Running head: MODIFIED TBS ON CORTICOSPINAL EXCITABILITY IN HEALTHY ADULTS

Effects of a modified 30 Hz intermittent theta-burst stimulation (iTBS) protocol on corticospinal excitability in healthy adults

By

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Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the
Master of Science in Human Kinetics

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List of Abbreviations

aMT	Active motor threshold
AP	Anterior-posterior
cSP	Contralateral silent period
cTBS	Continuous theta bust stimulation
D-waves	Direct waves
DLPFC	Dorsolateral prefrontal cortex
EMG	Electromyography
FDI	First dorsal interosseous
GABA	γ-Aminobutyric acid
ICF	Intracortical facilitation
ISI	Inter-stimulus interval
iSP	Ipsilateral silent period
iTBS	Intermittent theta burst stimulation
I-waves	Indirect waves
LAI	Long-latency afferent inhibition
LM	Latero-medial
LICI	Long-interval cortical inhibition
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MEPs	Motor evoked potentials
MSO	Maximum stimulator output
MT	Motor threshold
NIBS	Non-invasive brain stimulation
NMDA	N-methyl-D-aspartic acid
PA	Posterior-anterior
PNS	Peripheral nerve stimulation
S1	Primary somatosensory cortex
SAI	Short-latency afferent inhibition
SICI	Short interval intracortical inhibition
TBS	Theta burst stimulation
TMS	Transcranial magnetic stimulation
V1	Primary visual cortex
VAS	Visual analogue scale
rMT	Resting motor threshold
rTMS	Repetitive transcranial magnetic stimulation

Abstract

Theta-burst stimulation (TBS) is a form of repetitive transcranial magnetic stimulation (TMS) developed to induce neuroplasticity. TBS usually consists of 50 Hz bursts at 5 Hz intervals. When applied intermittently, it can lead to facilitation of motor evoked potentials (MEPs), although these effects can be variable between individuals. Here, we aimed to determine whether a version of intermittent TBS (iTBS) consisting of 30 Hz bursts at 6 Hz intervals would produce less variable modulation. Nineteen healthy adults underwent single-pulse TMS to assess corticomotor excitability at baseline as reflected in MEP amplitude. 30 Hz iTBS was then administered and MEP amplitude was reassessed at 5-, 20- and 45-mins after the iTBS protocol. Compared to baseline, MEPs were significantly facilitated up to 45-min post-iTBS and most participants exhibited the expected facilitation. These observations suggest that 30 Hz/6 Hz iTBS may provide a sound alternative to induce consistent neuromodulatory effects over the commonly used 50 Hz/5 Hz protocol.

Acknowledgements

Prof. Tremblay, thank you for your continuous support, insight, and lessons over the past two years. You have gone above and beyond to ensure I do well in this program, and for that I am truly grateful. It has been a privilege to be your graduate student as you turn a new leaf in your career, I wish you a long and happy retirement! Thank you to my friends and fellow HK graduate students who helped with participant recruitment. To the individuals who did participate, thank you for your time, especially in the midst of a pandemic. Finally, thank you to Elisabeth Bruyère Research Institute and the Canadian Cancer Society for providing financial support for this study.

Summary

Theta burst stimulation (TBS) is a form of repetitive transcranial magnetic stimulation (TMS) developed to induce neuroplasticity. TBS usually consists of 50 Hz bursts at 5 Hz intervals, and when applied intermittently (iTBS, 2 s ON, 8 s OFF), it can lead to facilitation of motor evoked potentials (MEPs) elicited by single-pulse TMS. However, these effects can be quite variable between individuals with up to 50% of non-responders. In this thesis, our goal was to determine whether a modified iTBS protocol consisting of 30 Hz bursts repeated at a 6 Hz interval would lead to fewer variable responses. We also looked at whether individual susceptibility of TMS pulses to recruit early and late indirect waves (I-waves) could predict responses to 30 Hz iTBS. Participants (n=19, 13 females) first underwent TMS to determine MEP amplitude at baseline. Then, MEPs were evoked to assess early and late I-waves recruitment by stimulating with the coil positioned in different orientations (i.e., posterior-anterior (PA), anterior-posterior (AP), and latero-medial (LM); (Hamada et al. 2013). The 30 Hz/ 6 Hz iTBS intervention was then administered to the left motor cortex (600 pulses @ 80% active MT), and MEPs were reassessed at 5-, 20- and 45-min following completion of the iTBS protocol. As expected, MEPs elicited with the LM and PA orientations exhibited significantly shorter latencies than those elicited with AP orientation. Latency differences computed between AP and LM/PA stimulation were distributed along a continuum reflecting early (1-3 ms) and late I-wave (3.5-7.5 ms) recruitment. After iTBS, most participants exhibited the expected MEP facilitation (13/19; mean 143.3 ± 78.7%) with significant effects detected at 20 and 45 mins. Variations in latency differences were significant predictors of individual responses (r^2=0.24, p=0.03), but, contrary to expectations, smaller differences predicted greater facilitation. Overall, these findings confirmed that the 30 Hz iTBS protocol is effective in inducing lasting modulation in corticospinal excitability with improved

consistency of response across participants when compared to those reported by studies using the 50 Hz/ 5 Hz standard. Our results also point to the recruitment of early I-waves in response to TMS as a factor to predict the effects of 30 Hz iTBS.

CHAPTER I: INTRODUCTION

Transcranial magnetic stimulation (TMS) is a virtually painless method of delivering electrical stimuli to the brain through the intact scalp. TMS can indirectly excite or inhibit corticospinal neurons depending on the stimulation parameters, as indexed by changes in the amplitude of motor evoked potentials (MEPs). Repetitive TMS (rTMS) refers to a train of TMS pulses delivered at the same intensity over a cortical region. Unlike single-pulse protocols, it can induce changes in corticospinal excitability that outlast the stimulation period for minutes to even hours (Ziemann et al. 2008).

Theta Burst Stimulation (TBS) is a relatively new form of rTMS introduced in the mid-2000's. Originally described by Huang et al. (2005), TBS consists of pulses applied in bursts of three at high frequency (most commonly 50 Hz) with an inter-burst interval in the theta rhythm (4-10 Hz, usually 5 Hz). TBS is usually delivered in 20 successive trains either continuously over a 40 s duration or intermittently (2 s ON, 8 s OFF) over 192 s (600 pulses in total). When applied continuously (cTBS), TBS tends to inhibit corticospinal excitability, whereas when applied in an intermittent mode (iTBS), it tends to facilitate corticospinal excitability (Huang et al. 2005). TBS shows promise as a therapeutic tool in some neurological and psychiatric disorders (Di Lazzaro et al. 2006a; Talelli et al. 2007), such as major depression (Chen et al. 2019). Unfortunately, its use in clinical settings is limited by the considerable variability in response both within and between subjects (Lopez-Alonso et al. 2014; Vernet et al. 2014). This variability may reflect, at least in part, the use of non-optimal stimulation parameters. For example, current TMS devices are limited in terms of the maximal stimulation output (MSO) they can produce in 50 Hz repetitive mode, limiting the intensity that can be used for therapeutic interventions (e.g., 50% MSO for the Magstim Rapid^2). Such limitations have led authors (Goldsworthy et al. 2012) to propose modifications to the original TBS protocol (e.g., 30 Hz instead of 50 Hz bursts) to produce more

consistent effects on motor cortical excitability. However, the available literature regarding modified TBS protocols is scarce apart from a few studies documenting the effects of modified cTBS on corticospinal excitability (Jacobs et al. 2014; Tsang et al. 2014). Another factor contributing to the variability in response to rTMS protocols is advancing age, with the majority of the literature suggesting lower susceptibility to TMS-induced neuroplasticity in older adults (Todd et al. 2010; Bashir et al. 2014). To date, no study has investigated systematically how responses to modified TBS protocols can be influenced by advancing age.

In 2013, Hamada et al. proposed that variability to TBS protocols may also be related to individual differences in the recruitment of cortical interneurons in response to stimulation. They addressed this question by comparing MEP latency differences elicited by single-pulse stimulation using three different coil orientations: anterior-posterior (AP), posterior-anterior (PA), and latero-medial (LM). Conventional PA orientation (coil held ~45° with the handle pointing backward) is known to recruit early indirect waves (i.e., I_1) leading to MEPs at short latency, whereas stimulating with the coil held in the AP orientation (coil handle pointing forward at 225°) leads to prolonged latency, reflecting the recruitment of late I-waves (i.e., I_2-I_5). MEPs with the shortest latency are elicited with the coil held at 90° to the midline, which is thought to reflect either direct activation of corticospinal neurons (D-wave) or recruitment of early I_1-waves. Hamada et al. (2013) used this approach to deduce the susceptibility of TMS pulses to recruit late I-waves by comparing MEP latency differences in their participants (i.e., AP-LM vs. PA-LM). Their primary finding was that AP-LM differences in latency contributed to about 50% of the variability in participants' responses to both standard cTBS and iTBS protocols (Hamada et al. 2013). Moreover, participants who displayed the largest AP-LM difference also showed the expected inhibition and facilitation in response to either cTBS or iTBS, while those with minimal latency differences

demonstrated the opposite pattern (Hamada et al. 2013). These observations indicated that individuals in whom TMS pulses could recruit late I-waves (i.e., largest AP-LM differences) were also those that exhibited the expected response to either cTBS or iTBS. However, whether such a relationship also holds when using different bursting frequency and inter-burst intervals for TBS applications remains to be seen.

In the present study, our goal was to investigate TBS-induced neuroplasticity further to determine whether a modified 30 Hz/6 Hz iTBS protocol would lead to more consistent changes in corticospinal excitability across participants, relative to the changes in corticospinal excitability reported using the 50 Hz/ 5 Hz protocol. In the next section, we will review the relevant literature on TMS applications and TBS protocols.

CHAPTER II: LITERATURE REVIEW

In the following sections, the fundamental mechanisms and physiology of TMS will be reviewed. Next, we will review TMS protocols, including single-pulse, paired-pulse and rTMS. The review will then focus on TBS and transition into the various issues associated with TBS, emphasizing the high variability in response both within and between subjects. Finally, the relevant literature regarding the individual factors that contribute to this variability will be reviewed.

2.1 Basic mechanisms and physiology of TMS

For many years, neurophysiologic investigations based on electrical stimulation were practically confined to animal studies. In humans, the available stimulation methods were limited by their invasive nature, involving removing the scalp and skull to expose the brain to stimulating electrodes. When non-invasive electrical stimulation methods were introduced, only a tiny fraction of the applied electrical current was found to penetrate the scalp and stimulate the brain effectively. Instead, most of the current flowed on the scalp, activating cutaneous nociceptors and producing contractions in the scalp muscles, which was reported as very uncomfortable by volunteers (Di Lazzaro et al. 2018).

It was not until 1985 that Barker and colleagues developed transcranial magnetic stimulation (TMS). TMS stimulates the brain electrically but uses a magnetic field to “carry” the stimulus into the brain (Di Lazzaro et al. 2018). The magnetic field is produced by placing an electromagnetic coil on the scalp and passing a powerful and rapidly changing magnetic current. The scalp and skull’s low impedance to the passage of the magnetic field allows the current to pass through effectively, with relatively little pain. Upon reaching the cortex, the magnetic field then induces an electrical current (i.e., an eddy current) that flows in loops parallel to the surface of the brain when the stimulating coil is held tangentially to the scalp and produces action potentials or

excitatory (or inhibitory) postsynaptic potentials in stimulated neurons (Terao and Ugawa 2002), with preferential activation of horizontally oriented neural elements (Abbruzzese and Trompetto 2002).

