Theta Burst Stimulation of the Human Cerebellum

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ABSTRACT

Background

Repetitive Transcranial Magnetic Stimulation (rTMS) of the cerebral cortex has been previously applied as a non-invasive therapy for neurological conditions due to its potential to modify cortical excitability through neuroplasticity. Cerebellar stimulation has the potential to modify cortical excitability because of its predominantly inhibitory connections with the motor cortex and other cortical areas. Previous studies have shown that cerebellar rTMS, including theta burst stimulation (TBS) can modulate excitability in the motor cortex, but the findings have been variable. The aim of this study was to investigate the effects of 30Hz cerebellar TBS on motor evoked potentials (MEP) and TMS-evoked cortical potentials (TEP).

Method

Combined TMS and electroencephalography (TMS-EEG) was carried out on 16 healthy participants, aged 21-30 years. Each subject was studied in three separate sessions, in which 30Hz intermittent TBS (iTBS), continuous TBS (cTBS) or sham stimulation at stimulus intensity of 80% or 90% of active motor threshold (AMT) was applied to the right cerebellar hemisphere. Each session consisted of active and resting MEP and TEP recording from the left motor cortex before and after TBS. EEG recordings were analysed offline using EEGLAB software and independent component analysis (ICA) was used to remove artifacts. TEP were extracted and averaged. Mean N100 waveform amplitudes were measured before and after each treatment protocol. Post stimulation values for all parameters were compared using mixed model ANOVA, with pre-treatment values as covariates.

Results

The TBS protocol at 90% of AMT stimulus intensity produced a significant decrease in amplitude of the resting MEP after cTBS compared to sham TBS, $F(2,13)= 4.87$, $p=0.035$. Cortical silent period (CSP) was increased following iTBS, compared to sham, $F(2,13)= 4.87$, $p=0.026$. The effects of 80% TBS on MEP were not significant The mean N100 amplitude was significantly greater after iTBS than sham TBS using 80% or 90% stimulation $F(2,348)=197.80$, $p < 0.001$ and $F(2,455)=6.17$, $p = 0.02$, respectively.

Conclusion

The study demonstrated that 30Hz cerebellar cTBS at 90% AMT produced a reduction in overall excitability of the contralateral motor cortex, as shown by reduced resting MEP amplitude. Although iTBS produced an increase in the CSP and the N100 amplitude, both thought to reflect intracortical inhibition, there was no significant effect of iTBS on MEP amplitude. As this measure is dependent on the net effect of inhibitory and facilitatory networks in the cerebral cortex, it is possible that an increase in intracortical inhibition cancelled out the inhibitory effects. These findings provide further evidence that cortical excitability can be modulated through cerebellar TBS. Cerebellar TBS has potential as a therapeutic modality for a number of neurological conditions where there is abnormal cortical excitability, including epilepsy, dystonia and Parkinson's disease. Future investigation of its effects is required before it can be recommended.

PREFACE

Transcranial magnetic stimulation (TMS) is a non-invasive method of brain stimulation, which involves the induction of a painless electrical current in the brain by means of a coil applied to the surface of the scalp. It has been used in neurological diagnosis and more recently as a form of treatment.

The motivation of this study was to investigate theta burst stimulation (TBS), a particular type of TMS applied to the cerebellum, as a potential treatment for epilepsy. 25-30% of patients with epilepsy are resistant to treatment with medication and alternative therapies are few. Other forms of brain stimulation have been trialed in the past, but with variable success.

The cerebello-thalamo-cortical pathway has been shown to have inhibitory effects on the cerebral cortex and therefore cerebellar stimulation would appear to be a logical approach to epilepsy treatment. But before proceeding with clinical trials, the effects of cerebellar TBS on cortical excitability require further investigation in healthy individuals.

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LIST OF ABBREVIATIONS

AAMP- Active motor evoked potential amplitude

AgCl- Silver chloride

AED Anti-epileptic drug

ADHD- Attention deficit hyperactivity disorder

AMT- Active Motor Threshold

AL-Active latency

ALS- Amyotrophic lateral sclerosis

BC- Basket cell

CBI- Cerebellar Inhibtion

Cerebello-thalamo-cortical pathway- Pathway between the cerebellum and the thalamus to the motor cortex

CF- Climbing fibes

CNS- Central nervous system

CS- Conditioning stimulus

CSP- Cortical silent period

cTBS- continuous Theta Burst Stimulation

C3- Electrode placement three

DBS- Deep Brain Stimulation

DC-Direct current

DCN-Deep cerebellar nuclei

Dentato-thalamo-cortical pathway- Pathway between the dentate nucleus and the thalamus to the motor cortex

DLPFC -Dorsolateral prefrontal cortex

ED- Epileptic discharges

EEG- Electroencephalography

EP- Evoked potential

EPSP- Excitatory postsynaptic potential

FDA- Food and Drug Administration

GABA- γ-Aminobutyric acid

GABA-A- γ-Aminobutyric acid receptor A

GABAβ- γ-Aminobutyric acid receptor β

GC- Granule cell

ICA- Independent component analysis

ICF- Intracortical facilitation

ICI- Intracortical inhibition

IO- Inferior olive

IGE- Idiopathic generalised epilepsy

ISI-Inter stimulus interval

iTBS- intermittent Theta Burst Stimulation

JME- Juvenile myoclonic epilepsy

LICI- Long Intra Cortical Inhibition

LID-Levodopa induced dyskinesia

LTD- Long-term depression

LTP- Long-term potentiation

MEP- Motor evoked potential

MF- Mossy fibers

MRI- Magnetic resonance imaging

MS-Mili seconds

MSO-Maximum stimulator output

MT-Motor threshold

mV-Milli volts

NMDA- N-methyl-D-aspartate receptor

NS-Non-significant

N15- Negative component peaking at 15ms

N45-Negative component peaking at 45ms

N100- Negative component peaking at 100ms

PF- Parallel fibers

P30- Positive component peaking at 30ms

P60- Positive component peaking at 60ms

P200- Positive component peaking at 200ms

RAMP-Resting motor evoked potential amplitude

RL-Resting latency

RMT- resting motor threshold

rTMS- repetitive transcranial magnetic stimulation

SD- Standard deviation

SC- Stellate cell

SICI- Short Intra Cortical Inhibition

SI- Stimulus Intensity

Sham- Placebo

TBS- Theta Burst Stimulation

tDCS- Transcranial direct current stimulation

TEP- Transcranial Magnetic Stimulation-Evoked Potentials

TMS- Transcranial Magnetic Stimulation

TMS-EEG-Transcranial magnetic stimulation combined with Electroencephalography

TS-Test Stimulus

USA- United States of America

VL-Ventro-lateral

μV-Microvolts

CHAPTER 1. LITERATURE REVIEW

There are a number of neurological diseases that are refractive to current treatment regimes. The motivation of this study was to explore an alterative therapy that has been around for a number of years, to investigate whether using this approach could highlight new evidence that could be applied as a potential treatment for these conditions.

Transcranial Magnetic Stimulation (TMS) is a non-invasive technique that is used as a diagnostic tool for a number of neurological conditions and repetitive TMS (rTMS) has been used as a form of treatment (Curra et al., 2002; Kobayashi & Pascual-Leone, 2003). The central nervous system (CNS) is reliant on the balance of the excitatory and inhibitory circuits. Each neuron and synapse forms a complex system that mediates all aspects of interneuronal communication. Every motor and non-motor process is highly specialised and dependent on these neural circuits.

Neurotransmitters and cellular receptors interact to facilitate and determine the balance between these excitatory and inhibitory networks. This process is controlled by ion channels controlling the flow of ions or by intracellular connections through secondary mechanisms. Inhibition is primarily mediated by the action of γ-aminobutyric acid (GABA) on the GABA-A and GABA-B receptors and excitation is facilitated by glutamate action on N-methyl-D-aspartate receptor (NMDA) and non-NMDA receptors (Masson, Sagne, Hamon, & Mestikawy, 1999).

Disruption in these circuits can result in altered excitability as seen in a number of neurological conditions, including epilepsy, stroke, amyotrophic lateral sclerosis (ALS), dystonia, Huntington disease, Parkinsonian disorders, cerebellar diseases, and tremor. Drug therapy has been applied to treat this imbalance in a number of conditions. Antiepileptic drugs (AED) have been administered to treat the changes associated with cortical excitability in epilepsy, although there are a number of patients who are refractive to this approach, which has initiated the search for alternative therapies.

Recently there has been increased interest in the application of rTMS as a therapeutic treatment for refractive epilepsy**.** Clinically, rTMS has demonstrated promising results in a few studies, although the clinical efficacy to treat the altered cortical excitability in epilepsy has yet to be determined (Brighina, Daniele, Piazza, Giglia, & Fierro, 2006; Fregni et al., 2006).

1.1. TMS as a method for assessing cortical excitability

The use of TMS is based on the principle of electromagnetic induction. This is produced by passing a high electrical current through a coil of wire placed against the scalp, to produce a small electrical current in the underlying cerebral cortex, and is non invasive and pain free (Hallett, 2000) (Figure 1.1).

There are three main types of TMS:

Single pulse TMS involves single pulses, usually applied to the motor cortex to activate the corticospinal tract and elicit a motor evoked potential (MEP), recorded over muscle groups contralateral to the site of stimulation. MEP are used in diagnosis; to measure conduction times in the CNS, especially in diagnosis of multiple sclerosis (Sahota et al., 2005; Rossini, 1998). They are also useful to assess cortical excitability: the amplitude of the motor response reflects the net effect of the excitatory and inhibitory networks in the cortex. When a single pulse is administered to the motor cortex, the lowest TMS stimulus intensity required to produce this response is referred to as the motor threshold (MT). This measure has been also described as a reflection of excitability of the corticospinal neurons and those interneurons that synapse on the corticospinal neurons of the motor cortex. MEP can be recorded while the muscles are at rest (resting MEP) or while contracting (active MEP). Following active MEP there is a brief period of muscle silence (suppression of the electromyogram), which is referred to as the cortical silent period (CSP). The CSP has been shown to reflect intracortical inhibition. (Kobayashi & Pascual-Leone, 2003)(Figure 1.2).

Paired pulse TMS is another useful technique to examine the cortical excitability and inhibitory circuits of the motor cortex. This type of stimulation involves applying a sub threshold conditioning stimulus (CS), which is then followed by a supra-threshold test stimulus (TS). The inter-stimulus interval (ISI) is of importance, as it determines the level of inhibition or facilitation. MEP amplitude is reduced if the CS is 1-4ms before the TS, and this phenomenon is referred to as short interval intracortical inhibition (SICI). Long interval intracortical inhibition (LICI) is demonstrated by a long ISI (50-200ms), which causes a reduction in the MEP amplitude. If the ISI is between 7 and 20ms, the MEP amplitude is increased, also known as intracortical facilitation (ICF). It has been proposed that the excitatory postsynaptic potentials (EPSPs) that transmit glutamate are where this facilitation originates and in comparison inhibition is thought to be due to the action GABA as a neurotransmitter (Kobayashi & Pascual-Leone, 2003).

The third type of TMS is *repetitive TMS*, which is proposed as a form of therapy and has been shown to modify cortical excitability in a number of studies (Fierro et al., 2007; Helfrich et al., 2012). The application of this therapy will be discussed in a later section.

The interaction between TMS and intracortical inhibition has been explored in a number of studies, and there is evidence that the CSP is affected by the intensity of TMS and reflects intracortical inhibition (ICI). There is evidence that the CSP shortened with SICI, pharmacological research has suggested that it is mediate by GABA-A (Kojima et al., 2013). Administration of GABA-B receptor agonists such as baclofen increased CSP and LICI. These studies have contributed to a better understanding of cortical excitability and intracortical inhibition (Tremblay et al., 2013).

TMS has been used to investigate changes in MEP, CSP, ICF, SICI and LICI in a number of neurological conditions. For example, TMS has been used in dystonia, which is a movement disorder, the precise mechanism of which is unknown. TMS has been used to show that patients with dystonia involving the hand and facial muscles have a shortened CSP (Filipovic, Rothwell, & Bhatia, 2010). These findings are consistent with evidence that suggests there is a decrease in GABA-ergic inhibition in patients with dystonia (Siebner, 1999). The introduction of TMS and paired pulse paradigms has provided an insight into this and a number of other neurological conditions.

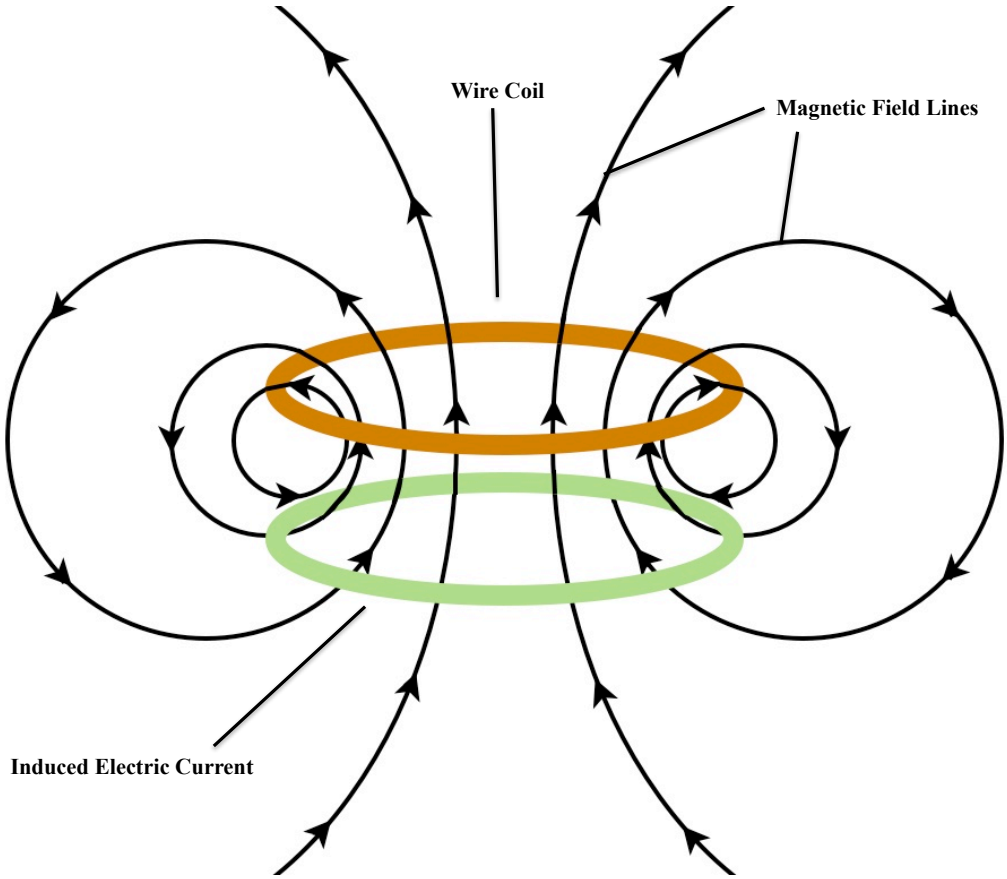


Figure 1.1. Induced currents in the brain by the TMS coil. A demonstration of the induced currents in the brain and the direction of current flow following the application of the magnetic coil (Diagram by A Harrington and D Kingma, 2014).

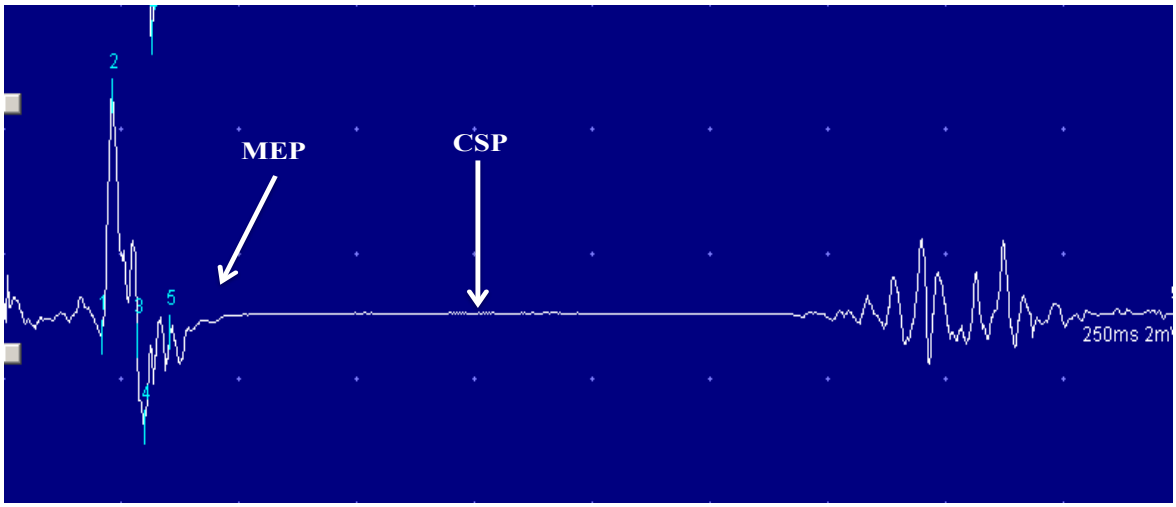


Figure 1.2. MEP following TMS over the left motor cortex of a healthy control. The arrows indicate the MEP and the CSP. The measurement of the CSP onset and offset times can vary between investigators; the onset in some studies is recorded from the beginning of the MEP onset, TMS onset or the appearance of the TMS artifact. Similarly differences occur with the offset measurement (Rabago, Lancaster, Narayana, Zhang, & Fox, 2009). For this study we measured CSP as the time interval from the stimulus to the reappearance of the EMG activity.

