked to explore the effect of handedness more. Whilst there was some variation in scores for right handers there was only one slightly ambidextrous left hander in the study. To explore how handedness interacts with the symmetry of IHI and IHF one should explore this question with a larger sample with a wide variety of handedness scores (ranging from -100 to 100, 100% left handed to 100% right handed).

Future work should aim to characterise some of the measures I have used in this thesis in healthy developing children and adolescents to determine how TMS-measured characteristics of the motor system change with age. Whilst there has already been work demonstrating the trajectories of many TMS-measured motor excitability (Garvey et al., 2003; Garvey & Mall, 2008) work is limited and would benefit from replication and expansion of measures taken including varying intensities of conditioning stimuli, given that the evidence suggests that children have lower gain (shallower IO slopes and increased RMT). Exploring the use of ds-TMS to measure IHF and IHI in young people with TS would be a next step following from **Chapter 5**, however, the use of smaller coils would be of benefit.

7.4.3 ^{1}H-MRS

Whilst there were no significant relationships between 1H-MRS measurements of GABA and TMS-measures of inhibition (or excitation) it would be

interesting to explore how GABA, Glu and Gln from the PMC relates to physiological measures in TS. The motor system is perturbed in TS and the evidence from this thesis and elsewhere (Church et al., 2008; Worbe et al., 2012) demonstrate that the connectivity and output of the system are characteristically immature. TMS studies in patients have shown reduced SICI compared to healthy controls (Gilbert et al., 2004; K. F. Heise et al., 2010; Orth, Amann, et al., 2005; Ziemann et al., 1997), increased RMT and reduced IO slopes in young people with TS (see **Chapter 3** and **4**). Assessing whether GABA, Glu, Gln measured in this region relate to these measures of motor excitability would be an interesting next step. In addition, Naa and Choline have been used to assess issues of myelination in development (Filippi, Uluğ, Deck, Zimmerman, & Heier, 2002; Peden et al., 1990). In light of neural network immaturity in young people with TS, this would be an interesting avenue to explore issues of white matter development (which may be more sensitive than DTI). Furthermore, we have already seen that there is evidence for increased GABA in the SMA in patients with TS (Draper et al., 2014); how these perturbed levels of GABA relate to the neurochemical levels in basal ganglia and motor excitability at rest have yet to be explored.

In relation to previous ^{1}H-MRS studies that have measured GABA in TS it is clear that there is a trend to interpret ^{1}H-MRS GABA as related to specific receptors such as $GABA_A$ receptors (Puts et al., 2017; Tinaz et al., 2014). The work in **Chapter 6** shows that this is unlikely and that the measurements could be somewhat independent. More work needs to be done exploring GABAergic measurements using multi-modal techniques such as pp-TMS, ^{1}H-MRS and serum levels of neurotransmitters both in health and disease.

7.5 Conclusions

In summary, this thesis has used various TMS techniques and ^{1}H-MRS to explore the excitability, connectivity and neurochemistry of the PMC and its primary role in TS. I have primarily explored the resting and active brain in a group of young people with TS and how these measures change over the course of neurodevelopment. My findings show that there is a developmental delay in the motor networks of young people with TS, which normalises to a comparable level with healthy developing individuals in adulthood. TMS measures of excitability show an increased RMT and general reduced gain of motor output characterised by shallow IO curves and increased MEP variability. Whilst these measures are particularly deviant in the younger group, they are seemingly no different in the older group of a cross-sectional sample and change as a function of age.

I further found that there was reduced gain during motor execution which is largely a result of baseline differences in motor output. When motor execution excitability is corrected to baseline, patients with TS ramped up their motor excitability to a greater extent, likely compensating for an immature neuromotor system. Patients' tic severity was inversely related to the increase in excitability demonstrating that those with more severe tic severity were least able to modulate motor excitability.

Patients largely showed the same patterns of change in excitability when preparing volitional movements (but not executing) and were successfully able to reduce motor excitability during the inhibition of responses. However, the extent to which they could suppress MEPs during response inhibition was positively related to tic severity. Therefore, patients that were able to modulate motor excitability (and thus suppress more) had reduced tic severity. This could be an indication that

thalamocortical pathways are less disinhibited in patients with reduced tic severity or that those with reduced tics are able to engage $GABA_A$-ergic inhibitory mechanisms to a greater extent than those with severe tics. This appeared to have the consequence of longer response times, whereby greater ability to suppress MEPs was related to slowed response times (only in patients). Furthermore, there was some evidence that patients that had increased motor excitability compared to baseline during motor preparation (and not execution) had less severe phonic tics.

I secondarily explored ds-TMS, pp-TMS and ^{1}H-MRS to explore the effective connectivity, excitatory and inhibitory physiological mechanisms and the neurochemistry of PMC. I found that whilst IHI can be readily activated using a supra-threshold CP in the left-to-right direction there was less evidence of IHI in the right-to-left direction. Furthermore, whilst there was some evidence of facilitation when using sub-threshold CPs this was not robust. I found evidence that there is directionality to interhemispheric interactions whereby testing left-to-right is not the same as right-to-left. The interhemispheric effect is likely related to intracortical circuits in the test hemisphere and ambient levels of neurochemical GABA. Females were found to have a greater interhemispheric influence, which is likely related to the influence of sex hormones. There were no sex differences, however, found when assessing pp-TMS and ^{1}H-MRS measures. I explored the relationships between ^{1}H-MRS Glu, GABA and Gln with physiological measures of excitation and inhibition using TMS and found no evidence that GABA was related to any pp-TMS measures that measure GABAergic receptor activity. ^{1}H-MRS is thus likely not sensitive at measuring synaptic changes in GABA and most likely reflect ambient tonic levels of GABA. Whilst Gln did not relate to any TMS measures, Glu were related to several. There appeared to be a hub of TMS measures that were all interrelated and related to Glu. The measurements most related to Glu were ICF (10ms) and perhaps

paradoxically LICI. A great overlap of mechanisms underlying TMS measures were found and explored in detail. Finally, it was shown the ^{1}H-MRS was sensitive enough to uncover relationships between neurochemicals which are known to be linked such as the positive relationship between Glu and Naa and the negative relationship between GABA and Gln. These latter experiments are useful for the interpretation of research in TS literature. For instance, ^{1}H-MRS measurements of GABA likely do not relate to $GABA_A$ receptor activity in the healthy brain; an increase in ambient levels of GABA are consistent with an increase in GABAergic tone. Whilst it is clear that the pathology of TS involves GABA, ^{1}H-MRS measurements of GABA should not be interpreted as related to specific receptors or synaptic activity.

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