As shown in Figure 2.1, the TMS coil is usually made of one or two wound loops of copper wires enclosed in plastic or ceramic. The geometry and size of the TMS coils can vary, each producing electric fields of different patterns and focality (Oberman 2014). With a circular coil, the strongest current loops are induced under the annulus of the coil and become weaker near the centre of the coil (Hallett 2000). To improve focality, a figure-of-eight coil was developed, which consists of two smaller overlapping coils, each passing current in the opposite direction. With a figure-of-eight coil, the maximum current lies near the coil centre but is slightly shifted in the direction of the coil current. Double-cone coils also consist of two coils, but the coils are joined at an angle of 90 to 100°. When the head is placed between the coils, double-cone coils can be useful for inducing current in deeper parts of the brain, such as the leg area of the motor cortex that lies deep within the interhemispheric fissure (Terao and Ugawa 2002).

TMS cannot induce a net current flow in the brain. The direction of the current must always be balanced, with equal current flow in each direction. Most “single-pulse” TMS machines therefore induce a quasi-monophasic stimulus pulse that consists of a quick rising high current (peaks in 100 μs) in one direction (i.e., along the axis of the overlapping segment of the figure-of-eight coil), followed by a slow (1 ms) smaller amplitude return current in the opposite direction (Lazzaro et al. 2008). Using a monophasic stimulator, neural stimulation occurs during the initial phase of high current. Biphasic stimulators have forward and reverse current phases with equal amplitude and duration, making them more balanced than monophasic stimulators. With these devices, stimulation occurs at the time when the current direction is reversed (Lazzaro et al. 2008)

When applied over the primary motor cortex (M1), TMS induces descending volleys that run along the corticospinal tract (Klomjai et al. 2015) to excite spinal motoneurons (Figure 2.1). Above a certain threshold intensity, this can result in contralateral muscle responses, most readily in intrinsic hand muscles. These responses are referred to as MEPs and can be detected using surface electromyography (EMG; Figure 2.1). In practice, specific measures such as the motor threshold (MT) and the peak-to-peak MEP amplitude can be used in single-pulse protocols to quantify the excitability of the corticospinal pathway (Bestmann and Krakauer 2015).

The resting motor threshold (rMT) is defined as the lowest level of stimulation required to evoke reliable MEPs at rest, usually defined as 50 μV in 5/10 consecutive pulses (Boroojerdi et al. 2001). Specifically, the rMT provides information regarding a central core region of neurons in the motor representation (Chen 2000; Herbsman et al. 2009) and depends on the excitability of cortico-cortical axons as well as excitatory pathways to corticospinal neurons (Habib et al. 2018). The stimulation intensity is generally based on the rMT, and it has become standard practice for determining the intensity of TMS for non-motor regions in which there is no measurable physiological response (Sandrini et al. 2011; Dayan et al. 2013).

The MEP amplitude is the most common measure of corticospinal excitability. As shown in Figure 2.1, the amplitude is typically measured from peak-to-peak and is represented in units of micro- or millivolts (Farzan 2014). It is a compound signal comprised of both cortical and spinal-segmental contributions, resulting from a series of descending corticospinal volleys with different generators (Bestmann and Krakauer 2015). Latency is another essential feature of the MEP. It is defined as the time between the TMS pulse delivery as indicated by the stimulus artifact, and the appearance of the EMG evoked response in the target muscle (Farzan 2014). MEP latency reflects the conduction time for neural impulses to travel from the motor cortex down to peripheral

muscles, which is determined by the conduction velocity of the fastest corticospinal neurones, the time for summation of descending volleys at the spinal motoneuron level, and the neuromuscular conduction in the periphery (Bestmann and Krakauer 2015).

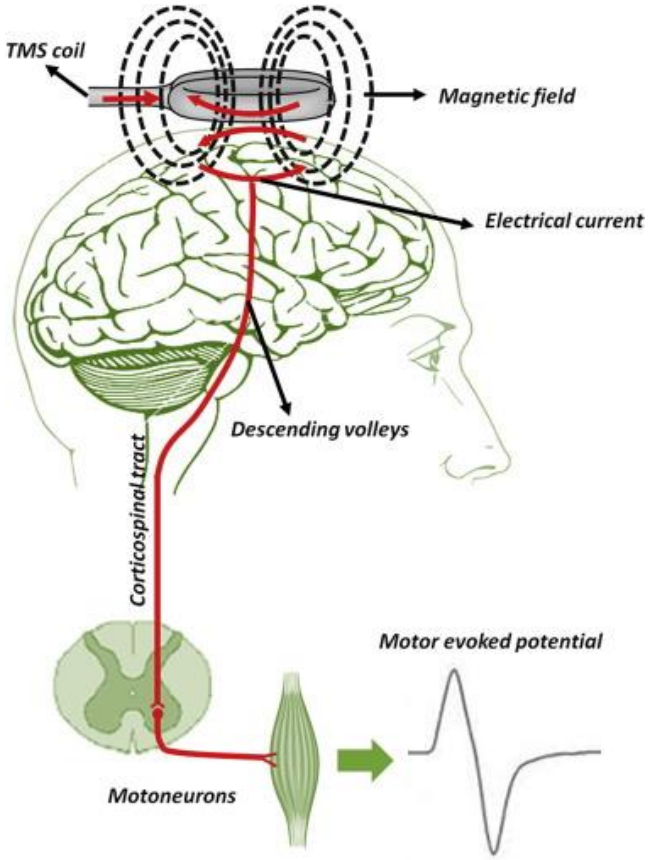

Figure 2.1 Simplified scheme of mechanism of action for TMS applied to the motor cortex (from *Rodríguez-Labrada R, Velázquez-Pérez L, Ziemann U (2018) Clin Neurophysiol 129:1688-1698* with permission).

2.2 TMS protocols

Single-pulse TMS is most used to probe the physiology of the motor cortex. Besides the measures described in the previous section, other measures can be derived with single-pulse protocols. This includes the contralateral silent period (cSP) and the ipsilateral silent period (iSP; Figure 2.2). The cSP is characterized by the suppression of background EMG activity during voluntary contraction of the target muscle. The cSP is obtained when a suprathreshold TMS pulse is delivered to the contralateral motor cortex (Wassermann et al. 1991; Meyer et al. 1995) during active contractions. Conversely, the iSP involves a transient suppression of EMG activity following the delivery of a suprathreshold TMS pulse to the motor cortex during voluntary contraction of an ipsilateral target muscle (Fuhr et al. 1991). The use of single-pulse TMS has also been extended to non-motor areas such as the visual system. For example, suprathreshold stimulation of the occipital cortex (mainly primary visual cortex, V1) produces bright spots of light in the visual field, known as phosphenes, as well as transient scotomas (Amassian et al. 1989).

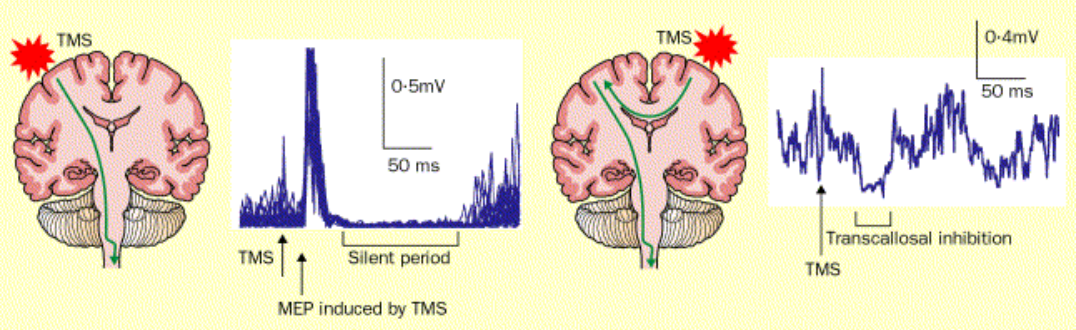

Figure 2.2 Contralateral and ipsilateral silent period (from *Kobayashi, M., & Pascual-Leone, A. (2003). The Lancet Neurology, 2(3), 145-156,* with permission).

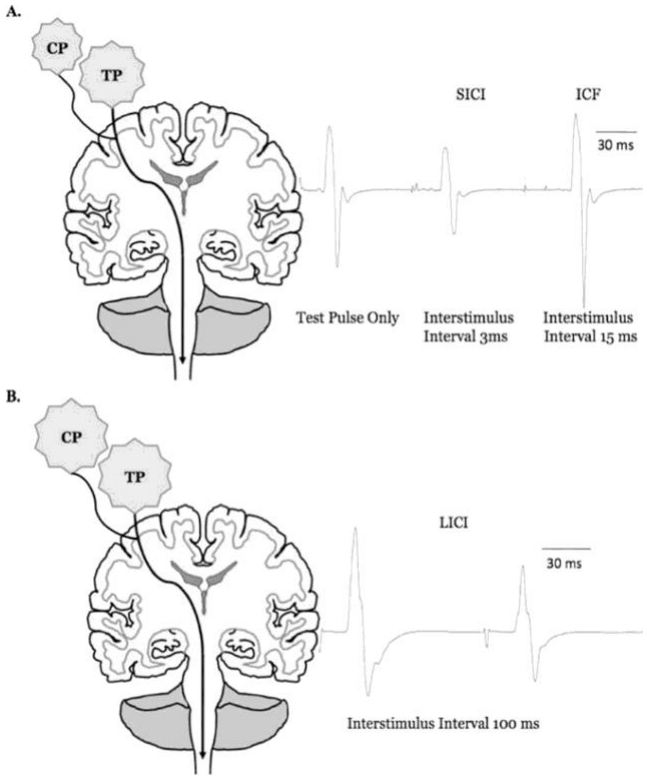

Figure 2.3 Paired-pulse TMS protocols (CP, conditioning stimulus; TP, Test pulse*;* from *Clark BC, et al. (2010). Muscle Nerve 42:363-372,* with permission).

Paired-pulse TMS protocols consist of two consecutive pulses applied through the same coil at a specific inter-stimulus interval (ISI) to probe different intracortical excitatory and inhibitory circuits (see Figure 2.3). Paired-pulse protocols can be applied to probe a single region (usually M1) or to probe circuits connecting brain areas (Reis et al. 2008). In the protocol termed short-interval intracortical inhibition (SICI), a subthreshold stimulus (e.g. 70 % MT) precedes a test stimulus by 1 to 5 ms. This results in a noticeable suppression of MEPs that is likely mediated by the activation of $GABA_A$ receptors. When the ISI between the subthreshold and threshold stimulus increases between 10 to 30 ms, the test results in MEP facilitation. This facilitation is referred to as intracortical facilitation (ICF). Long-interval cortical inhibition (LICI) involves two suprathreshold stimuli (e.g., 120% MT) with an ISI of 50-200 ms leading to inhibitory effects (Di Lazzaro et al. 2018).

In the motor system, inhibitory or facilitatory effects on MEP sizes can be produced by pairing TMS with peripheral nerve stimulation (PNS). One form of MEP inhibition termed short-latency afferent inhibition (SAI) is induced when the PNS (e.g., the median nerve at the wrist) precedes the TMS pulse at the motor cortex using 18-24 ms ISIs (Tokimura et al. 2000) see Figure 2.4). Another period of late inhibition, termed long-latency afferent inhibition (LAI) can also be produced when long ISIs are used (180-220 ms) between peripheral and cortical stimulation (Devanne et al. 2009). The magnitude of SAI is known to be cholinergic-dependant, and thus, SAI is considered a marker of cholinergic function in the motor cortex (Di Lazzaro et al. 2005a).