1.2. TMS-EEG as a method for assessing cortical excitability

Assessing cortical excitability through MEP is an effective tool although this method is limited to the motor cortex. When investigating cortical excitability in neurological conditions there is a need to examine other areas of the cortex where the disease may originate or spread. In order to assess cortical excitability in other parts of the brain, TMS combined with EEG (TMS-EEG) has demonstrated promise (Fitzgerald, 2010). TMS-evoked potentials (TEP) have been used as an index of cortical excitability in a number of studies.

Several components have been demonstrated using single-pulse TMS in the motor cortex; for example the N15 (EEG component that is a negative peak at approximately 15ms post-stimulus), P30 (EEG component that is positive, 30ms post-stimulus) and others such as N45, P60, N100, and P200 (Ilmoniemi & Kicic, 2010). These components can vary between individual subjects depending on whether they are patients or healthy controls and the experimental setup (Kicic, 2009). It has been demonstrated that the N100 component of the TEP is a reflection of cortical inhibition. The N100 is a negative trough that occurs approximately 100msec following the TMS stimulus (Figure 1.3) (Ilmoniemi & Kicic, 2010).

The amplitude of N100 and the CSP duration displayed a significant correlation, supporting previous evidence that the N100 relates to inhibition (Kimiskidis, Kugiumtzis, Papagiannopoulos, & Vlaikidis, 2013). As previously described there is a relationship between CSP and LICI and intracortical inhibition (Tremblay et al., 2013). Studies have shown a relationship between that the N100 component and LICI, and it has been suggested that is mediated by GABA-B inhibition. CSP measure is limited to surface EMG where as N100 is associated with activation of inhibitory cortical circuits (Rogasch, Daskalakis, & Fitzgerald, 2013).

A previous study investigated TMS-evoked EEG potentials in attention deficit hyperactivity disorder (ADHD) children to identify a marker that could be applied to real-time monitoring of the rTMS effect. In this particular trial 1Hz rTMS was applied to the primary motor cortex of children with ADHD and the TMS evoked N100 potential was studied. Following 1Hz rTMS the N100 potential was reduced and there was no reduction after sham stimulation. The results also showed that the N100 potential was a more sensitive marker than rTMS-induced changes in MEP amplitude. The authors of the study suggested that based on these findings the N100 component is a suitable marker of intracortical inhibition (Helfrich et al., 2012)

In contrast, a recent study has investigated the effects of rTMS on the TEP in the primary motor cortex of healthy controls. Following 1Hz there was a significant increase in the P60 and N100 components of the TEP. These results are in line with previous studies that have also demonstrated that there is a relationship between the N100 component and cortical inhibition, therefore making it a reliable marker (Bonnard, 2009; Nikulin, Kicic, Kahkonen, & Ilmoniemi, 2003) (Casula et al., 2014).

EEG has the ability to measure the effects of TMS in the brain (Blinowska, 2006). The use of EEG combined with TMS has excellent sensitivity in non-motor areas with increased spatiotemporal specificity, which allows the study of interhemispheric connections as well as cortical excitability (Komssi, Kahkonen, & Ilmoniemi, 2004)

The reproducibility of TEP was highlighted in the study conducted by Lioumis and colleagues (2009). TMS was applied at varying intensities over the participants' left motor and prefrontal cortices, and repeated at one-week intervals. The authors concluded that responses for both left and prefrontal cortex cortical stimulation areas were highly reproducible, and a reliable means of investigating cortical excitability (Lioumis, Kicic, Savolainen, Makela, & Kahkonen, 2009).

TMS-EEG has limitations. It is designed to record activity from the cerebral cortex but includes unwanted electrical activity known as artifact. Artifact can arise from within the patient (physiologic) such as eye blink or from an external source (extraphysiologic), which can come from equipment such as the TMS coil. The main artifact associated with combining TMS with EEG is due to the large electromagnetic pulses; TMS induces a large artifact within the EEG recording. There are ways to limit the artifacts when recording, including careful preparation of the electrodes to ensure that impendences are low (<5Ω) during recording (Ilmoniemi & Kicic, 2010).

Independent component analysis (ICA) has been used to remove artifacts from the EEG recording. ICA is capable of extracting independent sources from a mixed signal. It can solve the "cocktail party problem" where the speech of a single speaker needs to be separated from the background noise and is very effective in separating EEG signals and artifacts of different origin (Hyvarinen & Oja, 2000) (Figure 1.3).

Recent literature has been published to support the importance of removing artifacts using ICA. This study additional highlights the challenges faced when coming TMS-EGG when investigating cortical networks. The study applied ICA as a tool to remove these artifacts in order to assess TMS evoked changes in the dorsolateral prefrontal cortex (DLPFC). The results demonstrated that once the artifacts where removed using ICA the TEPs measure such as the N100, P60 could be identified. Overall, this provides evidence that ICA is a fundamental analysis tool that enables a contaminated TMS-EEG recording to be extracted for components without severely impacting the neural activity recorded (Rogasch et al., 2014).

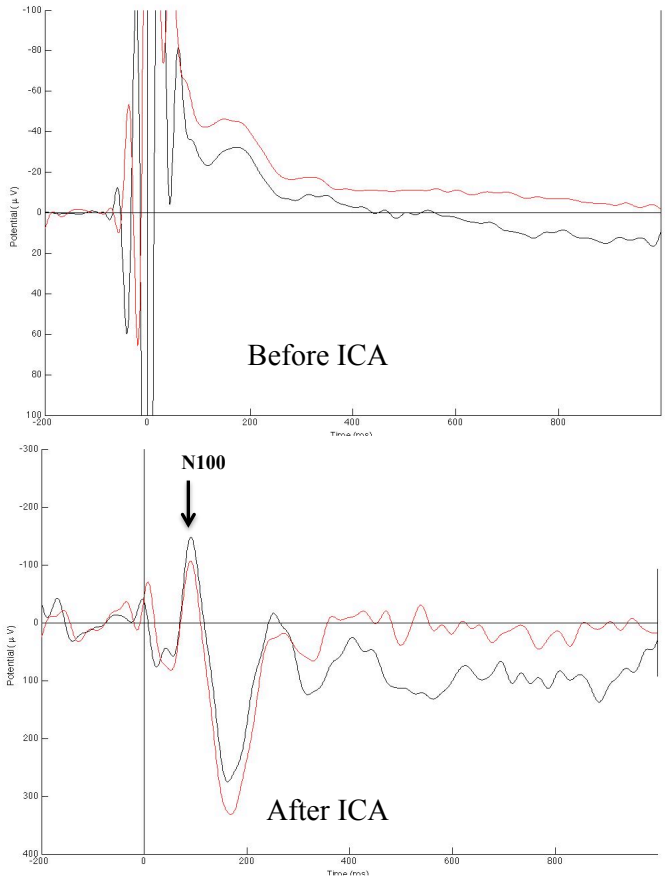


Figure 1.3 TMS-evoked potential (TEP) before and after ICA. The arrow indicates the N100 component. The top image displays the TEP before ICA, the image shows the large TMS artifact and the tail of the artifact hides the components. The bottom image demonstrates the TEP following ICA; it has removed a significant amount of the artifact, making it simpler to see the TMS components required for analysis.

1.3. Abnormal cortical excitability in disease

Epilepsy is a common neurological condition, defined as the occurrence of recurrent seizures. Seizures are divided into two main types: generalised and partial seizures. Generalised seizures are characterised electroencephalographically as having onset of paroxysmal activity over the entire cortex, while partial (also referred to as local/focal) seizures originate in a localised part of the cortex. Epilepsy is a complex disease with multiple mechanisms, but in most cases is thought to be caused by an

imbalance between the excitatory and inhibitory networks in the CNS (Valentin et al., 2008). There are a number of processes such as transmitter uptake, change in receptor function, extracellular ion homeostasis that can be altered, resulting in the imbalances which result in epilepsy (Badawy, Freestone, Lai, & Cook, 2012).

It has been proposed that epilepsy is characterized by a net increase in cortical excitability. A key point to understand about the epileptic brain is that it exists in a number of states. In a healthy individual there are a number of factors that influence the CNS to function normally. Neurons and the neural circuits mediate each and every action, behavior, emotion and response and are dependent on healthy excitatory and inhibitory circuits. Patients with epilepsy have ever changing states of cortical excitability (Badawy, Freestone, Lai, & Cook, 2012). The spontaneity of seizure occurrence is thought to occur because of the shift between the interictal (period between seizures) and ictal (state of seizure) states.

Some seizure types can be easily identified by the anatomy that they arise from, such as temporal lobe seizures, although studies have identified the complexity of the mechanisms associated with this condition. Seizure initiation has been investigated in numerous animal and human studies, which suggest that the epileptogenic foci (which refers to the alteration of normal neuronal network into a hyperexcitable state) play a role in loss of inhibition (Depaulis, 1994). This results in the neighboring neurons being excited, which facilitates spread of the seizure activity. The imbalance between the excitatory and inhibitory networks leads to the hyperexcitable states reported in the cortical motor areas in drug resistant patients. Hyperexcitable states occur when there is an increase in synaptic neurotransmission or a decrease in inhibitory neurotransmission (Lopes da Silva et al., 2003; Badawy et al., 2012).

The MT has been investigated in patients with generalised and focal epilepsy. There is evidence that patients with partial epilepsy on AED, showed an increase in the MT when compared to the controls (Tataroglu, Ozkiziltan, & Baklan, 2004). This increase was also demonstrated in patients with juvenile myoclonic epilepsy (JME), who were also on AED (Akgun et al., 2009).

In some subsets of patients with JME there was a decrease in MT (Brigo et al., 2012; Manganotti, Bongiovanni, Zanette, & Fiaschi, 2000). Additional studies have investigated patients with untreated generalised epilepsy and showed that their MT were lowered when compared to control subjects (Reutens., 1992,1993) (Table1.1). Cortical MT have been described as a reflection of the excitatory and inhibitory activity of the motor cortex. If individuals with epilepsy have an increase in cortical excitability, it would be expected that their MT be decreased suggesting a reduction in intracortical inhibition. There are a number of discrepancies between these studies as shown in Table 1.1. One explanation is that in the studies where there was an increase in MT, the patients were on AED. AED may act to increase the pathological low MT that would be expected in epileptic patients. The AED act to suppress seizures by modulating membrane excitability or by modifying excitability or inhibitory synapses. Another explanation is that each of these studies was using different TMS protocols that could have led to the variability in the results.

The CSP is another measure that is of interest because it has been described as a measure of inhibition. This measure has been explored in patients with both types of epilepsy and the results are variable; some studies demonstrated that the CSP was prolonged and others suggested that it was shortened in focal epilepsy on the affected side (Table 1.1). Findings with regard to ICI, ICF, SICI and LICI are also shown in Table 1.1. There is evidence that indicates that patients with JME have a decrease in early ICI in both hemispheres compared to controls and there was no difference in ICF (Manganotti et al., 2000), whereas other studies have demonstrated no change in this measure.

Similarly to the MT there is evidence to suggest that AEDs can affect ICI, although the effect does not appear to be as great. ICI and LICI have been identified as measures of intracortical inhibition and it has been suggested that they reflect the GABA-ergic mechanisms.

Studies have demonstrated a reduction in LICI in patients with both generalised and focal epilepsy, irrespective of drug therapy (Badawy, Curatolo, Newton, Berkovic, & Macdonell, 2006; Badawy, Curatolo, Newton, Berkovic, & Macdonell, 2007; Badawy, Macdonell, Berkovic, Newton, & Jackson, 2010).

A reduced LICI is expected as it indicates a reduction in inhibition in these epileptic patients. Overall the findings continue to add to the understanding of how complex epilepsy is and display that there are a number of challenges faced when targeting abnormal cortical excitability.

Imbalance between the excitatory and inhibitory circuits has also been proposed as a possible mechanism in other neurological conditions. Amyotrophic lateral sclerosis (ALS, also referred to as a motor neuron disease) is characterised by rapidly progressive muscle atrophy. The mechanism of motor neuron degeneration remains elusive, although altered cortical excitability has also been proposed as a cause (Bruijn et al., 1997) MEP findings have been variable. It has been reported that patients with ALS have a reduced MT and CSP, which supports the cortical hyperexcitability theory (Caramia et al., 1991; Triggs et al., 1999). Studies also showed a decreased SICI in ALS, which has been proposed as the cause of degeneration in the intracortical inhibitory circuits (Vucic, Cheah, & Kiernan, 2009)

A number of neurological conditions involve abnormal cortical excitability, which has become an increasingly popular concept that is beginning to extend the understanding of these complex conditions and suggests that the symptoms could be treated with the appropriate measures to restore homeostasis.

TMS measure	Generalised Epilepsy	Focal Epilepsy
Motor Threshold	• Decreased RMT in patients with JME (Brigo et al., 2012) • Mean MT lower in patients with IGE compared to controls (Macdonell, 2001). • Lower in untreated patients when compared to control (Reutens & Berkovic, 1992; Reutens, Berkovic, Macdonell, & Bladin, 1993). • RMT higher in patients with JME (Akgun et al., 2009) • Decrease in MT in JME patients without treatment (Manganotti et al., 2000).	• Increased MT in patients with partial epilepsy (all subjects were on AEDs) when compared to controls (Tataroglu et al., 2004). • RMT increased in two patients with focal epilepsy in the group overall there was no significant difference (Werhahn, 2000)
Cortical Silent Period	• Increased at all stimulus intensities in patients with IGE (Macdonell, 2001) • Decrease in MT in JME patients without treatment (Manganotti et al., 2000). • Primary generalised patients had prolonged SP irrespective of the number of drugs administered (Tataroglu et al., 2004) • In patients with myoclonic epilepsy there was a prolonged CSP; duration of CSP was not altered with drugs (Tataroglu et al., 2004). • Longer CSP in JME (Akgun et al., 2009) • Patients with untreated IGE CSP was normal (Badawy et al., 2007; Klimpe et al., 2009)	• Increase in SP in patients with cryptogenic partial epilepsy (Cincotta, 1998) • In patients with focal motor epilepsy SP shortened on the affected side (Inghilleri, Mattia, Berardelli, & Manfredi, 1998). • Patients with focal epilepsy outside the motor cortex on medication, SP shortened in hemisphere with focus when compared to unaffected side (Hamer et al., 2005) • Unmedicated patients with extra-temporal and temporal lobe epilepsy had no changes in either hemisphere in SP when compared to controls (Badawy et al., 2007; Klimpe, Behrang-Nia, Bott, & Werhahn, 2009; Werhahn, 2000)

Intracortical inhibition and facilitation	• Decrease or even absence in early ICI in patients with JME (Manganotti et al., 2000). • No difference in ICF in JME (Manganotti et al., 2000). • • Untreated IGE and treated progressive myoclonic epilepsy showed an overall reduction in LICI (Badawy et al., 2007; Badawy et al., 2010) • Two treated patients had an increase in SICI (Caramia et al., 1996).	• Decrease ICF in the abnormal hemisphere of patients, normal ICI (Werhahn, 2000). • In the normal side decrease in ICI and only a slight decrease in ICF (Werhahn, 2000). • In patients with untreated focal epilepsy the affected hemisphere had a decrease in LICI (Badawy et al., 2006; Badawy et al., 2007; Badawy et al., 2010).

Table 1.1. Overview of intracortical TMS findings in epileptic patients. Each section summarises studies for each TMS measure, across the two types of epilepsy Abbreviations: RMT = resting motor threshold; MT = motor threshold; CSP = cortical silent period; JME = juvenile myoclonic epilepsy; IGE = idiopathic generalized epilepsy; AEDs = anti epileptic drugs; ICI = intracortical inhibition; ICF = intracortical facilitation; SICI = short-interval intracortical inhibition; LICI = long term intracortical inhibition; TMS = Transcranial magnetic stimulation; rTMS = repetitive Transcranial magnetic stimulation; EEG = electroencephalography

1.4. TMS as a treatment for disease

The brain has a remarkable ability to be 'plastic'- to develop and adapt. Studies have explored the role of synaptic plasticity in neurological conditions and the potential for modulation by rTMS. Long-term potentiation (LTP) and long term depression (LTD) are important phenomena that relate to changes in synaptic strength. LTP and LTD

have significance in synaptic plasticity, and have importance in clinical applications. LTP is defined as the long lasting enhancement of synaptic transmission between two neurons, where as LTD is the reduction in efficacy of the neuronal synapses (Bliss & Cook, 2011; Badawy et al., 2006).

The modulation of neuronal synapses by non-invasive stimulation is an emerging therapy and it has been previously applied successfully as a therapy for neuropsychiatric conditions. rTMS has been used as a neuromodulation technique. This is the application of trains of magnetic stimuli with the aim of modifying cortical plasticity rTMS pulses when applied to the brain are given at constant intensity, but the frequency of the stimulus can vary between 1-20s or more (Bliss & Cook, 2011). The frequency of the stimuli determines the level of excitation or inhibition that occurs in the neuronal circuitry within the cortex. It has been shown that a frequency greater than 5Hz (high frequency TMS) induces an excitatory effect whereas 1Hz or less (low frequency) induces an inhibitory effect (Kobayashi & Pascual-Leone, 2003).

rTMS was first reported as a successful treatment in depression. Patients that suffer from depression have a reduced level of activity in the left dorsolateral prefrontal cortex (DLPFC). Neuroimaging studies have shown that in the left DLPFC there is hypometabolism in depressed patients (Aleman, 2013; Fitzgerald et al., 2009). Therefore excitatory rTMS using a stimulation frequency of 5Hz or greater has been investigated as therapeutic approach in this region. Low frequency rTMS has been used to treat the right DLPFC on the basis that there may be an imbalance between the two prefrontal regions. The clinical trial on treatment-resistant depression supports that rTMS has antidepressant effect which was observed as a significant reduction in the Montgomery-Asberg Depression Rating Scale over the course of the trial. rTMS has been approved by the FDA as a treatment for depression in the USA (Fitzgerald & Daskalakis, 2011).

Subsequently a different rTMS protocol known as TBS was introduced. It requires lower intensity and less stimulation time to induce long lasting effects that can modulate cortical excitability in the motor cortex. Previous animal studies had demonstrated that, when applied over the motor cortex, TBS induced LTP (Hess & Donoghue, 1996).

Huang and co workers (2005) were the first to apply different patterns of TBS to the motor cortex to investigate neuroplasticity (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005).

There are two main TBS protocols:

Intermittent TBS (iTBS) has been shown to have a facilitatory effect, resulting in a transient increase in the MEP as well as an increase in short intracortical inhibition (SICI) (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). iTBS consists of theta frequency (usually 5Hz) bursts of 3 TMS stimuli (usually at 50Hz) in 2 s trains occurring every 10s.

In contrast, continuous TBS (cTBS) has been shown to decrease the SICI and intracortical facilitation (ICF) with suppression of the MEP amplitude (Huang et al., 2005). cTBS involves one continuous train of theta frequency bursts of 3 stimuli at 50Hz. In both iTBS and cTBS, 600 stimuli are usually applied (Huang et al., 2005; Koch et al., 2008).

Preliminary results suggest that TBS can be applied to neurological conditions such as Parkinson's disease and chronic stroke to alter cortical excitability (Carrillo et al., 2013; Koch et al., 2014). TBS displays a promising advantage over standard rTMS protocols.

The use of lower stimulus frequency, such as 30Hz has an advantage compared to 50Hz TBS because of its ability to be delivered at higher stimulation intensity, using stimulators with limited capability. Only a small number of studies have explored the application of 30Hz stimulation and its ability to alter cortical excitability in motor and non-motor regions in the cortex (Nyffeler, 2008; Wu, Shahana, Huddleston, & Gilbert, 2012).

Wu and colleagues (2012) investigated the motor cortex in 18 healthy adults to explore the effects of iTBS and cTBS at 30Hz frequency. The results showed an increase following iTBS in MEP amplitude whereas cTBS had the opposite effect. Overall the safety of 30Hz TBS was consistent with other TBS studies, and the direction of change observed were similar to what other studies had shown with 50Hz stimulation (Wu, Shahana, Huddleston, & Gilbert, 2012).

Previously we studied the effects of 1Hz rTMS and 30Hz iTBS on the motor cortex of healthy individuals and showed that there was a reduction in the N100 component with iTBS. This has provided evidence that there is potential for 30Hz TBS to modulate the cortical excitability, although other avenues need to be explored to determine the optimal stimulation area and parameter (unpublished).

Low frequency rTMS and cTBS have been shown to reduce the cortical excitability of the motor cortex (Chen et al., 1997). This particular effect of rTMS has been explored in a number of studies to inhibit the epileptic activity. The clinical efficacy of rTMS in epilepsy has been under debate due to the inconsistency in the results. Tergau and co-workers investigated the application of 0.33Hz rTMS in a small sample size of nine patients with focal intractable epilepsy. They demonstrated a reduction in the number of seizures and the severity of symptoms recorded in eight of the patients (Tergau, Naumann, Paulus, & Steinhoff, 1999). Additionally Cantello and colleagues (2007) studied a larger sample of drug resistant epileptic patients using the same low frequency stimulus of 0.3Hz in a 5-day cycle, which was applied at the resting motor threshold (RMT) of each patient. The results were variable and only one third of the patients displayed a decrease in the interictal EEG abnormalities. Even though these results were not significant there was evidence to suggest that the rTMS was having an effect and was a safe method (Cantello et al., 2007).

Further studies have explored low frequency rTMS in the patients with refractive epilepsy and abnormalities of cortical development. The stimulation parameter frequency of 0.9Hz was reported to have a tendency to reduce the number of seizures,

but this was not statistically significant (Kinoshita et al., 2005). Additional research has demonstrated that five consecutive low frequency rTMS sessions were able to cause a significant decrease in the number of seizures in these refractive patients. Interestingly, the cortical inhibition following the rTMS in these patients lasted for a duration of two months (Fregni et al., 2006).

Another study investigated epileptic patients with partial seizures to explore whether rTMS could reduce epileptic discharges (ED) in patients that had epilepsy controlled by AEDs and patients that were refractive to drug therapy. EEG was used to examine the TMS effect. The stimulation frequency varied between 0.3 and 15Hz and was applied in trains of 1-10 biphasic stimuli. The study explored the effect of rTMS treatment on the duration of the epileptic discharges (ED), and the duration of the ED was reduced following rTMS. Overall the study demonstrated that with frontal lobe epilepsy, ED in patients could be modulated using TMS. The study highlighted that the ability of rTMS to modulate the ED was through superficial stimulation and that patients with deep epileptogenic foci in areas such as the temporal lobe, may not be suitable for rTMS treatment and further research was required to enable a suitable 'deeper' stimulation paradigm (Kimiskidis et al., 2013).

The relationship between 1Hz rTMS and the N100 component as been recently investigated by Casula and co workers (2014) who applied 1Hz rTMS to the primary motor cortex of healthy controls. Following rTMS there was an increase in the P60 and N100 component. Overall this study has confirmed the reliability to the N100 in TEP as an indicator of cortical inhibition (Casula et al., 2014).

Although a number of these studies have demonstrated the potential for rTMS, including TBS to induce changes in the cortical excitability of patients with refractive epilepsy, the ability of rTMS to reduce seizures is variable, depending on the stimulation parameters (Cantello et al., 2007). These results warrant further investigation because, even though the effects of low frequency rTMS were variable, other methods such as TBS could have greater effect and require less stimulation time to induce effective results that could have a benefit for these patients.

1.5. Other therapeutic forms of brain stimulation

Alongside TMS there are other forms of brain stimulation that have been used as a treatment for neurological conditions. There are a number of studies that have provided evidence that the motor cortex excitability can be modulated by transcranial direct current stimulation (tDCS). tDCS involves placing electrodes on the scalp over the brain region of interest, and a constant low direct current is passed through the electrodes causing an intracerebral flow of current, which either decreases or increases neuronal excitability. There are three different types of tDCS, anodal (positive stimulation), which increases neuronal excitability, cathodal (negative stimulation), which decreases neuronal excitability and sham, which is designed to mimic the tDCS (Kuo, Paulus, & Nitsche, 2014).

Deep brain stimulation (DBS) is another stimulation method that has provided therapeutic benefit to patients with Parkinson disease, dystonia, and essential tremor. DBS involves surgically implanting electrodes in deep structures such as the globus pallidus and subthalamic nucleus. A brain 'pacemaker', implanted in the chest wall is connected to the electrodes that interfere with the neural activity at the specified brain site. Even though DBS is applied in some cases, the procedure is invasive, which continues to highlight the demand for alternative therapies such as TMS which is non invasive. DBS however is now part of standard treatment for Parkinson's disease, whereas the use of rTMS in neurological disorders remains experimental (Limousin & Martinez-Torres, 2008).

1.6. Cerebellar stimulation as a therapy

The application of TMS to the cerebral cortex has been highlighted in previous studies as an effective way to modulate cortical excitability. For a number of neurological conditions such as epilepsy, the source of the problem may be outside the motor cortex or involve extensive portions of the cerebral cortex. The cerebellum has extensive connections with the cerebral cortex through direct and indirect pathways through which cerebellar stimulation could potentially modulate cortical excitability in a variety of cortical areas.

Previously the cerebellum was described as simply a motor structure, because damage to it resulted in impaired motor function (Holmes, 1917, 1939). Its primary motor function is to modify the cortical output to the descending motor pathway to enable the movements to be accurately executed and adapted. The cerebellum is essential for producing postural adjustment to stabilise and organise balance, fine movement coordination, equilibrium and muscle tone. However the cerebellum has extensive involvement in non-motor functions as well (Strick, Dum, & Fiez, 2009).

The ability of the cerebellum to modulate non-motor regions has been demonstrated in a number of studies (Strick, Dum, & Fiez, 2009) (Kelly, 2003; Schmahmann, 1996). It is remarkable that the cerebellum can contribute to such a diverse range of behavior from the execution of motor control to learning, language, emotion, working memory and pain. The cerebellum has a significant number of output projections to these non-motor regions including the pre frontal cortex and the posterior parietal cortex.

The cerebellum consists of two symmetrical hemispheres separated by a midline structure, the vermis. The cerebellar cortex contains almost all the neurons in this structure, and the deep cerebellar nuclei (DCN) constitute the main output component of the cerebellum. There are a number of nuclei that comprise the DCN. The dentate nucleus is the largest of these, and receives input from the lateral cerebellar hemisphere (Voogd, 1998).

The cerebellar cortex contains three main layers of cells. The inner layer of granule cells receives input from the mossy fibers and projects to the middle layer of Purkinje cells. The Purkinje cell layer is the only layer of the cerebellar cortex that has inhibitory projections to the dentate nucleus. The outer layer (molecular layer) contains the dendrites from the Purkinje cells and the axons from the granule cells (Figure 1.4) (Voogd, 1998).

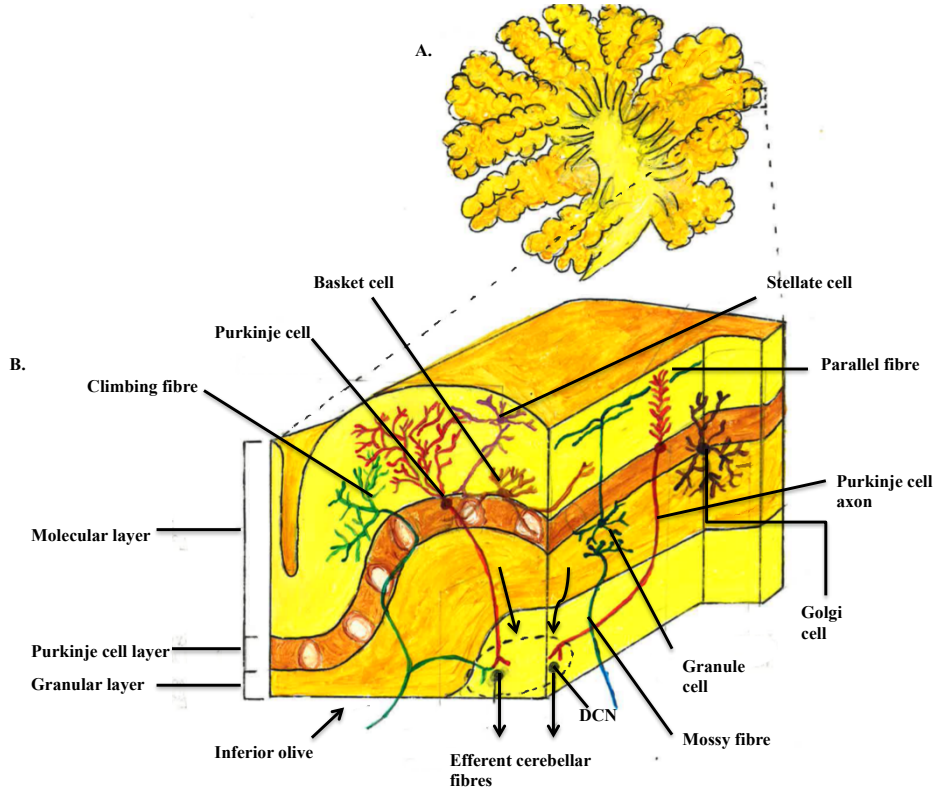


Figure 1.4. Diagrammatic representation of cerebellar cortical structure. **A.** Midsagittal cross-section of the human cerebellum. **B.** Cut away section of an individual cerebellar cortical lobule, which demonstrates the microstructure organization of the cerebellar cortex. The cerebellar cortex has three cellular layers and the positioning of the cells and their input and output are illustrated here (Diagram by A. Harrington, 2014).

The two main types of afferent fibers are mossy fibers and parallel fibers, which receive input from other parts of the CNS. Mossy fibers are extensively positioned throughout the cerebellar cortex and have excitatory synapses on the Purkinje cells. The cerebello-thalamo-cortical pathway is of particular importance because of its potential to modulate the motor cortex through stimulation of the cerebellum. This pathway has been extensively investigated in a number of stimulation studies to establish an understanding of the relationship the cerebellum has with non-motor and motor regions in the cerebral cortex(Fierro et al., 2007; Koch et al., 2008; Oliveri, Koch, Torriero, & Caltagirone, 2005).

The Purkinje cell axons project to the DCN and have inhibitory synapses on cells in the DCN. The efferent pathway from the dentate nucleus exerts an excitatory effect on the ventrolateral thalamus. The ventrolateral nucleus has a number of efferent pathways to the motor cortex, which terminate at both excitatory and inhibitory neurons. The cerebellum has the potential to modulate motor function via this pathway (Figure 1.5).

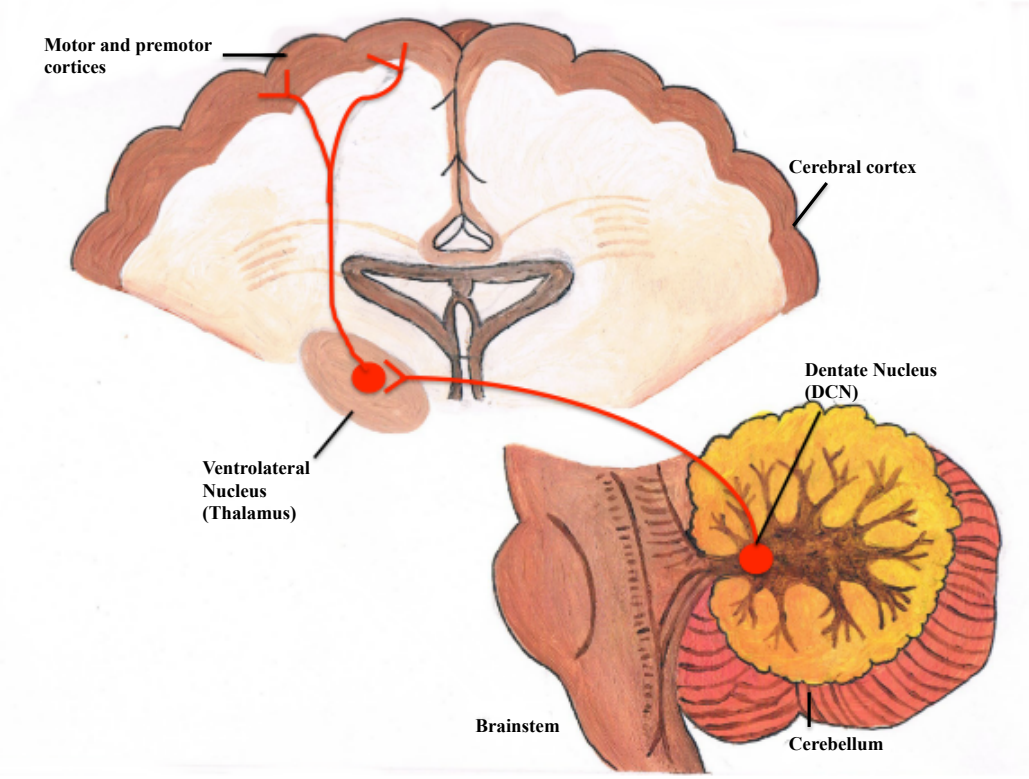


Figure 1.5. Diagrammatic representation of the dentato-thalamo-cortical pathway. The red lines indicate the pathway from the dentate nucleus of the cerebellum to the motor cortex, through the ventrolateral nucleus of the thalamus (Diagram by A.Harrington, 2014).