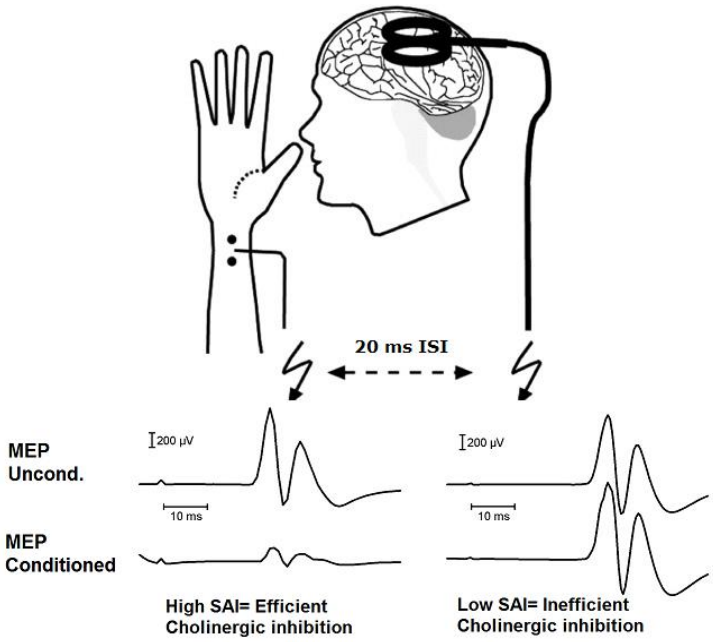

Figure 2.4 Short-latency afferent inhibition (SAI) in a young (highly efficient) and older (low efficiency) participant (adapted with permission from *Young-Bernier et al (2012). Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition with age. Neurobiol Aging 33:835 e831-811.*)

As mentioned earlier, rTMS refers to the delivery of consecutive trains of TMS pulses. Unlike single-pulse protocols, the induced effects of rTMS can outlast the stimulation period for several minutes to hours, providing insight into the role of the specific stimulated brain regions in plasticity and behaviour (Cárdenas-Morales et al. 2010; Dayan et al. 2013). The direction, magnitude and duration of these effects can be influenced by intrinsic factors such as the functional state of cortical neurons prior to or during stimulation as well as by extrinsic factors such as the frequency and intensity of the stimulation, or the pattern at which the stimuli are delivered (Ziemann et al. 2008). As shown in Figure 2.5, low-frequency rTMS (<1Hz) applied for longer

durations tends to produce inhibitory effects on corticospinal excitability (Gerschlager et al. 2001; Touge et al. 2001; Fitzgerald et al. 2006) while shorter exposure to high-frequency rTMS (> 5 Hz) tends to produce facilitatory effects (Pascual-Leone et al. 1994; Peinemann et al. 2000).

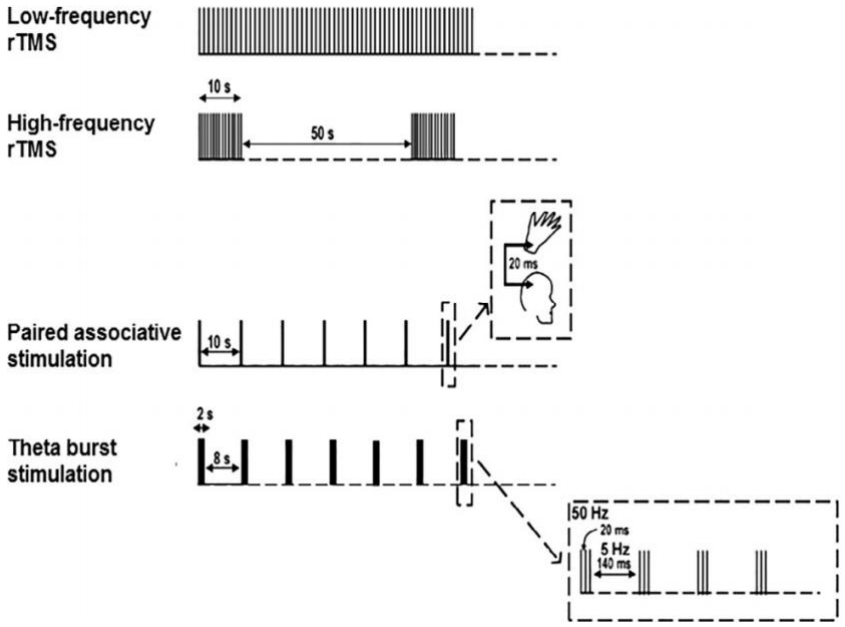

Figure 2.5 Schematic of rTMS protocols (from *Lefaucheur-J-P (2019) Chapter 37- Transcranial magnetic stimulation. In: Levin KH, Chauvel P (eds) Handbook of Clinical Neurology. Elsevier, pp 559-580*, with permission).

There is evidence to suggest that rTMS may promote effects on synaptic plasticity that relate to learning. As such, there has been a growing interest in the possibility of using rTMS as a therapeutic tool, assuming it might be able to enhance the natural ability of the brain to adapt in response to injury (Di Lazzaro et al. 2018). For example, rTMS has been explored as a treatment option to promote recovery post-stroke, given that stroke recovery is based on plastic changes in the central nervous system that can compensate the loss of activity in affected brain regions (Lefaucheur 2006).

Interestingly, rTMS has also been investigated as a therapeutic tool to manage a variety of psychiatric illnesses including, but not limited to, major depressive disorder, schizophrenia, obsessive compulsive disorder, post-traumatic stress disorder, and bipolar disorder (George et al. 1999; Wassermann and Lisanby 2001; Fitzgerald et al. 2002a; Fitzgerald et al. 2002b). In fact, rTMS-devices for the treatment of therapy-resistant depression have been approved by the USA, Food and Drug Administration (e.g., NeuroStar TMS Therapy System™; October 2008).

2.3 Theta-burst stimulation (TBS)

In 2005, a new rTMS protocol was introduced that involves the application of patterned bursts of stimuli. This protocol, known as theta-burst stimulation (TBS), mimics two characteristics of hippocampal physiology: complex-spike discharges of pyramidal neurons (i.e., 3-5 pulses at the gamma range 50-100 Hz) and the rhythmic modulation of excitability of those cells during theta rhythm (i.e., 5 Hz). In rodents, TBS with this pattern of stimuli induced long-term potentiation (LTP) when applied to the motor cortex or hippocampus (Larson et al. 1986; Hess et al. 1996). In the human motor cortex, Huang and colleagues (2005) demonstrated similar LTP-like effects using MEPs to monitor changes in corticospinal excitability. The TBS protocol consisted of pules applied in short bursts of three at 50 Hz repeated at a 5 Hz interval. As mentioned earlier, when applied continuously for 20-40 s, TBS tends to depress excitability, as reflected in MEP amplitude. On the other hand, when TBS trains are applied intermittently every 2 s for 10 s for a total of 20 cycles (600 pulses), the opposite effect is observed (Figure 2.6).

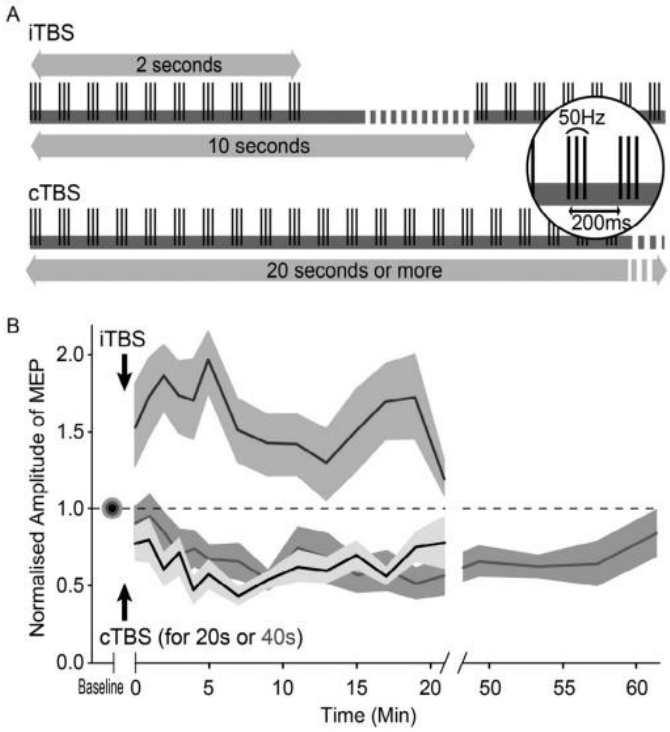

Figure 2.6 Schematic illustration of TBS protocols. Lasting MEP facilitation is commonly observed following iTBS, whereas lasting depression is observed following cTBS (from *Suppa et al. (2016) Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects. Brain Stimulation 9:323-335,* with permission).

Evidence derived from epidural recordings of descending volleys elicited in the pyramidal tract of patients with implanted electrodes suggests that changes in MEP amplitude following TBS are largely cortical in origin (Di Lazzaro et al. 2005b). The first volley, known as the direct wave, results from direct activation of corticospinal axons (hence the term D-wave). The subsequent waves are considered indirect, originating from the trans-synaptic activation of corticospinal neurons (Rothwell et al. 1991). These indirect waves are termed in the order of their appearance (i.e., I_1, I_2 and I_3). From epidural recordings, it was shown that cTBS affected

the early I_1-wave amplitude with a decrease. In contrast, iTBS affected primarily later I-waves (i.e., I_2, I_3), leading to an increased amplitude (Di Lazzaro et al. 2005b; Di Lazzaro et al. 2008). While the basic mechanisms underlying TBS-induced facilitation and inhibition are still being investigated, modulation of N-methyl-D-aspartate (NMDA) receptors (Huang et al. 2005; Di Lazzaro et al. 2008) and of γ-aminobutyric acid receptor (GABAr) activity may be involved in mediating changes in cortical excitability (Thickbroom 2007). As such, TBS effects are thought to occur via changes in synaptic efficacy within M1 via LTP- and LTD-like mechanisms, as observed in animal models.

As mentioned earlier, TBS is also being explored as a potential intervention to treat certain neurological conditions, such as multiple sclerosis (Centonze et al. 2007), stroke (Di Lazzaro et al. 2006b), and tinnitus (Plewnia et al. 2012). Like other rTMS protocols, there has been a particular interest in using TBS to manage major depressive disorders. Standard rTMS protocols for depression typically target the dorsolateral prefrontal cortex (DLPFC) using either high-frequency rTMS (5–20 Hz) over the left side (George et al. 2000) or low-frequency rTMS (1 Hz) over the right side (Klein et al. 1999). TBS paradigms may be applied similarly, to the left and right DLPFC using iTBS and cTBS, respectively.

A significant advantage of TBS protocols over other forms of rTMS protocols is shorter durations of administration (e.g., 3 minutes of iTBS versus 30- 40 minutes 10 Hz rTMS applied to the left DLPFC; (Blumberger et al. 2018). Moreover, the lower stimulation intensity used with TBS (i.e., of 80% instead of 120% rMT used in rTMS) may allow for more comfortable applications in a therapeutic setting. Given these advantages, TBS protocols are currently under intense investigation to determine their therapeutic efficacity compared to approved rTMS protocols (Bakker et al. 2015; Blumberger et al. 2018).