A number of animal studies have explored the excitatory and inhibitory cerebellar projections. Activation of non-pyramidal and pyramidal neurons in the motor cortex was shown following the stimulation of the cerebellar structures such as the DCN in the cat model (Noda & Yamamoto, 1984). Further studies explored the

microstimulation of the cerebellar nuclei of a conscious monkey; the results highlighted a pattern of both facilitation and inhibition in the motor cortex (Holderfer, 2000). These animal studies have demonstrated that there is connectivity between the cerebellum and areas of the cortex. This has provided a basis for exploration of cerebellar connectivity in human studies.

The first study to demonstrate the reduction in motor cortex cortical excitability following single pulse stimulation of the cerebellum was by Ugawa and colleagues (1995). The study investigated the effects of the cerebello-thalamo-cortical pathway on the motor cortex by applying a conditioning stimulus (CS) (either electrical or magnetic stimulation) to the cerebellum followed by a test stimulus (TS) to the motor cortex. There was suppression of MEP evoked by the TMS, which is believed to be due to an activation of the Purkinje cells, which resulted inhibition of cells in the dentate nucleus and thus reduced tonic cerebral cortical facilitation via the dentato-thalamic-cortical pathway. This inhibitory process has also been referred to as cerebellar inhibition (CBI) (Ugawa et al.,1995). Under normal conditions there appears to be a tonic excitatory activity in the dentato-thalamo-cortical pathway, which is interrupted by cerebellar stimulation, despite the presence of both excitatory and inhibitory fibers demonstrated in animal studies.

The effects of cerebellar tDCS on the primary motor cortex have been explored by Galea and colleagues (2009). This study investigated the CBI that is mediated by the dentate-thalamo-cortical connections. tDCS was applied to the right hemisphere of the cerebellum to assess the changes in the motor cortex, CBI and the brainstem. The different stimulation groups were anodal, cathodal and sham. There was a decrease in the CBI following cathodal tDCS whereas the opposite effect was observed following anodal stimulation. The cathodal decrease of the CBI was dependent on stimulation intensity and lasted up to 30 minutes. Overall the study demonstrated the effect of cerebellar tDCS on the corticospinal excitability and its potential for treating patients with motor and cerebellar dysfunction (Galea, Jayaram, Ajagbe, & Celnik, 2009).

Another study that investigated the circuitry between the cerebellum and the motor cortex using TMS was carried out by Daskalakis and colleagues (2004).

The experiments explored the effect of cerebellar stimulation on the intracortical networks as measured by paired pulse stimulation of the motor cortex. Increasing the TS intensity led to an increase in SICI and a decrease in all other measures. In the presence of CBI, SICI was reduced whereas ICF increased. Finally, there was a decrease in CBI and LICI when there was an increase in the TS intensity, suggesting that there are a number of similarities between these two measures. Even though both CBI and LICI appear at low intensities to inhibit the same neurons, their duration of activation appear to be mediated by different mechanisms (Daskalakis et al., 2004). The overall findings were variable, suggesting that there were both excitatory and inhibitory changes that occurred in the motor cortex following the stimulation of the cerebellum, in keeping with animal studies (Holderfer, 2000; Noda & Yamamoto, 1984).

rTMS has been applied to the cerebellum in healthy controls, and studies have demonstrated that the application of rTMS can produce long lasting modulation of the corticospinal excitability (Minks, 2010). Low-frequency 1Hz rTMS applied to the right cerebellar hemisphere decreased ICF at 10 ms ISI for up to 20 minutes to the contralateral motor cortex (Fierro et al., 2007). In contrast, Oliveri and colleagues (2005) found that cerebellar 1Hz rTMS increased ICF at 15ms for up to 30 minutes. There was no change in the CSP following cerebellar rTMS (Oliveri et al., 2005).

The application of TBS to the cerebellum to modulate intracortical circuits of the motor cortex in healthy subjects was investigated by Koch and colleagues (2008). They applied cTBS and iTBS to the cerebellum and examined the SICI, LICI and the MEP amplitudes before and after TBS. Following cTBS there was a decrease in the MEP amplitude using stimulus intensities of 80% and 90% of AMT, with an additional reduction of SICI (3ms) and an increase in LICI. iTBS at 80% produced an increase in MEP amplitude and a decrease in ICF and LICI (100ms). The modulation of LICI and SICI could reflect modulation of the GABA circuits. Even though the results were variable, they demonstrate that TBS applied to the cerebellum can modulate the cerebello-thalamo-cortical pathways and have a facilitatory or inhibitory effect on the motor cortex (Koch et al., 2008).

Subsequently Popa and colleagues (2010) studied the effects of 1Hz rTMS, cTBS and iTBS on CBI. cTBS and 1Hz rTMS over the right cerebellum exerted a significant suppression of CBI to the contralateral motor cortex for up to 30 minutes. This effect was not observed following iTBS. The study suggests that the intracortical inhibition observed over the contralateral motor cortex is due to transynaptic activation of the Purkinje cells and the parallel fibers in the cerebellar cortex. The activation of the Purkinje cells results in inhibition of the dentate nucleus, and the dentate-thalamo-cortical connections, which facilitate cortical activity under normal conditions (Popa, Russo, & Meunier, 2010).

A number of studies have explored the behavioral effects of rTMS on the cerebellum (Colnaghi et al., 2011; Fierro et al., 2007; Gerschlager, Christensen, & Bestmann; Rothwell, 2002). Cerebellar TMS has been demonstrated to have an effect on the perception of time, memory and the motor system. rTMS has been used in studies to investigate the performance of finger tapping, which was combined with auditory and visual cues following cerebellar stimulation. The results suggest that rTMS interfered with the intracortical neural networks, by increasing the variability in the finger tapping and changed excitability in a localised region of the cerebellum. This further identified the important behavioral relationship that the cerebellum has with neural processing (Del Olmo, Cheeran, Koch, & Rothwell, 2007). TMS has been applied to examine time perception, which is important in motor and cognitive functions. The cerebellum has an important role in timing and 1Hz rTMS impaired the subjects' perception of time when it was applied over the left cerebellum. The Purkinje cells have a crucial role in LTD and it has been suggested these cells are also important in learning dependent timing. The authors proposed that 1Hz rTMS to the cerebellum interfered with the physiological activity of these Purkinje cells to induce inhibition, similar to LTD (Koch et al., 2007).

The cerebellum's involvement in non-motor systems has been regularly explored throughout the literature. The ability for the cerebellum to be modulated by TMS and the impact that this has on other non-motor areas is integral to the treatment potential for a number of neurological conditions.

The role of the cerebellum as a target to abort seizures has been explored in animal studies. Cooke and Sinder (1955) demonstrated that surface electrical stimulation over the cerebellum in a cat model could abort seizures (Cooke & Sinder, 1955). This was also observed in a cat seizure model in a study conducted by Hutton and co workers (1972). The cerebellar vermis and dentate nucleus was stimulated, resulting in seizure inhibition (Hutton, Frost, & Foster, 1972).

One of the first studies to apply electrical cerebellum stimulation in patients with refractive epilepsy was Cooper and colleagues (1976). Initially the cerebellar cortex was stimulated using implanted electrodes with high frequency electrical stimulation, which produced seizure reduction in patients with drug resistant epilepsy. The inhibitory effects that were demonstrated during these studies have been attributed to the effect of the Purkinje cells on the deep cerebellar nuclei, and the cerebello-thalamo-cortical pathway (Cooper, Amin, Riklan, Waltz, & Poon, 1976).

The above literature suggests that electrical stimulation of the cerebellum aborts seizures, although the exact mechanism behind this process still remains unclear. While direct electrical stimulation of the cerebellum is a promising treatment, it is limited and requires surgery. On the other hand, the application of a non-invasive, relatively pain free treatment such as TMS could be advantageous in treating epilepsy (Fountas, Kapsalaki, & Hadjigeorgiou, 2010).

Cerebellar stimulation was explored in the treatment of refractive patients with multi-focal epilepsy (Brighina, Daniele, Piazza, Giglia, & Fierro, 2006). The study used high frequency cerebellar rTMS that was applied contralateral to the epileptic focus, and each patient underwent 20 sessions in which 5Hz rTMS was applied in two trains of 50 stimuli that were separated by a 50s interval. The study concluded that whether the patients had a single or multiple epileptic foci, rTMS over the cerebellum significantly reduced the patient's seizure frequency. The study's findings were limited due to small sample size and some participants displayed little reduction in seizures during the post-TMS phase. Although this study suggested a beneficial effect and provides evidence that this treatment has potential, this requires further research.

Cerebellar stimulation has also been investigated in other conditions. Recently, Brusa and co workers (2012) investigated cTBS of the cerebellum and its ability to reduce levodopa induced dyskinesia (LID) in patients with Parkinson disease. The study confirmed that there was a reduction of LID following weak bilateral cerebellum cTBS. The study further addressed whether the cTBS effect was due to changes in other interconnected brain areas or whether it was due to a direct modulation of the cerebellum (Brusa et al., 2012).

1.7. Aims and objectives

The aim of the present study was to investigate the effects of 30Hz cerebellar TBS on the contralateral motor cortex, using single pulse TMS and the combination of TMS and EEG to assess cortical excitability in healthy volunteers. Although previous studies have investigated MEP and paired pulse measures of cortical excitability following cerebellar rTMS and TBS, this has not been investigated using TMS-EEG.
Our hypotheses were:

a) That 30Hz TBS applied to the cerebellum would have similar effects on MEP amplitude to 50Hz TBS. ie. iTBS will increase MEP amplitude, and cTBS will reduce MEP amplitudes obtained from the contralateral motor cortex.

b) That changes in the N100 waveform of the TEP would reflect changes in intracortical inhibition. Ie. iTBS would decrease and cTBS would increase the N100 amplitude.

CHAPTER 2. METHODS

2.1 Participants

Sixteen healthy volunteers, nine male and seven female, aged 18-30 years, were recruited within the University of Otago by advertisement. Thirteen participants were right handed and three left-handed. The exclusion criteria were a medical history of neurological disorders, including epilepsy, and contraindications to transcranial magnetic stimulation: cardiac pacemakers, electronic implants, and metal aneurysm clips. The study received prior approval from the Central Regional Ethics Committee New Zealand and written informed consent was obtained from each participant.

2.2 Electromyography (EMG)

Surface EMG was recorded from the first dorsal interosseous (FDI) muscle of the right hand using 1cm diameter AgCl disc electrodes with the active electrode placed over the muscle belly, a reference electrode over the metacarpophalangeal joint of the forefinger and a ground electrode on the dorsum of the hand. The electrodes were connected to an electromyography system (Medelec Synergy). Motor evoked potentials (MEP) were recorded using a time base of 250 ms, a sensitivity of 5 mV per division, high pass filter of 3 Hz and low pass filter of 10 kHz.

2.3 Transcranial Magnetic Stimulation (TMS)

A Magstim Rapid^2 magnetic stimulator and air-cooled 70mm figure of eight coil was used for TMS. A Magstim placebo coil, which mimics the typical "click" of the genuine coil, but without the magnetic stimulation, was used as a control condition. The coil was positioned over the left motor cortex with the handle projecting posteriorly, at a 30-40 degree angle to the midsaggital line. The resting motor threshold (RMT) was defined as the lowest stimulus intensity required to produce a MEP of at least 50 microvolts (μV) in greater than 50% of trials, with the FDI fully relaxed. The active motor threshold (AMT) was defined as the lowest intensity required to produce MEP of at least 200 μV in greater than 50% of trials, while the participant exerted a 2 kg force on a pinch grip dynamometer (B&L pinch gauge).

Active and resting MEPs were ecorded using a stimulation intensity of 110% of RMT. MEP amplitudes were measured peak to peak, MEP latency was measured from the beginning of the stimulus to the start of the MEP and the cortical silent period was measured as the time from stimulus to the resumption of EMG activity.

2.4 Theta burst stimulation (TBS)

30Hz TBS was applied to the right cerebellum at an intensity of 80% for the first eight subjects and then at 90% for the last eight subjects. The point of stimulation was 3 cm lateral to the midline and 1 cm below the inion. The TMS coil was positioned vertically with the handle placed upwards. This position has been previously shown to optimize inhibition of the contralateral motor cortex (Ugawa et al., 1995; Werhahn et al., 1996). cTBS consisted of 30Hz stimulation, in 3-pulse bursts repeated every 200ms (5 bursts per second) to a total of 600 pulses. (Wu et al 2012) For iTBS two-second trains of TBS were repeated every 10 seconds for a total of 600 pulses. Sham TBS was applied using either the cTBS or iTBS pattern, but using the placebo coil. The iTBS and cTBS stimulation patterns were alternated between participants for the sham sessions.

2.5 Electroencephalography (EEG)

EEG was recorded using an EasyCap 32 channel electrode cap (Easycap GmbH, Herrsching, Germany) connected to a Synamps RT EEG system (Compumedics Neuroscan, Texas, USA). The electrodes were positioned on each individual's head according to the 10-20 electrode placement system. An additional electrode was placed below the eye, to identify blink artifact. Electrodes were referenced to an electrode placed on the vertex, posterior to the Cz electrode. Each electrode placement was preceded by cleaning the scalp with alcohol and NuPrep (skin prep gel). The impedances of the overall EEG cap was then checked to ensure that they were they were less than 5Ω. Neuroscan Acquire software was used to record the EEG in DC mode at a sampling rate of 20 kHz, with a high pass filter of 500 Hz. Timing of the magnetic pulses was relayed to the EEG system via a trigger signal from a Digitimer Neurolog System, which also triggered the Magstim Rapid^2 stimulator. The Neurolog System was, in turn, controlled by an E-Prime program, which produced a signal at pseudorandomised intervals ranging between 3 and 5

seconds. The EEG recordings were down-sampled to 1 kHz offline, using Neuroscan Edit software. They were then exported to EEGLAB (Delorme and Mackeig, 2004), a Matlab-based program (Mathworks®). Portions of the EEG containing excessive artifact were removed and pre- and post-TBS recordings were combined into one file for each session. Independent component analysis (ICA) was carried out using the EEGLAB 'runica' command. The EEG was then epoched from 200 ms prior to the TMS stimulus to 500 ms post-stimulus. The epochs were baseline corrected using the -200 to -50 ms pre-stimulus interval as the baseline. Components containing blink or TMS artifact (approximately 25% of components) were removed. The residual EEG was then processed using principal component analysis and further artifact-containing components were removed. The channel event related potentials were then extracted and averaged. A low pass filter of 30Hz (24 dB/Oct) was used for display purposes. The TMS-evoked potentials obtained before and after each TBS or sham treatment were averaged across subjects and inspected for differences. The N100 amplitude was calculated as the mean amplitude of the waveform between 90 and 110 ms after the magnetic stimulus. The N100 at C3 was used for statistical analysis as this was the electrode closest to the stimulating coil.

2.6 Experimental Design

The study involved two experiments. In experiment 1, the first eight participants received TBS at a stimulus intensity of 80% of AMT; in experiment 2, the second eight participants received TBS at 90% of AMT. Each participant underwent three sessions that were carried out at least one week apart. In each session one of the three TBS protocols was applied to the right cerebellar hemisphere: 30Hz iTBS, 30Hz cTBS or sham TBS. The order of the sessions was counterbalanced between participants. The participants were blinded to each session. Follow up questions were asked to ensure they were not aware of the treatment administered.

In each session, the electrode cap was applied and electrode impedances were checked. Electrodes were also applied to the right FDI, and the active and resting motor thresholds were determined. Six to eight resting MEP and a similar number

active MEP were obtained from the left motor cortex. The stimulation intensity that was applied over the cerebellum was calculated based on the subjects average resting MEP obtained from the left motor cortex. This was measured for every subject as the threshold can vary depending on the age and gender of each the subject, and factors like skull thickness. This is a commonly used method for determining stimulus intensities to be used for treatment. In some studies a correction has been used to compensate for the different depth of the cerebellum, but we did not have neuroimaging on the participants, so did not do this. The relatively small number of MEP was used because the experimental procedures were already quite long, EEG recording then commenced and each participant received 28-32 single pulses of TMS to the left motor cortex at a stimulus intensity of 90% RMT, at intervals of 3-5 seconds. 28-32 single pulses were used based on previous literature and our own previous experience.

One of the three TBS protocols was then applied to the right cerebellar hemisphere. After delay of two minutes a further 28-32 pulses of TMS were applied to the FDI 'hotspot' of the left motor cortex, while EEG was recorded. Finally, motor thresholds and MEP were again recorded 5-10 minutes after the end of TBS. Three participants in experiment 1 and six participants in experiment 2 underwent additional MEP recording at 20 and 30-minute after the end of the TBS treatment. This was performed in some of the later participants, when the procedures had become more efficient and less time had been required to set up the electrodes. Earlier in the study, the length of time taken was such that the experiment would have taken too long and exceeded what had been stated in the information sheet.