TBS protocols have a similar safety profile as other rTMS protocols and could be even safer in some respects than other higher frequency protocols (Rossi et al. 2009; Oberman et al. 2011). For instance, while transient headache and neck pain (the most common adverse effects) have been reported in up to 40% of patients undergoing high-frequency rTMS (Rossi et al. 2009), such effects are seen in less than 3% of subjects receiving TBS (Oberman et al. 2011). Other reported minor adverse effects of TBS include worsening in patients with tinnitus, nausea in a patient with Parkinson's disease, light-headedness or vagal responses in healthy control subjects, and unilateral eye pain and lacrimation in one healthy control subject (Rachid 2017). The most severe reported adverse event is a seizure, which has only been reported once in more than 4,500 sessions, resulting in a crude risk of 0.02% (Oberman et al. 2011). This is comparable to high frequency rTMS protocols, where the incidence of seizure is less than 0.1% (Oberman et al. 2011). Thus, in terms of safety, TBS protocols present no more risks for participants to experience a major adverse event, while reducing substantially the risk of minor adverse events.

2.4 Issues with TBS protocols

As we mentioned in the Introduction, one major lingering issue with rTMS protocols, including TBS protocols, is the high levels of inter-individual variability, which impairs its therapeutic applications. In the original study by Huang and colleagues (2005), the effects of TBS (either continuous or intermittent) were reported to last up to 1 hour following stimulation with little variability between participants. However, subsequent investigations, while corroborating the overall effects reported by Huang et al. (2005), have also highlighted the large inter-individual variability of responses (Di Lazzaro et al. 2011; Lopez-Alonso et al. 2014). For instance, Goldsworthy et al. (2012) reported that MEP modulation in response to cTBS lasted only 10 min and that many individuals either showed no change or even showed the opposite response to the

stimulation (i.e., facilitation instead of inhibition). More recent systematic investigations of TBS-related effects on MEP modulation have confirmed the poor test-re-test reliability (Vernet et al. 2014) and the high inter-individual variability of responses (Lopez-Alonso et al. 2014). To address this variability issue, the same group of investigators involved in the original study by Huang et al. (2005), proposed that variations in response to TBS might reflect individual differences in the recruitment of cortical interneurons (Hamada et al. 2013). As explained earlier, using differences in MEP latency elicited by changing the coil orientation, these authors showed that the effect of TBS was more consistent in individuals in whom TMS pulses could recruit later I-waves and, thus, specific cortical interneuron populations. Overall, these results highlighted the need to consider both individual and methodological factors when examining individual responses to rTMS protocols.

As noted in the previous section, some individuals seem more prone to exhibit lasting changes in cortical excitability in response to rTMS protocols because of physiological and anatomical reasons. Another factor known to influence the variability of responses to TBS is variations between protocols. In this regard, it is important to stress that almost all studies investigating TBS effects either at the physiological level or for therapeutic purposes have relied on the original standard “50 Hz/5 Hz” protocol described by Huang and colleagues in 2005. However, subsequent investigations have examined whether slight modifications of the TBS protocol parameters could lead to more consistent effects. For instance, in two different reports, Nyffeler (2006; 2008) showed that reducing the cTBS frequency from 50 to 30 Hz produces more consistent behavioural effects in the oculomotor system when applied to the frontal eye field and posterior parietal cortex. Along the same line, Goldsworthy et al. (2012) showed that cTBS, when applied to M1 using 30 Hz trains repeated at 6 Hz, evoked longer-lasting suppression in MEPs

than the standard 50 Hz trains at 5 Hz. Further, these authors also showed that MEP suppression induced by 30 Hz/6 Hz cTBS was still present when actively contracting the muscle, indicating a more robust depression in excitability than that seen with the standard protocol (Goldsworthy et al. 2012). Following these first investigations, other studies have confirmed the potential advantage of the modified 30 Hz cTBS protocol in depressing corticospinal excitability (Wu et al. 2012a; Jacobs et al. 2014; Tsang et al. 2014).

Another major advantage of decreasing the frequency of TBS from 50 Hz to 30 Hz is the greater intensity that can be used for stimulation. For instance, The Magstim® Rapid^2 inherent limitations in terms of output require counter-balancing intensity and burst frequency (Wu et al. 2012a). As such, the Rapid^2 device does not permit stimulation intensity greater than 50% MSO when using 50 Hz burst-frequency, which limits its application considerably in individuals with high rMTs. Using an intensity equivalent to 80% aMT, an individual with an aMT of 65% (a common finding in seniors) could not receive adequate stimulation (i.e., 80% of 65= 52% MSO). This is an important consideration given the strong influence of TMS intensity on neuromodulatory effects (Hamada et al. 2007). For instance, Sasaki and colleagues (2018) compared the effects of standard cTBS at different intensities. Their results showed that cTBS-induced MEP inhibition could be reversed in some participants by increasing the intensity from 80 to 100% MT. Similarly, Doeltgen and Ridding (2011) compared the effects of varying the TMS intensity on responses to standard cTBS with 300 total pulses. Interestingly, they demonstrated that cTBS led to depressed MEPs when applied at an intensity of 65% rMT, whereas 70% rMT cTBS produced an opposite facilitatory effect (Doeltgen and Ridding 2011).

Aside from comparative studies, most investigations have adhered to the original protocol described by Huang et al. (2005) and used a stimulation intensity equivalent to 80% the active

motor threshold (aMT[1]). However, some studies have elected to use another stimulation intensity (Chung et al. 2016). For example, Jacobs et al. (2014) delivered 30 Hz cTBS over the primary somatosensory cortex (S1) and M1 using an intensity of 55% rMT. They stated that this intensity was chosen to limit the influence of current spread between adjacent cortical areas. Contrary to other studies that reported no effects using a lower stimulation intensity (i.e., 60% rMT) their result showed cTBS over SI facilitated MEPs while cTBS delivered over M1 suppressed MEPs (Jacobs et al. 2014). In this case, the change from 50 Hz to 30 Hz may have compensated for the use of lower stimulation intensity.

While the effects of the modified 30 Hz TBS protocol have been investigated for the continuous mode, there is comparatively little information regarding the intermittent mode. In the prefrontal cortex, Chung et al. (2019) found significant variability in response to iTBS following both 30 and 50 Hz iTBS, with individualized iTBS resulting in more robust changes compared to the standard protocol. In the cerebellum, Hammond-Tooke and Harrington (2015) found evidence to suggest that 30 Hz iTBS enhanced activity in both inhibitory and facilitatory cortical networks. Another more recent meta-analysis by Chung et al. (2016) concluded that modified 30 Hz TBS protocols tended to produce more persistent and even larger effects over time when compared to the standard 50 Hz protocol.

To our knowledge, only one report by Wu et al (2012a) investigated the effect of 30 Hz iTBS on corticospinal excitability in young adults. Their results show that the modified 30 Hz iTBS protocol produced similar facilitation of MEPs as the standard protocol. Another study

[1] The active motor threshold (aMT) is determined using the same approach as for rMT. The addition of a light contraction will produce slightly lower values for the aMT. In general, the aMT corresponds to 90% of the rMT, so an intensity of 80% aMT corresponds to about 70% rMT.

examined whether a modified version of 30 Hz iTBS consisting of only 300 pulses (i.e., half of the standard 600 pulses) was safe and tolerable when applied to children and adolescents (Wu et al. 2012b). Their results showed that healthy children demonstrated similar MEP facilitation in response to the modified 30 Hz iTBS with 300 pulses, as reported in healthy young adults by Wu et al. (2012a).

2.5 Influence of individual factors

Besides methodological factors, factors at the individual level can also contribute to the variability of responses to rTMS protocols. The following section will focus on the individual differences in TMS ability to recruit specific cortical interneuron populations and how that may influence TMS-induced neuroplasticity.

2.5.1 Effect of age on rTMS-induced neuroplasticity.

Age is known to be one of several factors that can contribute to inter-individual variability in response to TBS (Ridding and Ziemann 2010; Freitas et al. 2011; Young-Bernier et al. 2014). Much of the current literature suggests that the efficacy of rTMS protocols tends to be reduced in older adults due to age-related changes in brain functions and notably changes in synaptic function (Doherty et al. 1993; Bergado and Almaguer 2002; Jankelowitz et al. 2007; Zhong and Gerges 2010; Rosso et al. 2013; Opie et al. 2018). For example, Bashir et al. (2014) showed that MEP modulation following rTMS differed significantly in both the dominant and non-dominant hands of younger individuals, while older individuals showed no significant differences in either hand. Similarly, Todd et al. (2010) reported that a group of younger subjects exhibited significantly reduced MEP amplitude following 10 min of 6 Hz rTMS compared to sham stimulation, whereas the older adults showed no effects.

Regarding TBS protocols, Freitas et al. (2011) showed that motor cortical plasticity induced by cTBS progressively declined with age. Another study by Opie et al. (2017) showed that prior priming stimulation to enhance TBS effects (both continuous and intermittent) was ineffective in old adults, whereas an increased response was observed in young adults. These effects were not dependent on the type of priming protocol. Other studies have reported conflicting results regarding the effects of older age on neuroplastic responses to iTBS, finding either no difference (Young-Bernier et al. 2014) or only a slight difference in MEP facilitation (Di Lazzaro et al. 2008a). Given the reported effect of the 30 Hz TBS protocol, it seems imperative to investigate whether such a modified protocol would lead to better response profiles in senior individuals. To date, however, no studies have explored this possibility.

2.5.2 Recruitment of cortical interneurons

A significant issue when interpreting age effects on neuroplastic responses to rTMS pertains to whether the age-related variability is related to differences in intrinsic mechanisms of synaptic plasticity at the cortical level (Ridding and Ziemann 2010) or to the fact that individuals may exhibit differences in the ability of TMS to recruit specific populations of cortical interneurons, irrespective of their age (Day et al. 1989; Rothwell 1997).

As explained earlier, it has long been established that monophasic TMS pulses with a posterior-anterior (PA) current elicit the lowest threshold MEPs, with a latency that is approximately 1–2 ms longer than MEPs evoked by direct activation of corticospinal axons (Day et al. 1989). When the current direction is reversed (anterior–posterior, AP), the threshold is higher, and the MEP latency onset is 2–3 ms later (Day et al. 1989). Interestingly, the onset latency of MEPs evoked by AP stimulation is highly variable from one person to another, whereas the latency of MEPs elicited with conventional PA stimulation is relatively consistent between individuals

(D'Ostilio et al. 2016). As Hamada et al. (2013) proposed, the variability of MEP latency observed when using AP current with TMS may provide a basis to explain why specific individuals may be more susceptible to rTMS-induced effects and why others are not.

TBS is produced using biphasic stimulus pulses, which preferentially activates cortical cells during the second depolarizing phase of the current in the brain, with an AP current (Maccabee et al. 1998; Di Lazzaro et al. 2001). As mentioned earlier, Hamada et al. (2013) used these observations to investigate whether individual differences in MEP latency evoked with different coil orientations could provide clues regarding the variability of responses to TBS. By comparing latency differences in MEPs with AP, PA and LM current, they could deduce how easily TMS could recruit early and late I-waves in each participant. They postulated that individuals with MEP onset latencies close to D-wave latency (i.e., AP-LM:1-2 ms) would readily recruit early I-waves whereas those with later onsets (AP-LM:> 3 ms) would tend to recruit later I-waves (Day et al. 1989; Rothwell 1997; see Figure 2.7). Their results confirmed that individuals with preferential recruitment of late I-waves exhibited the most consistent pattern of MEP response to either cTBS or iTBS. Conversely, individuals with preferential recruitment of early I-waves exhibited more variable and often opposite responses to TBS applications.