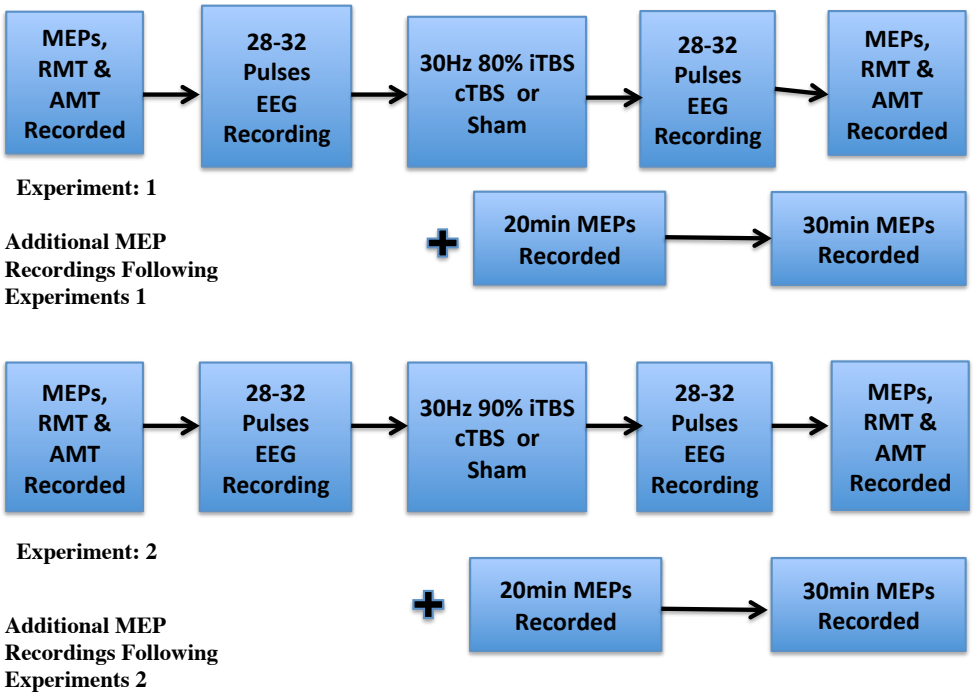


Figure 2.1. Schematic presentation of the experimental protocol. Experiments 1 and 2 differed in the stimulus intensity used for TBS treatment.



Figure 2.2. Setup for recording Motor Evoked Potentials and TMS Evoked Potentials. The figure of eight coil was placed on the participant's head over the left the motor cortex as described in the text. The 10-20 electrode cap was used to record the TEP.

2.7 Data Analysis

The MEP data from experiments 1 and 2 were analysed separately. The mean thresholds, amplitudes, latencies and cortical silent period, and N100 amplitudes before and after TBS were determined for each session. MEP amplitudes were measured peak to peak, MEP latency was measured from the beginning of the stimulus to the start of the MEP and the cortical silent period was measured as the time from stimulus to the resumption of EMG activity.

Analysis was carried out in SPSS statistics software, using a mixed model analysis of variance (ANOVA). Two methods were used: (a) In an initial repeated measures analysis, treatment type (iTBS, cTBS and sham TBS) and Time (Pre- or Post-treatment) were used as fixed effects factors, with subject as a random factor. (b) In the second analysis the dependent variable was the log of the post-treatment value for each parameter and Treatment Type and SI were fixed factors, with Subject and the log of the pre-treatment value as random factor and covariate respectively. In the case of the N100 amplitude, Electrode was used as an additional factor. Data was considered significant at $p<0.05$, two tailed.

CHAPTER 3. RESULTS

3.1 Motor Evoked Potentials

Appendix 1 shows the mean parameter values before and after TBS treatment for experiments 1 and 2.

Experiment 1: TBS at 80% AMT

There were a total of eight subjects in this experiment; four males and four females, with a mean age of 22 years (range 20-26years). Six of the subjects were right handed and two were left-handed. The procedure was well tolerated by all the subjects and all subjects completed all three sessions.

In the repeated measures mixed model analysis, there was an overall effect of Time: CSP was significantly larger after treatment, 188 (ms) compared to 178 (ms) pre-treatment $F(1,7) = 25.15$, $p = 0.001$. There were no other significant effects on MEP parameters.

With Regard to TEP, the appearance of the waveforms suggested an increase in the N100 amplitude at C3 following iTBS (Figure 3.3 and 3.4), while sham stimulation did not appear to produce any change in the waveform (Figure 3.6). For stimulation at 80% of RMT, mixed ANOVA with factors Treatment x Electrode, with pre-treatment N100 amplitude as a covariate revealed a significant effect on treatment on N100 amplitude, $F(2,348) = 197.80$, $p < 0.001$. Post-hoc analysis showed that values were significantly greater after iTBS than after Sham or cTBS $p < 0.05$ (Figure 3.7)

Experiment 2: TBS at 90% of AMT

This experiment included eight subjects: four males and four females, with a mean age of 22 years. One of the subjects was left-handed and all others were right handed. During this experiment, one participant withdrew from the iTBS session because of discomfort related to stimulation of cervical muscles. Two of the participants experienced slight discomfort in their cervical muscles following the higher intensity

stimulation, but completed all three sessions. An additional subject experienced neck spasm the night after the treatment, and one other subject experienced dizziness the day after their first session. It was uncertain whether this was due to the study procedures.

Otherwise, the procedures were well tolerated.

Using repeated measures ANOVA there was an effect of Time on resting MEP amplitude which was greater after treatment than before treatment, (1.543±248 vs. 1.161±118 mV), $F(1,60) = 5.53$, $p = 0.022$. Using the pre-treatment value as covariates, there was no significant effect of treatment type on active latency $F(2, 14) = 3.46$, p=0.06 or the active amplitude $F(2,9) = 3.98$, p=0.06. There was an effect of treatment type on resting amplitude, $F(2,14) = 4.28$, p=0.03. Resting amplitude was lower after cTBS than after sham TBS (Figure 3.1). There was also a significant effect of treatment type on CSP $F(2,13) = 4.87$, p=0.026. CSP was increased following iTBS, compared to sham (Fig. 3.2).

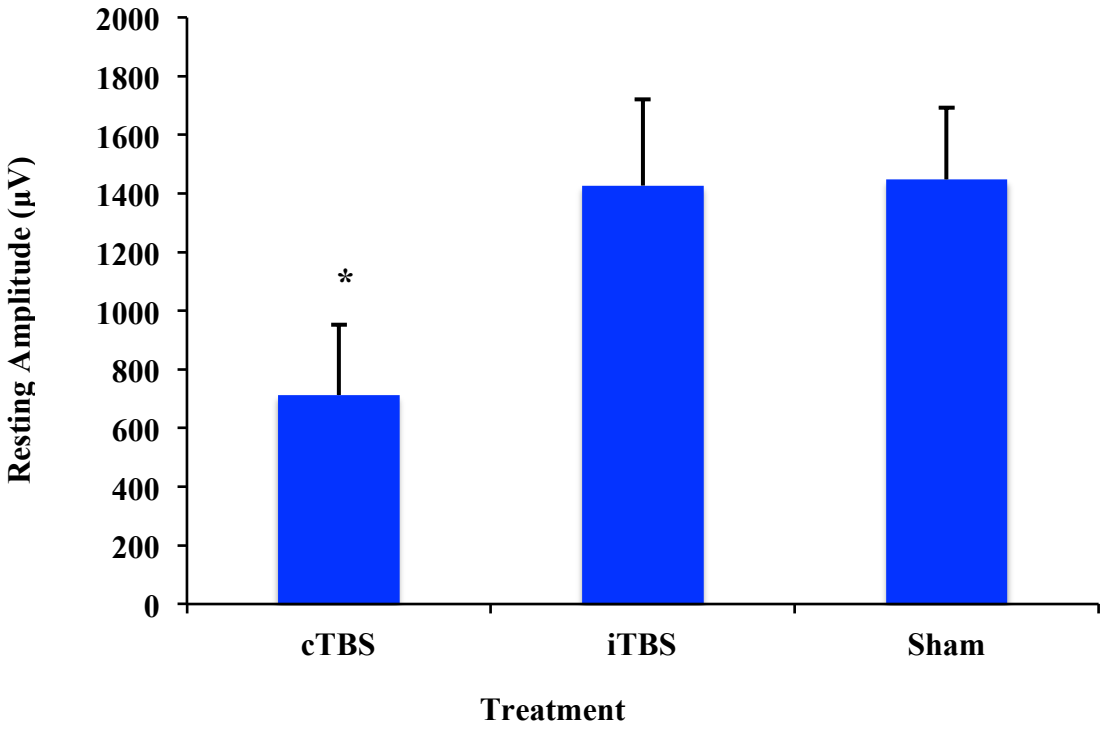


Figure 3.1. Effects of 90% cerebellar TBS on Resting MEP amplitude. Following cTBS the amplitude was less than after sham treatment. *p=0.03.

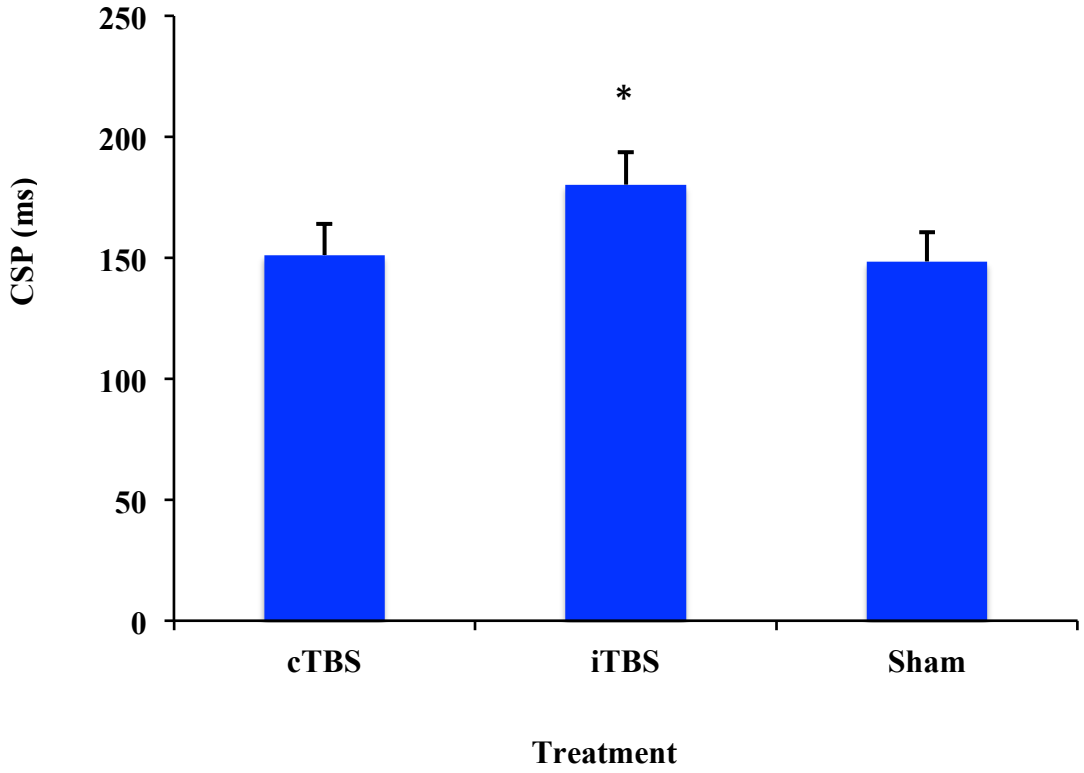


Figure. 3.2. Effects of 90% cerebellar TBS on CSP. Following iTBS there was an increase in the CSP compared to sham TBS. *p=0.026.

Mixed ANOVA with factors Treatment x Electrode, with pre-treatment N100 amplitude as a covariate revealed a significant effect of Treatment on N100 amplitude, $F(2,455) = 6.17$, $p = 0.002$. Post-hoc analysis showed that values were significantly greater after iTBS than after Sham or cTBS, $p < 0.05$ (Figure 3.7 and 3.8). There was no effect of Electrode, and the effects appeared to be widespread, rather than localized to a few electrodes, $F(26,452) = 0.66$, $p=0.90$ (Figure 3.9).

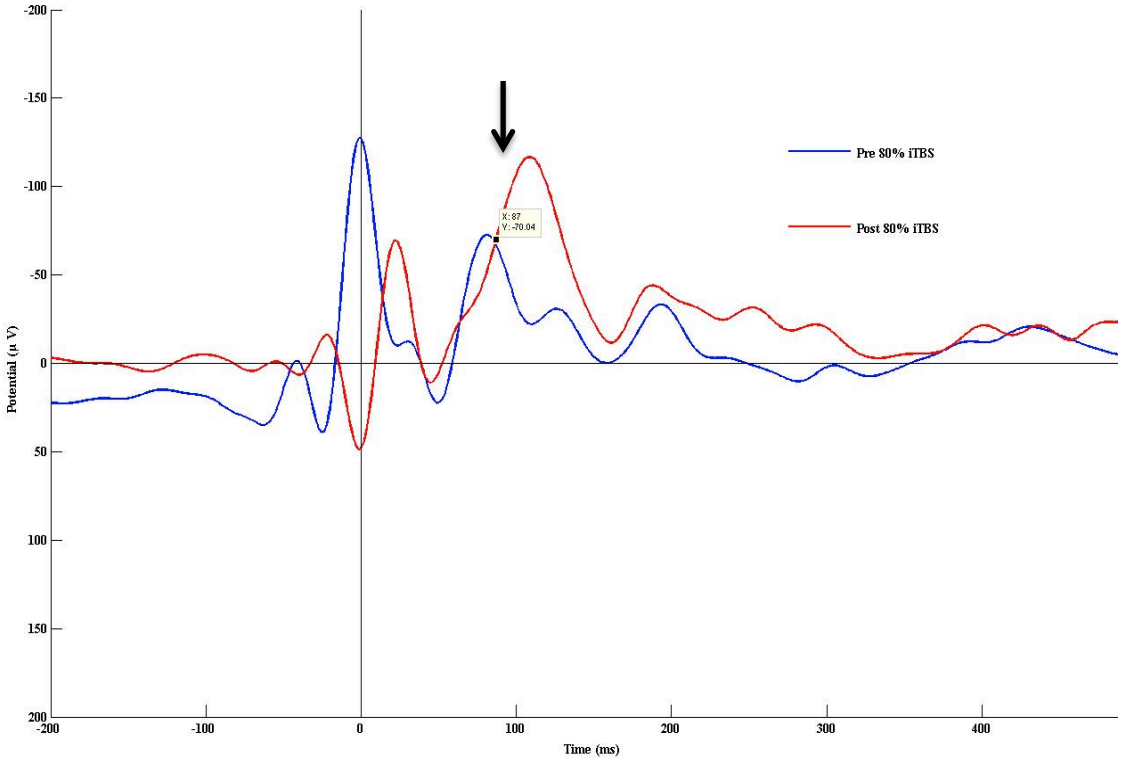


Figure 3.3. Effect of 80% cerebellar iTBS on the TMS evoked potential. The N100 potential was more prominent after treatment (red) than before (blue). The arrow indicates the position of N100 potential.

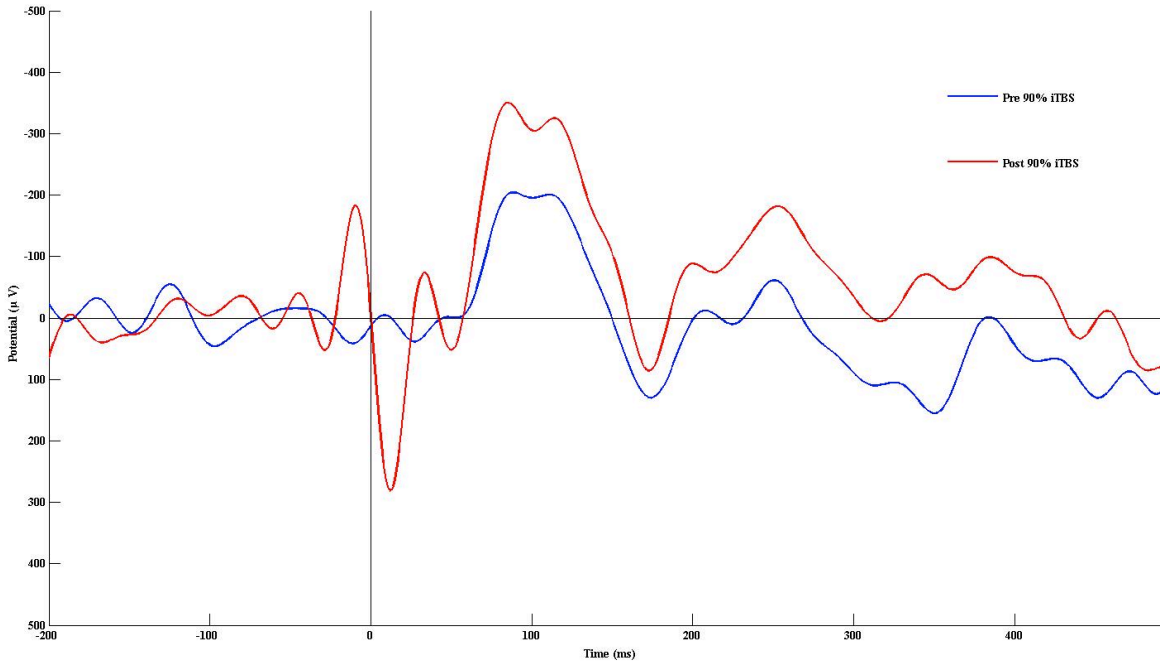


Figure 3.4. Effect of 90% cerebellar iTBS on the TMS evoked potential. The N100 potential was more prominent after treatment (red) than before (blue).