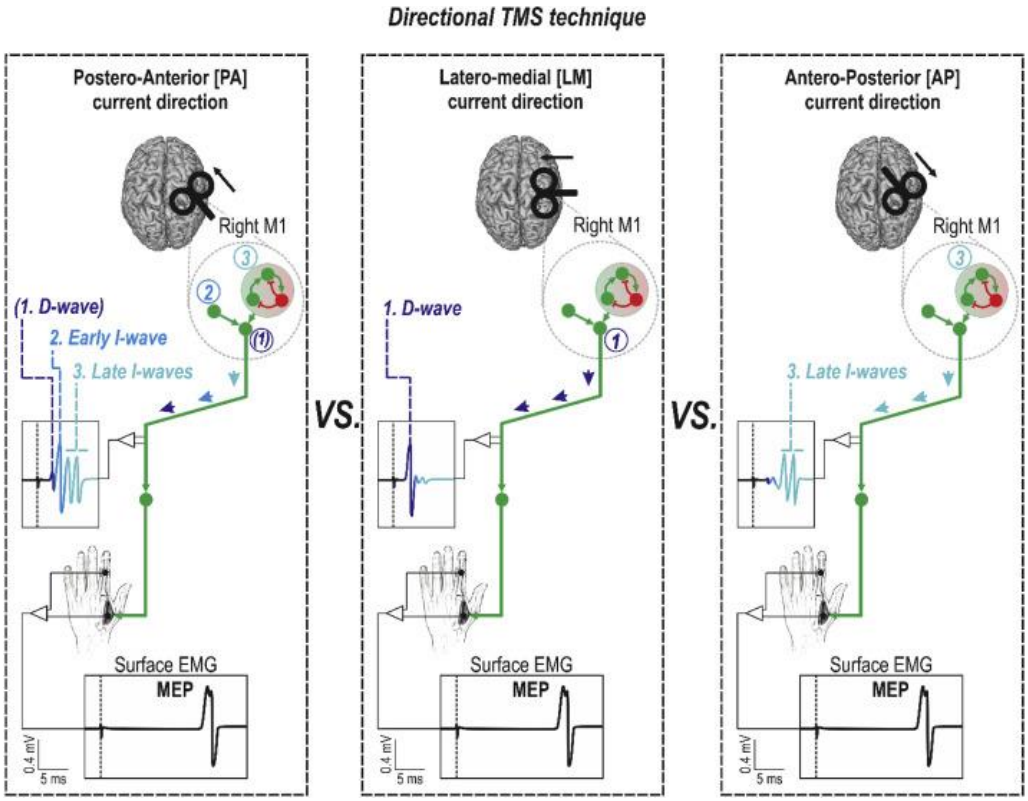

Figure 2.7 Schematic representations of coil positioning to elicit posterior-anterior (PA), anterior-posterior (AP) and lateral-medial (LM) directed currents (from *Derosiere G, Vassiliadis P, Duque J (2020). Advanced TMS approaches to probe corticospinal excitability during action preparation. NeuroImage 213:116746,* with permission).

From these observations, the authors concluded that variations in response to TBS protocols were likely due to individual differences in the population of neurons activated by the TMS pulse, rather than individual differences in intrinsic mechanisms of synaptic plasticity (Hamada et al. 2013). Such conclusions have not been reproduced for older participants, where changes in neurotransmitter levels and synaptic function could alter responses to rTMS protocols. Also, it is not known whether these findings can be extended to modified TBS protocols.

2.6 Objectives of the present work

The primary aim of the present study was to investigate further the effects of a modified iTBS protocol consisting of 30 Hz bursts repeated at 6 Hz intervals on corticospinal excitability in healthy young adults and seniors. Besides the influence of age, we aimed to determine whether 30 Hz iTBS effects are influenced by individual differences in the ability of TMS to recruit specific cortical interneurons using the approach proposed by Hamada et al. (2013) based on the latency of MEPs elicited using different coil orientations. It was hypothesized that the modified iTBS protocol would yield less variable response rates than those reported previously for the 50 Hz standard TBS protocol in both age groups. Lastly, we anticipated that independent of age, individuals in whom TMS pulses could recruit later-I waves, as reflected in large differences between latency elicited with AP and LM stimulation, would exhibit more robust and lasting facilitation than those in whom early I-waves were more readily recruited.

CHAPTER III: METHODS

3.1 Participants

A power analysis based on the systematic review of Chung et al. (2016; standardized mean difference of 0.71 for iTBS) indicated that a sample size of 30 would detect a difference between means of 0.67 mV with an 80% power at an alpha level of 0.05. Based on this analysis, our goal was initially to recruit 30 participants for this study, with one half (n=15) consisting of young adults (18- 35 years old) and the other half consisting of senior adults (age 60-75 years). However, due to the COVID-19 pandemic and related restrictions, we were not allowed to recruit seniors for the study. Thus, our recruitment targeted only adults < 55 years. Given these restrictions, we recruited 21 healthy adults (15 females; mean age, 25.3± 4.8 years; range, 19–40 years; 3 left-handed). All participants were considered healthy and were exempt from recent injuries in the upper extremities. Before testing, participants were screened with a health questionnaire to ensure that they had no contraindications to TMS. All procedures were approved per the Declaration of Helsinki by the institutional research ethics boards (Bruyère Protocol # M16-20-009; uOttawa, Office of Research Ethics and Integrity, protocol# H-10-20-6523) and all participants provided written informed consent before participation.

3.2 COVID-19 special safety measures

In the context of the COVID-19 pandemic, several precautionary measures were established for face-to-face research. Before testing sessions, participants were screened for the presence of symptoms using an application developed by the Institution (Bruyère COVID-19 Visitor Screening Questionnaire). Once passed the screening process, participants completed the verbal COVID Consent Form, and a testing session was scheduled for the on-site visit. At that time, potential participants were also informed about disclosing any new symptoms or contact with confirmed COVID-19 cases before scheduling an appointment. A reminder call was done 24 hours

before the testing day to ensure that participants were still free of symptoms and had no contact with COVID-19 cases. On the day of the testing, participants were required to wear a procedural mask and sanitize their hands before entering the research area. During testing, both the research staff and the participants wore a procedural mask and maintained physical distancing whenever possible. The touching of surfaces was limited. At the end of each testing session, research tools and surfaces were adequately sanitized.

3.3 Experimental protocol

A schematic of the experimental protocol is illustrated in Figure 3.1. Participants first underwent single-pulse TMS with the coil in the standard orientation (PA) placed over the left motor cortex to determine MEP amplitude at baseline (n=20 trials). Then, MEPs were elicited with the coil placed in different orientations (i.e., AP, LM, PA) to assess differences in latency. Afterwards, participants were moved to administer the 30 Hz/6 Hz iTBS protocol using a second stimulator for repetitive stimulation (Rapid^2, Magstim Corp.). Immediately after the iTBS block, participants were moved back to perform single-pulse TMS to assess MEP modulation post-iTBS at specific time points: 5-, 20- and 45-min. Safety and tolerability were assessed within 5 min after iTBS with standard questionnaires and the visual analog scale (VAS).

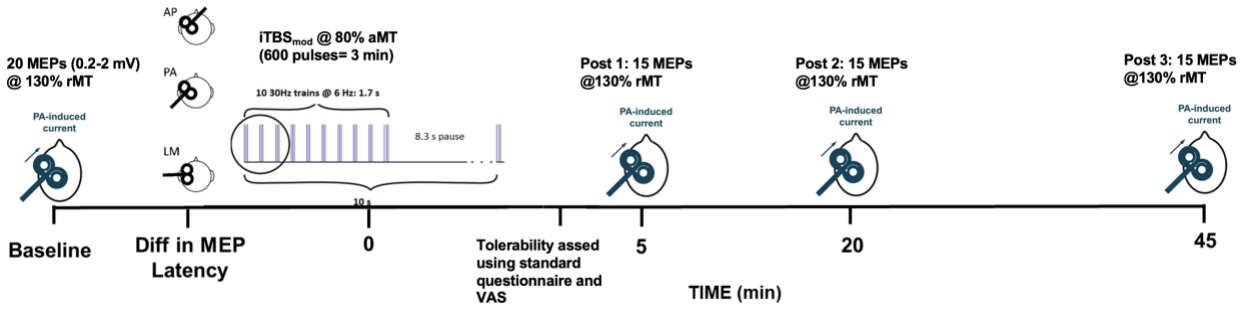

Figure 3.1 Schematic representation of the experimental protocol.

3.4 Baseline assessment of corticospinal excitability

Corticomotor excitability was assessed with participants comfortably seated in a recording chair with armrests. Movements of the head were restrained with a U-shape neck cushion. The right hand rested flat on a small wooden plate with two protruding blocks spaced to delimit the index and thumb finger position (Figure 3.2). Single-pulse TMS was delivered to the left motor cortex using a focal coil connected to a BiStim^2 stimulator (Magstim Corp., Whitland, Dyfed, UK) and targeted the motor hot spot of the first dorsal interosseus (FDI) muscle. MEPs were recorded using surface sensors (DE-2.1; Delsys Inc., Boston, MA, USA) placed over the right FDI. After amplification and filtering (Bagnoli™ 4 System; Delsys Inc., Boston, MA, USA, bandwidth = 6–450 Hz, gain = 1,000), electromyographic signals were digitized at a rate of 2 kHz (PCI-63203; National Instrument Corp., Austin, TX, USA) and transferred to a laboratory computer running custom software for off-line analysis. Prior to testing, the resting motor threshold (rMT) was determined using the Motor Threshold Assessment Tool software [2] (MTAT 2.0; Clinical Researcher, Knoxville, TN, USA) using a threshold amplitude of 50 μV for detecting MEPs. After rMT determination, baseline corticospinal excitability was assessed by applying consecutive pulses at an intensity equivalent to 130% rMT with 5-10 sec intervals between pulses. Twenty MEPs were recorded for each participant, avoiding extreme values (i.e., very small, <0.2 mV or very large MEPs, > 2 mV) to reduce inter-trial variability.

Figure 3.2 Experimental setup including the apparatus used to delimit the index and thumb finger position (right). Activation of FDI is achieved by pressing the index finger lightly against the top block.

3.5 Assessment of MEP latency with different coil orientations

Following the baseline assessment, single-pulse TMS was performed again to elicit MEPs with the coil placed in three different orientations to estimate D-wave and I-wave recruitment at the individual level (Hamada et al. 2013). Before testing, the active motor threshold (aMT) was determined while participants exerted a light contraction (about 10% of their max) of the right FDI by pushing the index finger against a wooden block (See Figure 3.2). The threshold amplitude was set at 200 μV for detecting the presence of MEPs with the MTAT software. The stimulator intensity was set at 110% of aMT for MEPs elicited with the coil positioned in the standard PA orientation

(i.e., with the handle pointing 45° backward). For the AP orientation (handle pointing 45° forward) and LM orientations (handle pointing downward), the stimulation intensity was increased to 140% aMT to ensure recruitment of D-wave (LM stimulation) and late I-waves (AP stimulation). For AP and PA stimulations, 15 MEPs were recorded, whereas ten were recorded for LM stimulation. These numbers were deemed sufficient to provide a reliable estimate of onset latency measures (Hamada et al., 2013). The order of testing with the different orientations was counterbalanced across participants.