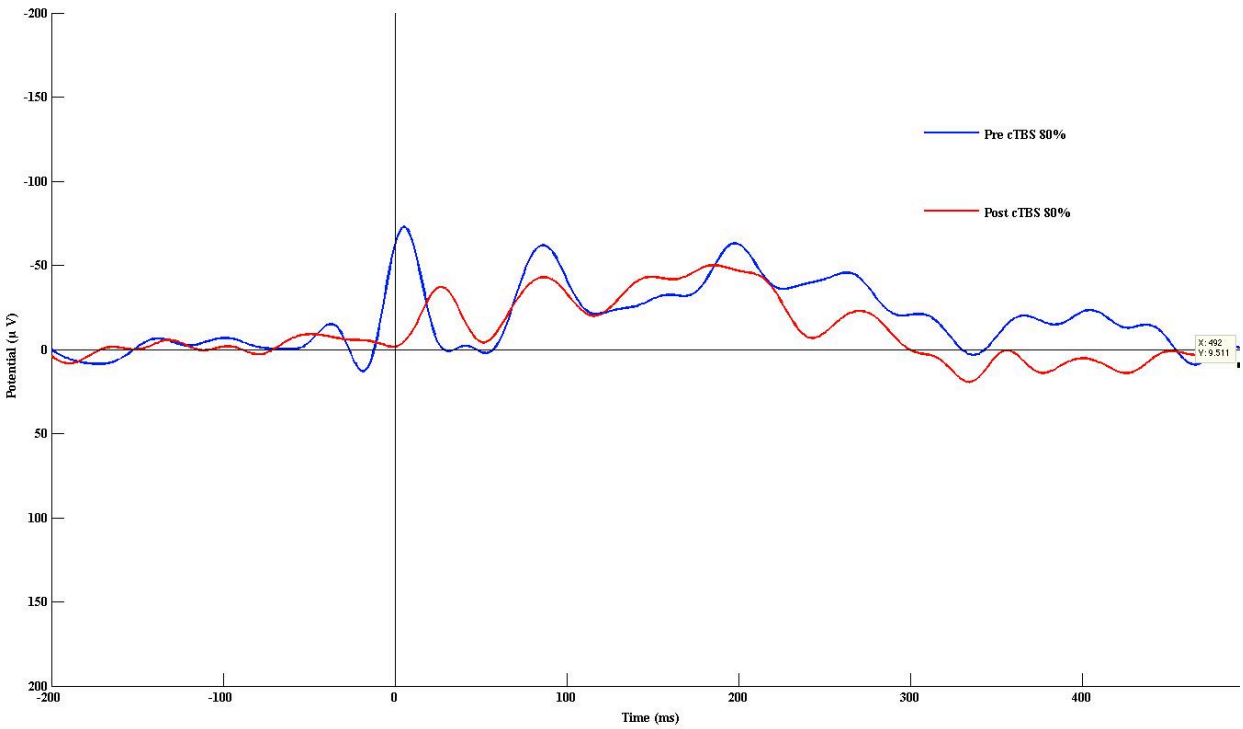


Figure 3.5. Effect of 80% cerebellar cTBS on the TMS evoked potential. The N100 potential appears to be less prominent after treatment (red) than before (blue).

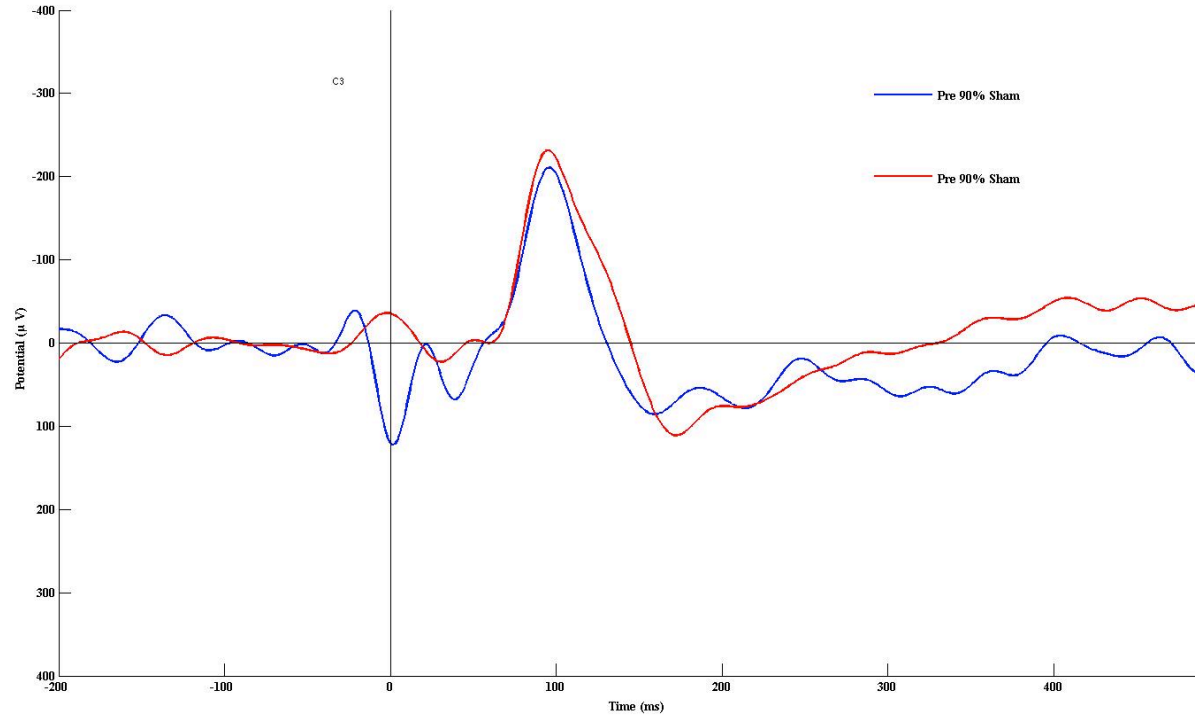


Figure 3.6. Effect of sham cerebellar TBS on the TMS evoked potential. The N100 potential was unaltered after treatment (red) compared to before treatment (blue).

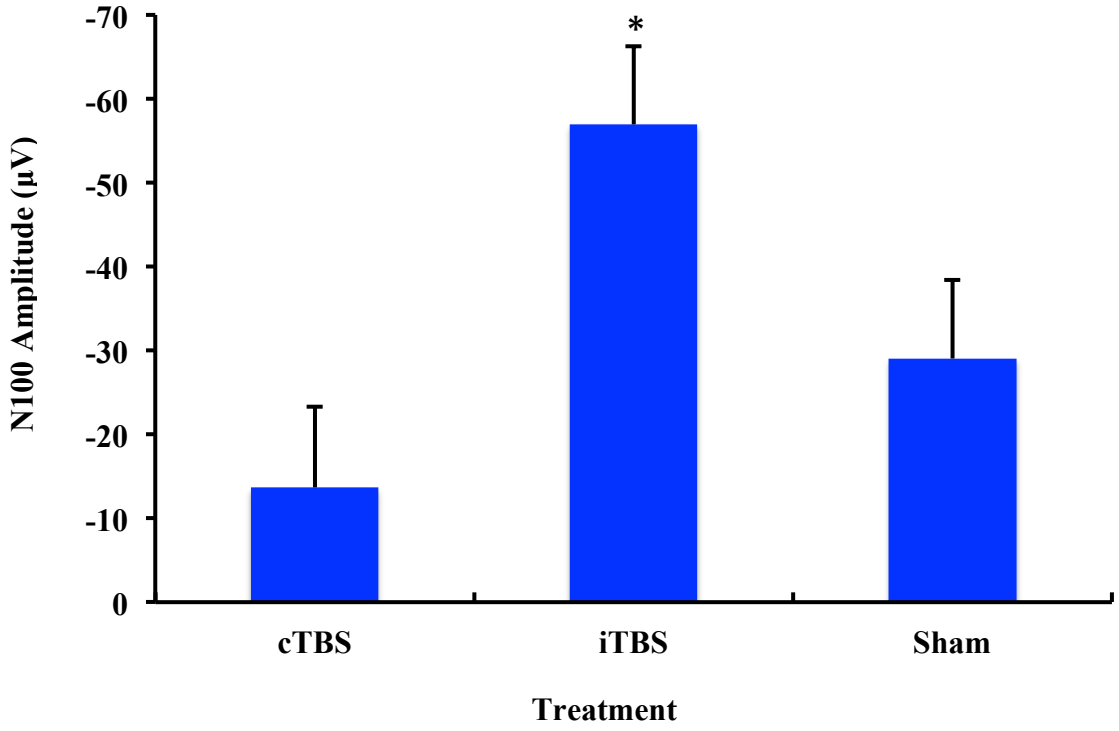


Figure 3.7. Effects of cTBS, iTBS and Sham on the N100 amplitude when applied over the lateral cerebellum at 80% RMT. Mean post-iTBS amplitude was significantly increased compared to Sham treatment. * p=0.002.

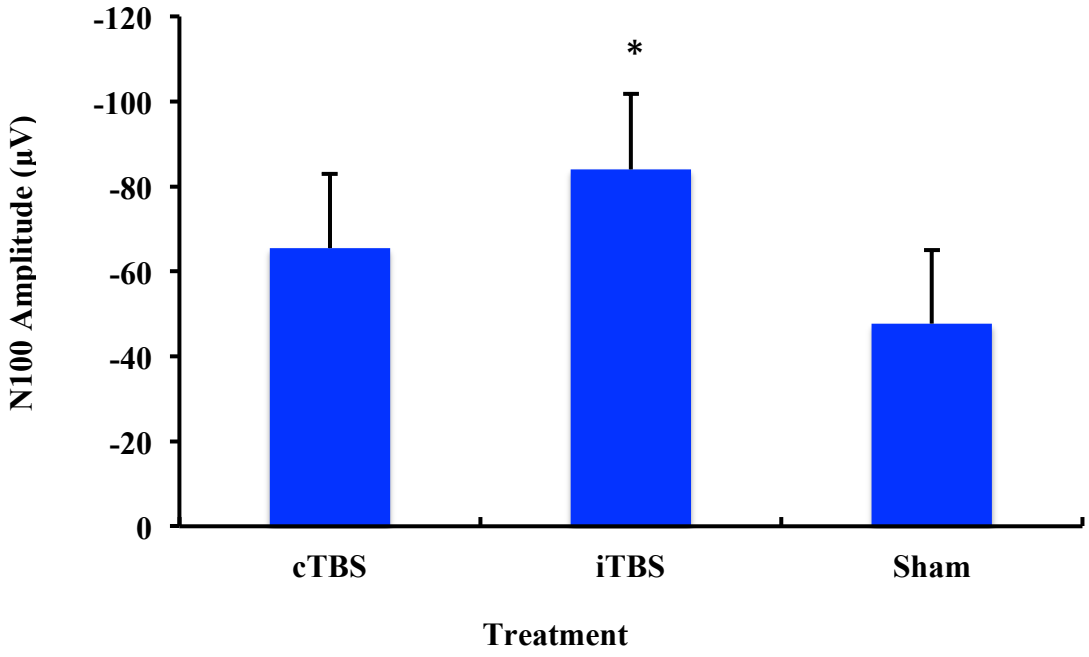


Figure 3.8. Effects of cTBS, iTBS and Sham on the N100 amplitude when applied over the lateral cerebellum at 90% RMT. Mean post-iTBS amplitude was significantly increased compared to Sham treatment. * p=0.002.

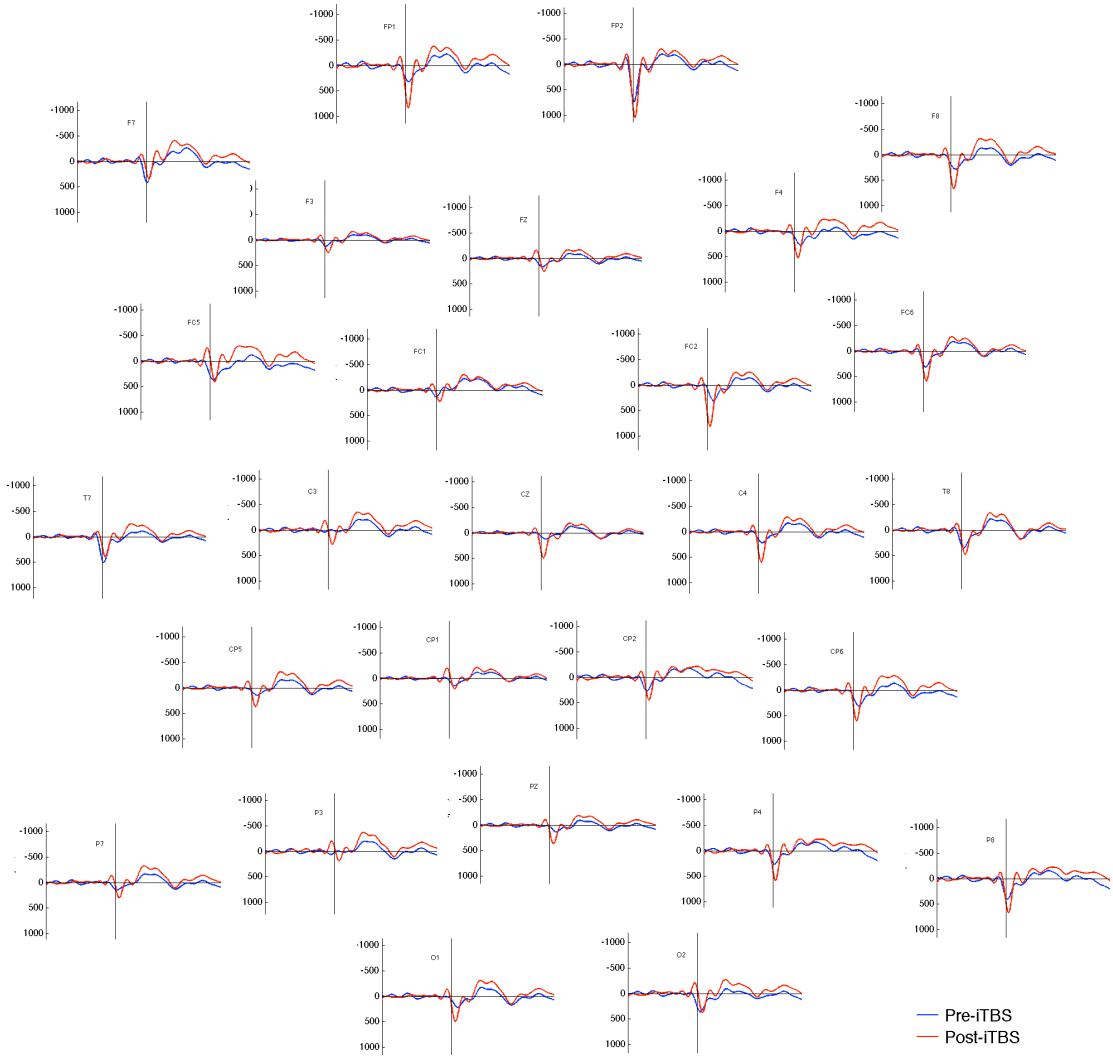


Figure 3.9. Effect of iTBS at 90% AMT on TMS-evoked potentials. EEG montage showing TMS-evoked potentials at all electrode sites. Pre-iTBS TMS evoked potential (blue) and post-TMS evoked potentials (red) are demonstrated. The N100 appears to be increased post-iTBS at most electrodes.

3.2 30 minute MEP results

30 minutes post stimulation there was no significant effect of treatment type on the CSP. There was a significant effect on active and resting MEP amplitudes following 90% iTBS. Active MEP amplitude remained elevated when compared to sham, $F(2,11) = 11.06$, p=0.05 (Figure 3.11). Resting amplitude at 30 minutes post iTBS possibly remained elevated, although this fell just short of significance, $F(2,11) = 11.52$, p = 0.05 (Figure 3.12). Thus, the effects on active and resting MEP amplitudes appeared to persist up to 30 minutes following TBS treatment, although the effect on CSP was no longer demonstrable.

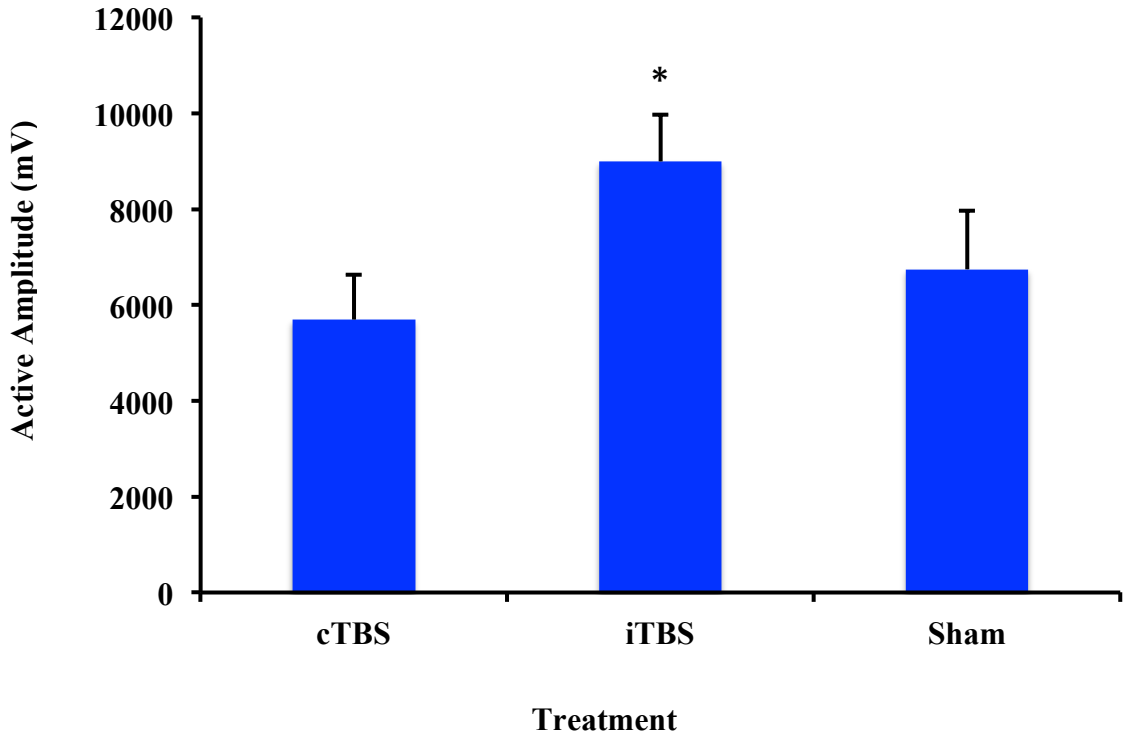


Figure 3.10 Effects of treatment on Active amplitude 30 minutes post stimulation. There was an increase following iTBS when compared to Sham. * p=0.05

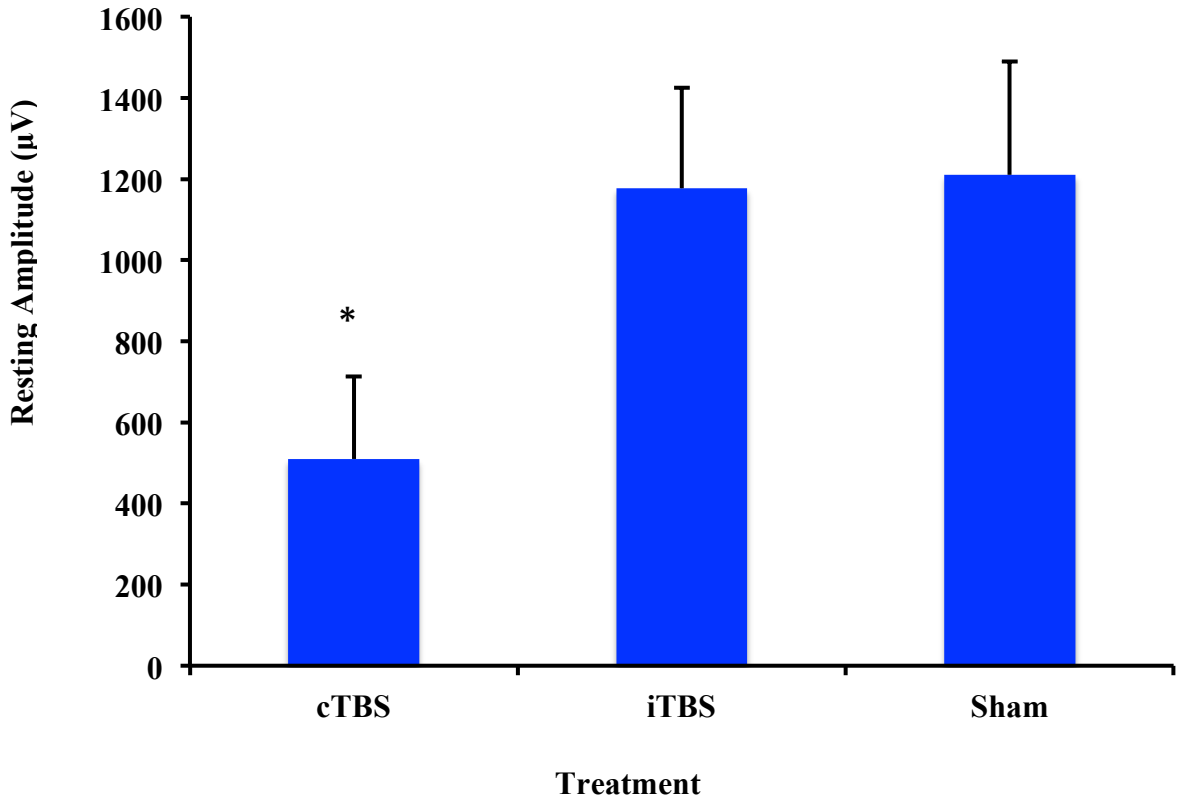


Figure 3.11 Effects of treatment on Resting amplitude 30 minutes post stimulation. There showed a decrease following cTBS when compared to Sham. * p=0.05

CHAPTER 5. DISCUSSION

In this study, we applied two different TBS protocols to the lateral cerebellum and demonstrated their effects on excitability in the contralateral human motor cortex, as compared with placebo. One protocol, iTBS, has previously been shown to produce corticospinal facilitation when applied directly to the motor cortex (Huang et al., 2005). The other, cTBS, has been shown to produce inhibition (Huang et al., 2005). In experiment 1, following iTBS and cTBS at 80% AMT there were no significant effects on MEP amplitude and CSP. In experiment 2, following cTBS at 90% AMT there was reduction in excitability in the contralateral motor cortex, compared to placebo, as demonstrated by reduced resting MEP amplitude. Following 90% iTBS there was increase in the CSP. Following both 80% and 90% iTBS there was an increase in the N100 potential. These results provide further evidence that cerebellar TBS can modulate excitability of the contralateral motor cortex, an effect mediated most likely via the cerebello-thalamo-cortical pathway.

4.1. Effect of cerebellar TBS on MEP

The MEP findings can be compared to those described by Koch *et al*. (2008) who demonstrated a reduction in resting MEP amplitude following 50Hz cTBS over the left lateral cerebellum using the same landmarks (1 cm below the inion and 3 cm lateral to midline) at 80 or 90% AMT and an increase in resting MEP amplitude following 50Hz iTBS at 80% AMT (iTBS at 90% AMT was not investigated). In contrast to our study, they also studied paired pulse measures of cortical excitability, and found a decrease in SICI at 3ms following cerebellar cTBS at 80 or 90% AMT and an increase in LICI at 100ms following cerebellar cTBS at 80% AMT.

Following 80% iTBS stimulation Koch *et al*. (2008) found a decrease in ICF and LICI at 100ms. It is likely that LICI and the CSP relate to the same inhibitory mechanisms and the decrease in LICI following iTBS in the Koch *et al*. (2008) study is therefore in contrast to our finding of prolonged CSP after iTBS at 90% AMT. Koch *et al*. (2008) did not study the CSP (Tremblay et al., 2013).

A possible explanation for the variation in these results obtained from each study could be due to the type of Magstim stimulator that was used. Koch et al. 2008 used a mono-phasic stimulator where the current study used a bi-phasic stimulator to record MEP it is possible that different neural circuits were activated by the magnetic fields. This contrast in findings warrants further research into the depth at which the stimulation occurs.

What is consistent between the two studies is that cerebellar cTBS decreased MEP amplitude. Measurements reflecting intracortical inhibition were less consistent, as our study suggested that iTBS caused increased inhibition (increased CSP and N100) while Koch *et al*. (2008) demonstrate decreased inhibition (LICI). It is likely that TBS modulates the intracortical circuits via the cerebello-thalamo-cortical pathway; this is because it is the main efferent pathway from the cerebellum to the cortex (Koch et al., 2008). A significant difference between our study and that of Koch *et al.* (2008) was that we used 30Hz rather than 50Hz TBS. Two studies have shown that 30Hz TBS produces similar neurophysiological effects to 50Hz when applied to the primary motor cortex (Wu et al., 2012; Gilbert, Shahana, Huddleston, & Wu, 2012). In a prior study, we demonstrated N100 effects using iTBS applied to the motor cortex (unpublished). However 30Hz TBS has not previously been reported in studies of non-invasive cerebellar stimulation. 30Hz stimulation has the advantage that it can be applied at adequate stimulus intensity using the Magstim Rapid^2 stimulator, whereas 50 Hz requires the Magstim Super Rapid^2 stimulator.

MEP amplitude is dependent on changes in the spinal motor neurons as well as the cortico-spinal neurons within the motor cortex; these changes can occur at a spinal and cortical level or a combination of the two. The effects of TBS are likely to occur at cortical level (Wassermann, 1998), however, and the amplitude of the MEP reflects the excitability of the motor cortex, which is determined by the balance between inhibition and facilitation (Day et al., 1989; Di Lazzaro, 2004). Without paired pulse TMS studies, it is difficult to determine whether an increase in the MEP amplitude is due to a decrease in inhibition or an increase in excitation or both, and conversely, a reduction in MEP amplitude could be due to a reduction in excitation or increased inhibition.

Variable findings are not unusual in TMS experiments. Like cTBS, 1Hz rTMS is reported to produce inhibitory effects when applied directly to the motor cortex. Previous 1Hz rTMS cerebellar studies have demonstrated variable results (Fierro et al., 2007; Gerschlager, Christensen, & Bestmann; Rothwell, 2002; Oliveri, Koch, Torriero, & Caltagirone, 2005).

Gerschlager *et al*. (2002) studied the effects of 600 stimuli at 1 Hz over the right cerebellum and showed a decrease in corticospinal excitability (decreased MEP amplitude) in the right arm. Fierro *et al*. (2007) studied SICI and ICF following 1 Hz rTMS to the right cerebellar hemisphere (900 pulses) and demonstrated a decrease in ICF in the left motor cortex. In contrast, Oliveri *et al*. (2005) carried out a similar experiment using 1Hz at a stimulus intensity of 90% of RMT and showed that ICF and MEP were *increased* in the contralateral motor cortex. CSP was unchanged. Studies of cerebellar TBS (ours and Koch *et al*.) appear to demonstrate that cTBS reduces MEP amplitude obtained from the contralateral motor cortex. The variability in these effects could be based again on the level of stimulation that occurs. The thickness of the participant's skull across all these experiments could vary significantly, additionally affecting the site of stimulation.

One further study provides evidence that cerebellar cTBS (600 stimuli) has the ability to effectively inhibit the cerebellar projections to the motor cortex. The study of Popa *et al*. (2010) showed that 1Hz rTMS (900 stimuli) and cTBS abolished the CBI, presumably because MEP amplitude cannot be reduced any further as it is already maximally inhibited. The study used similar stimulation location to our study, but the optimal position for stimulation was not based around manual measurements but was defined based on the help of magnetic resonance imaging (MRI) neuronavigation. Popa *et al*., (2010) studied the effects of 1Hz on the CBI paradigm, so they did not examine the effects on motor cortex excitability directly (Popa et al., 2010).

The relationship between CBI and LICI has been described by Daskalakis and colleagues (2004). CBI and LICI were both decreased as a result of increasing the TS, although the mechanism of action appears to be different as the time course of CBI (10ms) and LICI (100ms) are different. Interestingly CBI was reduced in the presence of LICI, which demonstrates that these measures may be activating subcortical sites. The study suggests that based on these results it is possible that at the level of the motor thalamus the inhibitory cerebellothalamic pathway is disrupted. Overall the study concludes that there is potential for excitatory and inhibitory changes in the motor cortex following cerebellar stimulation (Daskalakis et al., 2004).

The findings from the studies of cerebellar rTMS and TBS appear to be counter-intuitive. CBI as demonstrated originally by Ugawa *et al*. (1995) is thought to be due to excitation of the Purkinje cells, which results in inhibition of the dentate nucleus and the dentato-thalamo-cortical pathway. Why then should cTBS, which causes inhibition when applied to the cortex, cause excitation of the Purkinje cells rather than inhibition?

The distribution of cells in the cerebellar cortex is complex; the cellular structures stimulated could influence the effect on the cerebral cortex. We postulate that cTBS, which uses lower stimulation intensity than single pulse CBI experiments, may stimulate interneurons, such as the stellate and basket cells rather the Purkinje cells directly, and these may exert an inhibitory effect on the (deeper) Purkinje cells. This would explain why the present study as well as the study of Koch *et al*. (2008) demonstrated decreased MEP following cTBS.

Another issue not discussed so far is that there was evidence of an effect of time (pre- or post-stimulation) on CSP in experiment 1 and on resting MEP amplitude in experiment 2. It is possible that the methods we used to assess cortical excitability – MEP and TEP - themselves alter cortical excitability. A significant number of stimuli were applied in recording these measures, and although the stimulation frequency was generally low (3-7 seconds between stimuli) it is possible that these stimuli may have altered these parameters. One advantage of our study design is that we included a sham treatment, so that our findings were based on a comparison between active and

sham treatments the differences between real TBS and sham therefore due to TBS and not due to the procedures used to measure the effects of TBS.

Another concern in studies of cerebellar TMS is that the findings could have resulted from stimulation of the cervical nerve roots. This is an important issue to highlight as the nerves that innervate the spine, are close to the site of stimulation, as evidenced by occasional occurrence of neck twitching during stimulation. Previous studies have identified that single pulse stimulation over the cerebellum has both central and peripheral actions (Gerschlager et al., 2002). Studies using rTMS and TBS protocols have explored the same type of stimulation applied over the cervical roots. The findings suggested that it was unlikely that results were affected by stimulation of the brachial plexus (Popa et al., 2010). Thus it is doubtful that our findings were due to stimulation of the cervical nerves. However, a drawback of our study is that we did not formally explore this.

In addition to studying the immediate effects that cTBS and iTBS had on the cerebellum and the contralateral motor cortex, we explored the duration of the effect on the MEP, in a subset of participants. The findings showed that 30 minutes post-stimulation there was an apparent increase in the active amplitude after iTBS and a decrease in the resting amplitude following cTBS. Even though TBS appeared to be persistent or have delayed effects, it is difficult to draw further conclusions because the number of participants who had delayed testing is too small. We did not repeat the TEP, so the duration of those effects was not investigated. The duration of effect following TBS is important. Previous rTMS studies have confirmed that when applied over the motor cortex the MEP can be affected for several minutes after the end of the stimulation (Chen et al., 1997; Stefan, Kunesch, Cohen, Benecke, & Classen, 2000; Tsuji & Rothwell, 2002). Much longer lasting effects would be necessary for cerebellar TBS to be useful in treatment. Studies using rTMS in disease have demonstrated prolonged benefits in some conditions, usually by giving repeated treatments over a period of weeks.

4.2 Effects of TBS on TEP

MEP have provided useful measures of cortical excitability, although limited to the motor cortex. The combination of TMS and EEG provides an alternative method of assessing the effects of TMS in health and disease. Previous studies have shown that the N100, which is the negative waveform that occurs about 100 ms after the stimulus, is a measure of intracortical inhibition (Helfrich et al., 2012). There is recent additional evidence to confirm that the N100 is a reliable marker of cortical inhibition. Casula *et al*. (2014) demonstrated that following 1Hz rTMS to the primary cortex there was an increase in the P60 and N100 components and a decrease in the amplitude of the MEP (Casula et al., 2014). These findings are in contrast with our findings that following cerebellar iTBS there was an increase in the N100 amplitude in CSP both inhibitory markers. These findings were unexpected however, considering previous evidence that iTBS to the cerebellum results in increased MEP amplitude (Koch et al., 2008).

The explanation may be that iTBS activates both facilitatory and inhibitory networks, and the amplitude of the MEP depends on which process is dominant. There is evidence to suggest a correlation between the MEP amplitude and the CSP (Wehahn, 2007), although studies have been unable to reproduce similar correlation between the MEP amplitude and the N100 (Bender et al., 2005). The CSP and the N100 have both been described as a measure of inhibition and we found that iTBS increased both CSP and N100 amplitude (Rogasch, Daskalakis, & Fitzgerald, 2013; Nikulin, Kicic, Kahkonen, & Ilmoniemi, 2003).