3.6 30 Hz/6 Hz modified iTBS protocol

For the 30 Hz iTBS, participants were moved to another chair to allow for the rTMS application. The 30 Hz iTBS was delivered using a Magstim Rapid^2 stimulator (Magstim Co. Wales, UK) connected to a focal D70^2 high-efficiency coil. Before application, the aMT was re-assessed to account for the differences between the BiStim^2 and the Rapid^2 (e.g., different coil, monophasic vs. biphasic pulses). Once the aMT was determined, the stimulator intensity was set at 80% aMT in line with safety recommendations for TBS applications targeting the motor cortex (Oberman et al. 2011). The 30 Hz iTBS was delivered over the hand motor area and consisted of 10 trains of 30 Hz 3-pulse bursts applied at 6 Hz interval and repeated every 10 s (1.7 ON, 8.3 s OFF) for a total of 20 cycles (600 pulses over 192 s).

3.7 Post-iTBS changes in corticospinal excitability, safety and tolerability

Following the 30 Hz iTBS protocol, participants were quickly returned to the recording chair for single-pulse TMS. During the time between the end of the iTBS session and the first post-iTBS time point, participants completed an rTMS adverse events questionnaire to assess safety and tolerability. Participants were asked to rate on a scale of 0 to 5 (none, minimal, mild, moderate, marked, severe) if they experienced any of the following symptoms after the intervention:

headache, scalp pain, arm/ hand pain, other pain, other sensations (e.g., tingling, burning), weakness, loss of dexterity, vision/ hearing changes, ear ringing, nausea/ vomiting, rash/ skin changes, or others. The pain and discomfort associated with iTBS were also rated using the VAS. At 5 min post iTBS, 15 MEPs were elicited at rest (130% rMT) to assess changes in corticospinal excitability. The same measures were repeated at 20 and 45 min post-iTBS.

3.8 Analysis of MEP data

Analysis of MEP characteristics in terms of amplitude and latency was performed offline by the same investigator (KH) using custom software. MEPs were analyzed by first superimposing MEP traces recorded under each testing condition (i.e., baseline, MEPs AP/PA/LM orientations and post- iTBS). Peak-to-peak mean amplitude in mV and mean latency in ms was determined by visual inspection of individual traces. Individual measures of latency and amplitude were then reported in the database for further analysis.

3.9 Latency differences

As mentioned earlier, individual susceptibility to recruit early and late I-waves in response to single-pulse TMS was assessed by computing the latency differences between MEPs recorded with AP stimulation and with those recorded with LM or PA stimulation (Hamada et al. 2013). The latency difference was determined by subtracting the mean AP latency from the LM latency (i.e., AP-LM). As stated earlier, small differences (e.g., < 3 ms) reflected preferential recruitment of early I-waves (I_1-I_2), whereas large differences (e.g., > 4 ms) reflected preferential recruitment of late I-waves (I_3, I_4) in response to TMS.

3.10 Analysis of responses to 30 Hz iTBS

In line with previous studies (Hinder et al. 2014; Perellón-Alfonso et al. 2018), we computed MEP ratios to identify individuals who responded to the modified 30 Hz protocol.

Specifically, responders and non-responders were operationally defined using a cut-off of ±10% from baseline. A grand average was first computed by averaging the MEP amplitude in mV recorded at each time point post-iTBS. Then, MEP ratios were computed by expressing the grand MEP average relative to baseline MEP amplitude times 100 (i.e., $MEP_{grand\ avg}/MEP_{baseline}$) x 100). Using the 10% cut-off, individuals showing facilitation (i.e., MEP ratio > 110%) were considered responders, while those showing either depression (i.e., MEP ratio < 90%) or no modulation (90%<MEP<110%) were classified as non-responders. Note that due to a technical issue, MEPs were not recorded for participant KH-19 at the 5 min post-iTBS time point. In this case, we averaged the MEP amplitudes from 20- and 45-mins time points to fill the missing value.

3.11. Statistical analysis

D'Agostino-Pearson's test revealed that amplitude data at specific intervals post-iTBS were not normally distributed. As suggested by Nielsen (1996), amplitude data were log-transformed to normalize the distributions. After log-transformation, variations in MEP amplitude were entered into a one-way repeated measures analysis of variance (ANOVA) with *Time (0,5,20,45 min)* as the repeated factor. Dunnett's post-test was used for *post hoc* comparisons. The influence of *Sex* was not considered in this analysis for our sample of participants consisted mainly of females (15/21) and, given evidence that sex differences have little effect on responses to NIBS (Pellegrini et al. 2018). Latency data were normally distributed and did not need transformation. A one-way repeated measures ANOVA was also performed on latency data to compare differences at the different coil orientations (AP, PA and LM) using Tukey's post-test for *post hoc* comparisons. Finally, a linear regression analysis was used to determine whether latency differences were predictive of MEP modulation following iTBS. The significance level was set at

5%. All statistical tests and graphs were produced using GraphPad Prism version 9.01 (San Diego, CA, USA).

CHAPTER IV: RESULTS

4.1 Baseline measures of excitability and latency differences

Of 21 participants, 19 (13 females) completed the protocol without any issues. Two participants (female) had to be excluded after experiencing minor adverse reactions (i.e., lightheaded, nauseous) to single-pulse TMS. At baseline, the average rMT was 44.1 ± 8.8 % MSO, and the mean MEP amplitude was 1.1 ± 0.8 mV. The average aMT, as determined with the BiStim^2 stimulator, was 33.0 ± 5.7% % MSO.

Figure 4.1(a) shows the distribution of latency values measured with the different coil orientations. As expected, participants exhibited the shortest latency values in response to LM stimulation (mean, 19.8 ms). Latencies measured in response to PA stimulation were also short (mean, 20.8 ms) and only slightly longer than those measured for LM stimulation. Also, as expected, the most prolonged latencies were measured in response to AP stimulation in all participants (mean, 23.0 ms). The fact that latencies differed significantly at the different coil orientations was confirmed by the ANOVA ($F_{2,34}$, $p<0.001$). Post-hoc comparisons also confirmed that latencies measured with LM and PA orientations were significantly shorter than those measured with AP orientation (Tukey's post-test, $p<0.001$) and that there was no significant difference between the two ($p=0.19$) (Figure 4.1). Given that some participants (n=4) exhibited a shorter latency with PA stimulation than with LM stimulation, the PA latency was used to compute the latency differences in those cases. The distribution of latency differences (i.e., AP-LM/PA) computed in each participant is shown in Figure 4.1 (b). As evident in the figure, participants exhibited a relatively wide range of differences (1-7.5 ms) with a median value at 3.5 ms.

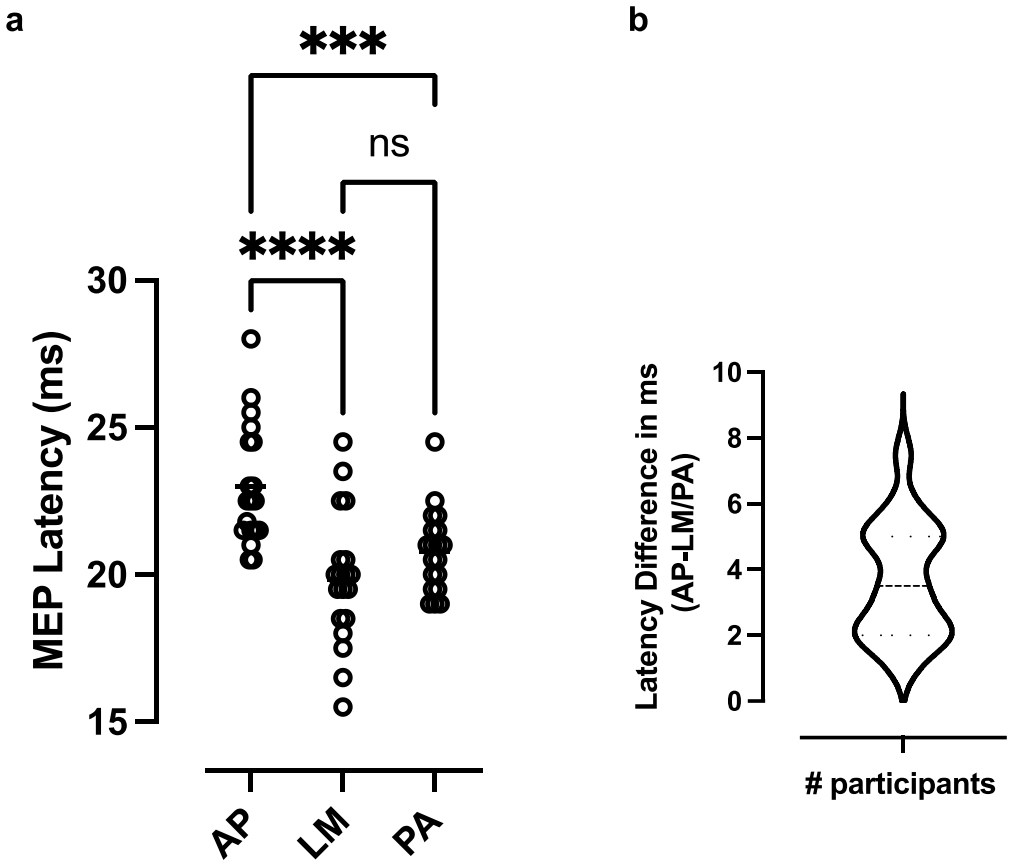

Figure 4.1 (a) Distribution of individual MEP latency values obtained when stimulating at different coil orientations (AP, Anterior-Posterior; LM, Latero-Medial; PA, Posterior-Anterior). (b) Distribution of latency differences computed between AP and either LM or PA stimulation in all participants (b). In (a) the level of significance from comparisons between latency values is indicated by the asterisks (***$p<0.001$, ****$p<0.0001$). In (b), the thick dotted line indicates the median, whereas the upper and lower quartiles are indicated by the thin dotted lines.

4.2 Tolerability and MEP modulation in response to iTBS

Only mild adverse events were reported in association with the iTBS protocol. About three-quarters of the participants (14/19) experienced mild side-effects (ratings 1-3/5), mainly during the

application in the form of scalp sensitivity (7/19), headache (6/19), and tingling or burning sensations (7/19). Most participants reported little to no pain (mean VAS score, 1.1± 1.5 cm), although one participant did report significant pain (VAS score, 6 cm). This high rating was likely related to the higher intensity used for iTBS in this participant as he also exhibited an unusually high aMT (67% MSO).

Regarding MEP modulation, the distribution of individual MEP log amplitude measured at each time point before and after iTBS is shown in Figure 4.2. It can be seen that MEPs tended to be enhanced post-iTBS with greater enhancement at 20 and 45 min. The fact that MEP amplitude was significantly modulated in response to iTBS was confirmed by the ANOVA ($F_{2, 42}$, p= 0.007). Post-hoc comparisons also confirmed that MEPs recorded at 20- and 45-min post were significantly larger than those recorded at baseline (Dunnett's *post*-test, p=0.02).

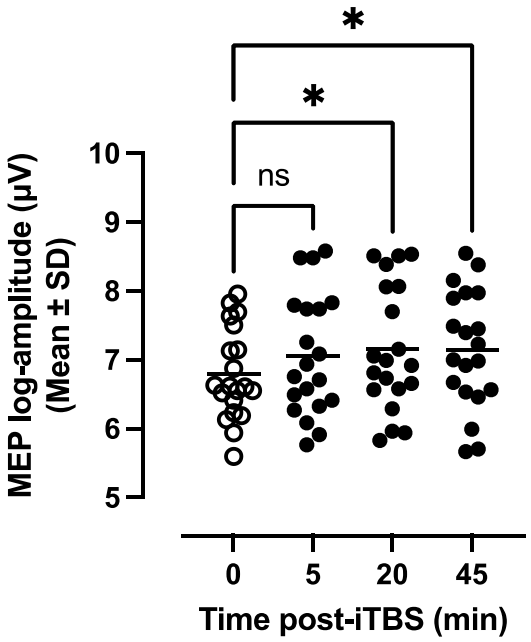

Figure 4.2 The distribution of individual MEP log-amplitudes measured at each time point before and after iTBS. MEP log-amplitudes recorded at 20- and 45-minutes post iTBS were significantly different from those recorded at baseline (Time 0) (Dunnett's post-test comparison, *p<0.05).

4.3 Variability of individual responses

Although many participants exhibited the expected MEP facilitation post-iTBS, some variability was also observed. This variability can be appreciated by inspecting Figure 4.3 (a), where individual changes in MEP amplitude relative to baseline are shown post iTBS. Of 19 participants, 68% (n=13) were classified as responders (112-388% facilitation), while the remaining 32% (n=6) were classified as non-responders showing either suppression (n=3, range, 65-73%) or no modulation (n=3, range, 96-104%). Typical examples of MEP modulation in responders and non-responders following iTBS are shown in Figure 4.3 (b).

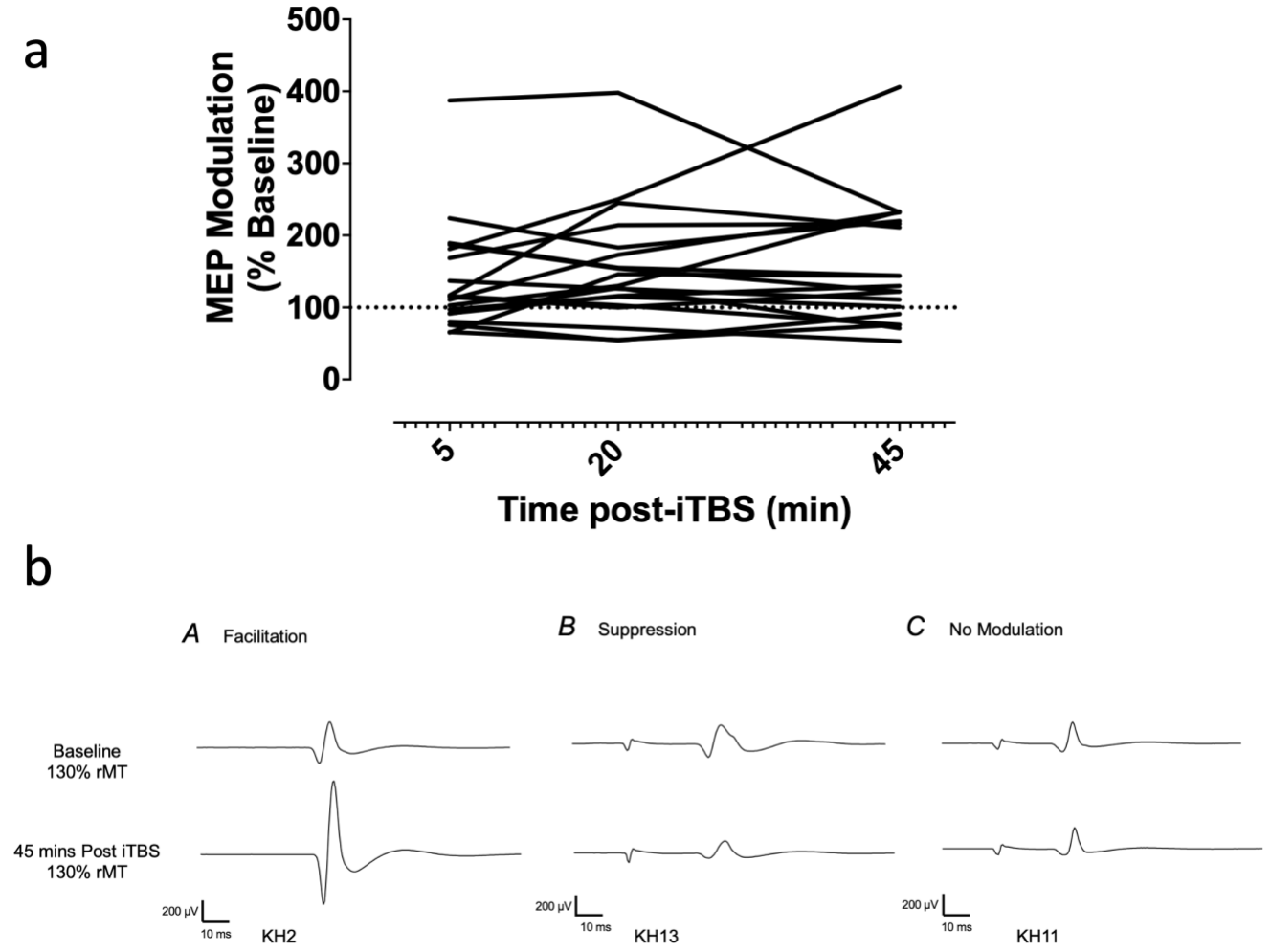

Figure 4.3 (a) Individual iTBS effects reflected as changes in MEP size relative to baseline. Each line represents the relative change in MEP amplitude (% baseline) recorded across time points post-iTBS for each participant. b) Typical examples of MEP modulation observed in response to iTBS in selected participants (A, facilitation; B, suppression; C, no-modulation).

4.4 Latency differences as predictors of responses to iTBS

Figure 4.4 (a) shows the relationship between individual latency differences and corresponding normalized MEP amplitude in response to iTBS. This relationship was inverse, with

large differences being associated with no modulation or depression, while small ones were associated with facilitation. The linear regression analysis revealed that latency differences were significant predictors of responses to iTBS, accounting for 24% of the variance in MEP amplitude (r^2=0.24, p=0.03). To further examine the inverse nature of the association, participants were regrouped based on the median latency difference into an 'early I-waves' (n=11, Difference <3.5 ms) and a 'late I-waves' (n=8, Difference >3.5 ms) group (Hordacre et al. 2017; Dam et al. 2021). As shown in Figure 4.4 (b), the early I-waves group tended to show larger MEP facilitation on average when compared to the late I-waves group. However, the difference was not significant when compared with the Mann-Witney test (U=32, p=0.31), given the variability and the small number of observations in each group.

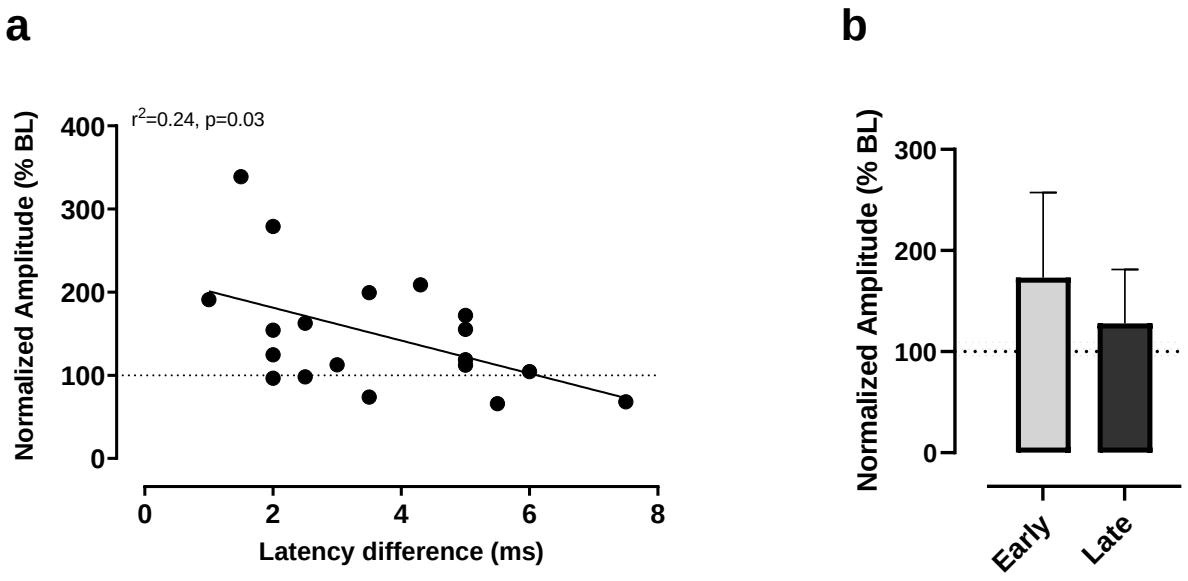

Figure 4.4 (a) The relationship between individual latency differences and corresponding normalized MEP amplitude in response to iTBS. (b) Comparison of MEP amplitude modulation in "early I-wave" and "late I-wave" groups. The "early I-wave" group consisted of individuals in whom latency differences were smaller than the median difference (<3.5 ms, n=9), whereas "late I-wave" group consisted of individuals in whom latency differences were larger than the median (>3.5 ms, n=10). Note the trend for larger MEP facilitation post- iTBS in the Early vs. Late group.

CHAPTER V: DISCUSSION

In the present study, we sought to determine whether a modified 30/6 Hz iTBS protocol could induce lasting modulation of corticospinal excitability and, at the same time, reduced the variability of responses. Our results showed that the 30 Hz iTBS effectively induced lasting modulation with significant MEP facilitation detected up to 45 min post-intervention. Further to this, our analysis of responders showed that these effects were relatively consistent, with more than two-thirds of the participants exhibiting the expected facilitation. Our regression analysis also revealed that small latency differences were associated with facilitation, a finding contrasting with previous reports. In the following discussion, we will address the significance of these findings for the applications of iTBS protocols in experimental and clinical settings.

5.1 Corticospinal excitability and latency differences at baseline

At baseline, our group of participants exhibited the expected variations in rMT and MEP amplitude for adults in their age range (19-40 years). More specifically, both the average rMT (mean, 43%) and MEP amplitude (mean, 1.1 mV) were in line with previous reports on the reliability of measures of corticomotor excitability (Brown et al. 2017; Hordacre et al. 2017). The range of latencies measured in our participants in response to stimulation at different coil orientations was comparable to that reported in previous studies (Davidson et al., 2016; Higashihara et al. 2020). The observation that some participants (4/19) exhibited a shorter latency with PA stimulation than with LM stimulation may have reflected individual differences at the anatomical or physiological level in the ability of TMS pulses to recruit D-wave or I_1 wave (Di Lazzaro et al. 2018). At any rate, the observed range of latency differences (1-7.5 ms) corresponded with that reported by Hamada et al. (2013; 0-6.5 ms).