We did not repeat the TEP after a delay, so the duration of the TEP effects was not investigated.

4.3 Mechanism of TBS effects on the Cerebellum

A number of animal and human studies have explored the cerebello-thalamo-cortical pathway and showed that the projections can terminate on either excitatory or inhibitory neurons. The axons of the Purkinje cells are the main inhibitory projection

to the dentate nucleus (which is the main output for the cerebellum), and projections from the dentate nucleus terminate in the thalamus, where a number of excitatory and inhibitory pathways project to the motor cortex. Depending on the location of stimulation and the layer of the cerebellar cortex activated the cortical effect could vary. However, single pulses to the cerebellum have generally been shown to reduce MEP elicited from the opposite motor cortex milliseconds later (Ugawa et al., 1995).

Previous studies that have used the paired pulse paradigm and rTMS suggest that stimulation of the cerebellum results in activation of this direct Purkinje output pathway causing either a reduced or increased amount of tonic facilitation of the contralateral motor cortex. We postulated that cTBS would have an inhibitory effect on the cerebellum (analogous to its inhibitory effect when applied to the motor cortex) and this would then result in excitation of the contralateral motor cortex, by disinhibiting the tonic facilitation maintained by the dentate nucleus. This would be consistent with the findings of Oliveri *et al*. (2005), using 1 Hz rTMS. However this was not demonstrated in the TBS study of Koch *et al*. (2008) or by the present study, where cTBS appeared to have an overall inhibitory effect on contralateral motor cortex excitability.

On the other hand, the stimulation intensities used in 1Hz TMS are higher than those used in TBS and it is possible that the results of stimulation are affected by stimulation intensity; higher intensities are likely to stimulate deeper levels of the cerebellar cortex. This may explain the contrary findings of Gerschlager *et al*. (2002) and Oliver *et al*. (2005).

In the study of Koch *et al*. (2008), iTBS had the opposite effect to cTBS, resulting in increased MEP amplitude obtained from the contralateral motor cortex. We did not demonstrate a significant effect of iTBS on MEP amplitude, but iTBS produced increased intracortical inhibition as shown by increased CSP and N100. The effect on excitability of the contralateral motor cortex is presumably mediated through the cerebello-thalamo-cortical pathway shown by the thick lines in Figure 4.1.

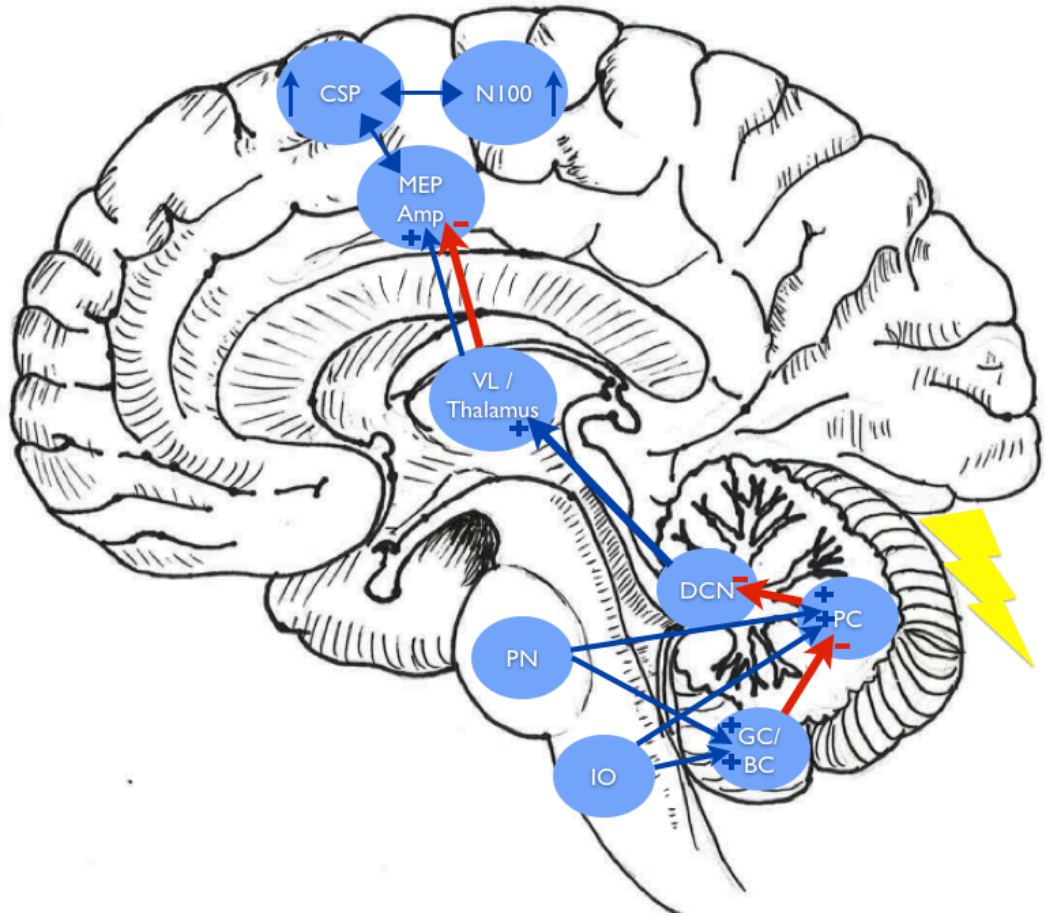


Figure 4.1. Cerebello-thalamo-cortical connections. The blue circles superimposed on a sagittal diagram of the brain each represent a population of neurons that mediates an inhibitory or excitatory process. The red lines represent inhibitory pathways whereas the blue lines represent facilitatory pathways. The thick lines represent the proposed cerebello-thalamic-cortical pathway as well as the inhibitory effect of the granule and basket cells on the Purkinje cells resulting in inhibition of the contralateral motor cortex. Thus excitation of granule and basket cells by iTBS would increase facilitation of the motor cortex, while cTBS would have the opposite effect GC = granule cells, PC = Purkinje cell, BC = basket cells, VL/Thalamus = Ventro-lateral thalamus, DCN = deep cerebellar nuclei, PN = Pontine nucleus, IO = Inferior olive (Diagram by Harrington., 2014).

The cerebellum is a very complex structure and structurally quite different from the motor cortex, so the effects of rTMS or TBS on the motor cortex are not necessarily the same in the cerebellar cortex. In the motor cortex iTBS appears to have a facilitatory effect and cTBS an inhibitory effect on pyramidal cells. In the context of the cerebellum the Purkinje cells are the main output to the dentate nucleus, and it is possible that cells other than the Purkinje cells could have been modified by TMS, with secondary effects on the Purkinje cells. For example, iTBS excitation of the granule cells or basket cells (directly or via the mossy fibers that come from the pontine nucleus via the pontocerebellar pathway) could cause inhibition of the Purkinje cells on which they synapse. This would in turn result in reduced (inhibitory) Purkinje output to the DCN causing more tonic facilitation of the contralateral motor cortex via the dentato-thalamo-cortical pathway (Figure 4.1.) It is also possible based on TMS protocols that at the level of the motor cortex, iTBS could be essentially an inhibitory pattern that acts on these inhibitory neurons directly.

Conversely, cTBS could be acting by inhibiting the cerebellar interneurons that synapse on the Purkinje cells, thus disinhibiting the Purkinje cells and decreasing the facilitatory effects of the dentate nucleus on the opposite motor cortex. This explanation, that it is the granule and basket cells that are primarily stimulated, seems credible, as they are more superficial than the Purkinje cells (see Figure 4.2). The cerebellum is not as close to the stimulating coil during TMS as the motor cortex and the stimulation intensity may very critical to the outcome measured. With higher stimulation intensity it is possible that direct stimulation of Purkinje cell could occur. This would reverse the effect compared to stimulation of the stellate cell and basket cell.

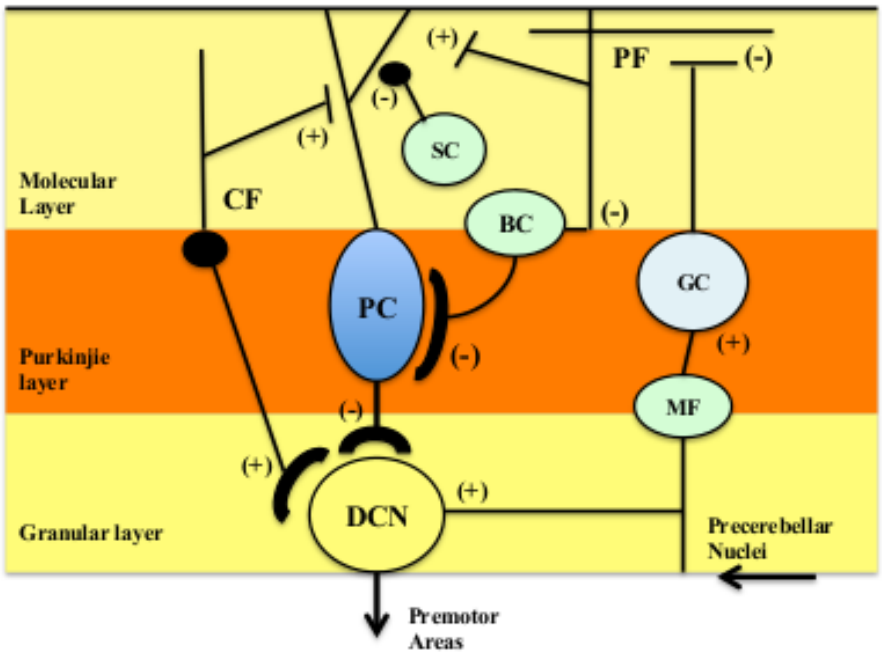


Figure 4.2. A basic illustration of the cortical layers of the cerebellum. The diagram shows the basic interaction of the cells within the cerebellum. Note that the stellate and basket cells have inhibitory synapse on the Purkinje cells. The plus and minus symbols represents the excitation (+) and inhibition (-) between each of the cells types. PC = Purkinje cell, SC = Stellate cell, GC = Granule cell, DCN = dentate cerebellar nucleus, MF = Mossy fibers, PF = Parallel fibers, BC = Basket cells, CF = Climbing fibers (Diagram by Harrington., 2014).

4.4. TBS as Treatment for Epilepsy and other Neurological Conditions

The current experimental findings and previous literature provides evidence that TBS has potential to be applied as a therapy for a number of neurological conditions. The ability to stimulate the cerebellum provides an alternative site for non-invasive brain stimulation as a means for altering cortical excitability in the motor cortex and other areas of the cortex. This may be useful in a number of conditions such as epilepsy and dystonia where the mechanism appears to be due to an increase in cortical excitability.

The MT have variously been shown to be increased or decreased in patients with generalized epilepsy when compared to controls, whereas in patients with focal epilepsy there was an increase (Brigo et al., 2012; Reutens & Berkovic, 1992; Tataroglu et al., 2004; Werhahn, 2000). Likewise while, the CSP has been demonstrated to be increased in patients with generalised and focal epilepsy, there was one study that demonstrated an increase, suggesting a reduced amount of inhibitory networks (Tataroglu et al., 2004; Akgun et al., 2009). Paired pulse studies have demonstrated evidence that ICI and LICI are decreased in both types of epilepsy. Thus the literature concerning cortical excitability in epilepsy is rather inconclusive and it is unclear whether the problem is due to reduced inhibition or increased facilitation. This raises the question: should the primary target for treatment be intracortical inhibition as shown by the CSP and N100 or the MEP amplitude? From the current experimental findings cerebellar iTBS appears to increase the CSP and the amplitude of the N100 potential, suggesting increased intracortical inhibition. Nevertheless it appeared to increase MEP amplitude in the study of Koch *et al*. (2008). In contrast cTBS effectively reduced the MEP amplitude in both our study and Koch *et al*. 2008.

Therefore, based on the current evidence, cerebellar cTBS might be the best TBS paradigm to trial in epileptic patients based on its overall ability to reduce excitability in the motor cortex, as reflected in the MEP amplitude. On the other hand iTBS appears to increase intracortical inhibition as reflected in the N100 amplitude. These results obtained are thus contradictory which makes it difficult to proceed with a clinical trial in patients with epilepsy.

CHAPTER 5. CONCLUSION

5.1. Relationship Between TBS and the Cerebellum

To our knowledge this is the first study using 30Hz TBS over the cerebellum to modify excitability of the contralateral motor cortex, and the first study to demonstrate the effects of cerebellar TBS on TEP. On the one hand it has shown that cTBS reduces overall excitability in the motor cortex as measured by MEP amplitude. On the other hand iTBS appears to increase intracortical inhibitory mechanisms, but without an overall change in MEP amplitude presumably because facilitatory networks were also activated. Although these results require replication, they provide a better understanding of TBS protocols that could be used therapeutically to treat epilepsy and other neurological conditions through modulation of the cerebello-thalamo-cortical pathway.

5.2. Future Research

Further studies are required to explore the effects of cerebellar TBS. A larger sample size and the use of paired pulse stimulation is required to examine whether the effects are due to increased inhibition or decreased excitation. Future studies should also explore the duration and time course of the effects. TBS protocols could be then applied to patients with dystonia or epilepsy where cortical excitability is altered. Although studies in healthy volunteers help to understand the effects of TBS, ultimately therapeutic use of TBS in these disorders will depend on the demonstration of clinical benefits, such as reduction in seizure frequency and improvement in function.

APPENDIX

Continuous TBS	80% Pre	80% Post	90% Pre	90% Post
RMT (% MSO)	73.3 +/- 9.0	74.4 +/- 9.2	65.5 +/- 6.3	66.9 +/- 6.9
AMT (% MSO)	48.4 +/- 4.7	47.1 +/- 6.3	43.4 +/- 4.7	44.4 +/- 5.4
RL (ms)	24.2 +/- 1.8	24.7 +/- 2.3	25.8 +/- 2.0	25.8 +/- 2.8
AL (ms)	20.8 +/- 1.6	20.6 +/- 2.1	22.3 +/- 1.6	22.6 +/- 1.9
RAMP (mV)	978.3 +/- 899.3	868.2 +/- 498.3	935.5 +/- 326.8	902.4 +/- 313.7
AAMP (mV)	5600.6 +/- 3623.0	6072.2+/-3136.1	7278.9 2692.9	7311.3+/- 2830.8
CSP (ms)	176.5 +/- 40.1	188.7 +/- 35.4	169.8 +/- 33.5	161.4 +/- 43.8

Intermittent TBS	80% Pre	80% Post	90% Pre	90% Post
RMT (% MSO)	74.6 +/- 10.2	71 +/- 8.4	68.3 +/- 7.1	65.6 +/- 6.4
AMT (% MSO)	49 +/- 4.7	46.3 +/- 3.9	41.7 +/- 3.1	42.7 +/- 8.2
RL (ms)	24.2 +/- 1.5	24.3 +/- 1.9	25.1 +/- 2.7	24.4 +/- 1.7
AL (ms)	20.7 +/- 1.6	21.0 +/- 1.4	21.2 +/- 1.6	21.8 +/- 1.9
RAMP (mV)	1328.3 +/- 592.4	1013.7 +/- 363.4	1486.2 +/- 954.1	2025.7+/- 1310.1
AAMP (mV)	7926.3 +/- 4919.5	5977.2+/-2370.2	8730.2+/- 2951.8	9199.5+/- 2781.3
CSP (ms)	185.5 +/- 39.6	174.3 +/- 47.1	168.6 +/- 25.5	186.8 +/- 33.0

SHAM	80% Pre	80% Post	90% Pre	90% Post
RMT (% MSO)	78.1 +/- 12.6	74.1 +/- 12.8	68.4 +/- 8.2	67.6 +/- 8.5
AMT (% MSO)	46.9 +/- 5.6	47.7 +/- 4.4	44.3 +/- 4.9	45.1 +/- 6.9
RL (ms)	24.3 +/- 1.5	24.1 +/- 1.3	25.0 +/- 1.8	25.2 +/- 1.8
AL (ms)	20.9 +/- 2.0	20.1 +/- 1.6	21.7 +/- 2.1	21.7 +/- 2.5
RAMP (mV)	857.5 +/- 475.1	1005.7 +/- 683.3	1058.1 +/- 425.8	1682.3 +/- 871.7
AAMP (mV)	7524.7 +/- 8578.7	5110.3+/-1064.0	8095.9+/- 1779.7	7719.2+/- 1599.5
CSP (ms)	173.8 +/- 40.4	203.8 +/- 28.5	160.5 +/- 44.3	159.4 +/- 36.1

Appendix.1. Motor evoked potential parameters before and after Theta Burst Stimulation. Mean values and standard deviation are shown for cTBS, iTBS and Sham at 80% and 90% stimulation. RMT = Resting Motor Threshold, AMT = Active Motor Threshold, AL = Active Latency, RL = Resting Latency, RAMP = Resting Motor Evoked Potential Amplitude, AAMP = Active Motor Evoked Potential Amplitude, CSP = Cortical Silent Period, MSO = maximum stimulator output, ms = milliseconds and mV = microvolts.

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