5.2 Tolerability, MEP modulation and variability of responses to 30 Hz iTBS

Regarding tolerability, the 30 Hz iTBS intervention was well tolerated by our group of participants and, more importantly, no serious adverse events were reported. While two participants had to be excluded, these exclusions were related to vaso-vagal reactions after experiencing single pulse stimulation, which is uncommon but can happen in susceptible individuals (Gillick et al. 2015). We surmised that these reactions were partly attributable to the pandemic context and that participants had to wear masks during testing. Concerning the iTBS protocol, while many participants (74%) reported adverse events, these were generally mild and consisted of the expected side effects of rTMS applications (i.e., headache, scalp pain and craniofacial discomfort). The overall level of pain perceived in association with the iTBS session was lower (mean, 1-cm) than that reported by Malm and colleagues (2020) following 50 Hz/ 5Hz iTBS in a group of clinically depressed patients (median VAS of 4 cm). However, in this study, iTBS targeted the prefrontal cortex (DLPFC) for a total of 2400 pulses and at 90% rMT, which may have accounted for the higher pain ratings. Interestingly, they did report that the painfulness of active iTBS decreased over time, converging on sham iTBS so that the two became equally tolerable towards the end. In the present study, only one participant did report a high level of pain, and as mentioned, this report was linked to high intensity of stimulation during iTBS, confirming that intensity is the main factor driving pain and discomfort during application.

In terms of MEP modulation, our analysis following iTBS showed that evoked responses were facilitated up to 45 min after application. The observation that significant facilitation was detected at 20 and 45-min post-iTBS and not at 5 min is consistent with a recent meta-analysis by Chung et al. (2016). In analyzing the results of 87 iTBS studies, these authors concluded that iTBS facilitatory effects on MEPs were greater at mid-time points (20-30 mins) than early time-points

(< 5 min) post-intervention. However, these authors also noticed that iTBS effects were more variable at later time points (i.e., >30 min post), which contrasts with the strong facilitation we detected at 45 min. On the other hand, another recent quantitative review by Wischnewski and Schutter (2015) concluded that iTBS increases excitability for up to 60 min, consistent with our current observation. In terms of the size of facilitation, on average MEPs were facilitated by about 40% over baseline (mean 143%), an increase larger than that reported by Wischnewski and Schutter (2016) in their quantitative review of iTBS effects. Such a difference reinforces our contention that the 30 Hz protocol was effective in eliciting robust facilitation. In agreement with this, Pedapati et al. (2015) reported similar large effects (up to 1.5-fold increase in MEP size) in children and adolescents in response to 300 pulses 30 Hz/6 Hz iTBS. Thus, in line with other reports on iTBS effects, our modified 30 Hz/6 Hz iTBS was highly effective in eliciting lasting MEP facilitation with an overall increase in corticospinal excitability above the level reported in previous studies based on the standard 50 Hz/5 Hz protocol.

Besides effectiveness, another advantage of the 30 Hz iTBS protocol, as mentioned earlier, is allowing a wider range of stimulation intensities. For instance, in our group of participants, three would not have received adequate stimulation if the standard 50 Hz protocol had been used, for their aMT dictated an intensity >50% of the stimulator output, which is not possible to deliver with the Magstim, Rapid^2.

In terms of variability, much like other iTBS reports, not every participant exhibited the expected facilitation following 30 Hz iTBS. As stated earlier, inter-individual variability has been a lingering issue in TBS studies for more than a decade now, with a growing number of studies reporting no change in cortical excitability or an “opposite” effect to what is expected (Goldsworthy et al. 2021). To our knowledge, only one recent study has observed a similar rate of

facilitatory responses (i.e., 68%; Guerra et al. 2020b). following standard iTBS. The majority of studies using the standard iTBS protocol have reported much lower response rates, including McCalley et al. (2021), who reported only 33% of participants displaying facilitation. It may be argued that high inter-individual response variability will persist regardless of the TBS protocol used in terms of bursting frequency and inter-burst intervals. For instance, protocols used to induce LTP and LTD in animal models are far more precise than rTMS protocols in the human scalp, which are more diffuse, leading to activation of large cortical networks comprised of a greater variety of cell types. Likewise, slice experiments suggested a blurred line between LTP and LTD, as both responses can be induced using identical stimuli on different parts of the neuron or under different experimental conditions (Hirsch and Crepel 1990; Nishiyama et al. 2000; Shen et al. 2003; Liu et al. 2004). At this time, we would also like to entertain the idea that reducing the variability of neurophysiological responses to TBS is not always required or desirable (Guerra et al. 2020a). For example, the variability in response to TBS and other NIBS paradigms may reflect natural properties of cerebral cortices and underlying physiological mechanisms (Bergmann et al. 2012; Keil et al. 2014; Guerra et al. 2016; Ferreri et al. 2017). A detailed understanding of these sources of variably will in turn, provide a basis for altered response to TBS in several neurological disorders (e.g., Suppa et al. 2016a, Suppa et al. 2017) and will aide in the design of more optimal interventions that are tailored to the individual.

5.3 Predictor of responses to iTBS from latency differences

In the present study, we found an inverse relationship between iTBS after-effects in terms of MEP modulation and latency differences. Participants with slight differences tended to show MEP facilitation, while those with large differences tended to show suppression or no response. Such a relationship contrasts with the positive association reported by Hamada et al. (2013), who

found that the larger the latency difference (i.e., greater recruitment of late I-waves) the greater the MEP facilitation. Before attempting to interpret this apparent contradiction, it is important to emphasize that not all TBS studies have found the positive relationship reported by Hamada et al (2013). For instance, Hinder et al. (2014) found no association between large latency differences (i.e., > 4 ms) and MEP facilitation following 50 Hz iTBS. In fact, in their report, 75% of the participants exhibiting MEP facilitation following iTBS exhibited small AP-LM latency differences (<4 ms), which is somewhat in line with the present observation linking MEP facilitation with small differences. More recently, Rocchi et al. (2018), in exploring predictors of responses to cTBS, found no correlations between AP-LM latency difference and cTBS aftereffects. Thus, not all studies are in agreement with the notion that preferential recruitment of late I-waves, as reflected in large AP-LM differences, are predictive of positive responses to iTBS.

The inverse relationship we found between AP-LM/PA latency differences and MEP modulation suggests that preferential recruitment of early I-waves was likely a critical factor in mediating the aftereffects of 30 Hz iTBS. Although speculative, it is conceivable that for 30 Hz/6 Hz protocol, the recruitment and modulation of early I-waves might be more critical than for 50 Hz/5 Hz. In this respect, it is worth noting that the superiority of the 30 Hz over the 50 Hz TBS protocol was initially described for cTBS, where Goldsworthy et al. (2012) showed that the 30 Hz protocol induced more significant and more lasting depression in MEPs. Given that the inhibitory effects of cTBS are thought to involve a reduction in the excitability of circuits generating early I-wave (Di Lazzaro et al. 2005b), it is tempting to suggest that 30 Hz/6 Hz combination might be more efficient in modulating early I-waves. Recruitment of early I-waves has also been implicated in other facilitation-inducing TMS paradigms. For instance, Di Lazzaro et al. (1999) showed that modulation of I_1 wave was critical in determining the magnitude of short-interval intracortical

facilitation (SIFC), a form of facilitation observed when a suprathreshold TMS pulse is paired with a second suprathreshold pulse delivered at 1-5 ms interval. Moreover, a recent study by Higashihara et al. (2020) found that individuals exhibiting short AP-LM latency differences (< 4 ms) also exhibited significantly higher SICF when compared to participants with large latency differences (> 4 ms). These findings confirm that facilitatory effects are more likely to be expressed in individuals in whom recruitment of early I-waves are easily achieved via TMS. Interestingly, in the report of Hamada et al. (2013), individuals who exhibited opposite responses to cTBS (i.e., MEP facilitation instead of depression) were also those that showed small AP-LM latency differences.

While recruitment of I-waves and individual susceptibility to TMS appears to be a critical factor in predicting TBS aftereffects, other factors might be important as well. In fact, in our group of participants, differences in latency explained about 25% of the variance in MEP amplitude modulation, leaving a substantial proportion unexplained. Pharmacological studies suggest that the LTP-like after-effects of iTBS (Huang et al. 2007) due to NMDA receptor-dependent glutamatergic transmission. One theory is that differences between individuals in baseline levels of glutamate and GABA, and hence the balance between cortical excitation and inhibition, may contribute to varying responsiveness (Krause et al. 2013; Krause and Cohen Kadosh 2014). On this basis, the same NIBS paradigm, whether it be iTBS or other forms of rTMS, may result in variable responses, such that some individuals reach optimal levels of excitation, while others show little to no effect. In addition, it has been suggested that the variable responses to TBS could be due in part, to genetic factors (Cheeran et al. 2008). Specifically, brain derived neurotrophic factor *(*BDNF) polymorphism has been associated with measures of cortical plasticity (Cheeran et al. 2008; Cheeran et al. 2009; Antal et al. 2010; Cirillo et al. 2012; Lee et al. 2013; Chang et al.

2014; Di Lazzaro et al. 2015) including both experience-driven and human cortical plasticity induced by iTBS (Kleim et al. 2006; Cheeran et al. 2008). Finally, other factors related to age differences, baseline excitability and time of day have been identified as potential factors to predict TBS effects (Corp et al. 2020).

5.4 Study limitations

This study, like all studies, is not without its limitations. Firstly, while our sample size was acceptable, a larger sample size would have been preferable, given that high variability is often congruous with TBS experiments in which sample sizes exceed 20 (Guerra et al. 2020a). Along the same line, some may argue that more MEPs (i.e., 20-30 trials) should have been collected at each post-iTBS time point as a means to provide a more stable measure of MEP amplitude with less within- and between-session reliability. However, by using 130% rMT, which has been shown to elicit more reproducible MEP amplitude (Brown et al. 2017), variability could be reduced in a more time-efficient manner that also minimizes the number of stimuli administered. The latter is an important consideration given that single TMS pulses have been shown to induce cumulative changes in MEP amplitude over a single experimental session (Pellicciari et al. 2016).

Additionally, as mentioned earlier, age-related differences could contribute to the variability of responses to 30 Hz iTBS (Ridding and Ziemann 2010; Freitas et al. 2011; Young-Bernier et al. 2014). Unfortunately, because of COVID-19 restrictions, the recruitment of seniors for in-person research was not allowed. Furthermore, although we were permitted to recruit adults up to 55 years old, there was limited interest in study participation from older adults in this age range. The pandemic undoubtedly may have contributed to limit participation, given that recent studies have reported progressively higher threat and fear perceptions of COVID-19 as respondents age (Niño et al. 2021).

Finally, we did not directly compare our modified iTBS protocol to the standard on the basis that there is already substantial evidence regarding the effects of 50 Hz/5 Hz protocol (e.g., Suppa et al., 2016; Chung et al., 2016). Also, we wanted to minimize the time on site for both the research staff and participants in light of local COVID-19 guidelines. The lack of a sham condition is another possible limitation, but the goal of this study was not to test efficacy but rather to investigate the effects of a modification to the standard iTBS protocol. Nevertheless, the addition of a sham condition could have helped to pinpoint the effects of expectations and anticipation on individual responses to 30 Hz TBS (McCalley et al. 2021).

5.5. Conclusions

In conclusion, the present study investigated the effects of a modified 30 Hz / 6 Hz iTBS protocol on corticospinal excitability. Our results showed that corticospinal excitability was increased for up to 45 min post-iTBS. Furthermore, these effects appeared less variable than those reported before for the standard protocol, with more than two-thirds of the participants showing the expected MEP facilitation. Also, our regression analysis of latency differences as predictors of iTBS effects pointed to a different mode of action for the modified protocol with modulation of circuits generating early, as opposed to late I-waves, as a preferential mechanism leading to MEP facilitation. Altogether, these results suggest that the modified 30 Hz iTBS might be a sound alternative to the standard protocol to induce neuroplasticity. This finding may have implications for the applications of TBS interventions in clinical populations.

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