Time Course of Corticospinal Excitability in Simple Reaction Time Tasks

Michael Kennefick

Thesis Supervisor

Dr. Anthony Carlsen

Thesis Submitted in Partial Fulfillment of the Requirements
for the Master of Science Degree in Human Kinetics

School of Human Kinetics
Faculty of Health Sciences
University of Ottawa

Statement of Contribution of Collaborators

I hereby declare that I am the sole author of this Masters' thesis. I performed all experiments and data analysis for this thesis with the guidance of my research supervisor, Dr. Anthony N. Carlsen.

The original conception of the experiments in this thesis were performed in collaboration with Dr. Dana Maslovat (Department of Kinesiology, Langara College) and Dr. Romeo Chua (Department of Kinesiology, University of British Columbia).

Editorial corrections were provided by my supervisor and all articles in this thesis are co-authored by him.

Acknowledgements

The past two years have been a journey filled with more questions than answers. This journey, undoubtedly, would not have been possible without the help, support, and guidance of several people. I would first like to thank my supervisor Dr. Tony Carlsen, who has developed a great sense of community within the lab. This has allowed everyone within the lab to talk about experiments, ideas, problems, but most importantly, to become friends. Dr. Carlsen has always found the time to help me when I needed it, but always in a way that challenged me to answer my own questions, and for this I am grateful. His guidance, mentorship, and support has been invaluable and has allowed me to grow as a researcher. Without his help, I never would have been able complete this journey.

I would also like to thank my family and my girlfriend, who have always supported and believed in me. To my parents, I would like to thank you for always encouraging me to make my own decisions, and to make it a point to offer guidance, but to not influence me when it came time to make these decisions. To Erinn, thank you for always believing in me, asking questions, and for some reason, always finding anything related to my research interesting.

Table of Contents

Table of Abbreviations

Definition	**Abbreviation**
Active motor threshold	AMT
Corticospinal excitability	CE
Electroencephalography	EEG
Electromyography	EMG
Flexor digitorum superficialis	FDS
Imperative stimulus	IS
Motor-evoked potential	MEP
Reaction time	RT
Repetitive TMS	rTMS
Resting motor threshold	RMT
Short-interval intracortical inhibition	SICI
Transcranial electric stimulation	TES
Transcranial magnetic stimulation	TMS
Warning signal	WS

List of Figures and Tables

Abstract

The process of movement execution can be separated into two sections; the foreperiod and the response time. The foreperiod represents the time between the warning signal (WS) and the presentation of the imperative “go” signal, and the response time incorporates both the reaction time (RT) and the movement time (Schmidt & Lee, 2011). Transcranial magnetic stimulation (TMS) was used to probe corticospinal excitability (CE) which has been measured in a variety of RT tasks during both the foreperiod and the response time periods. The purpose of the two studies in this thesis was to measure when and at what rate changes in CE occur in both simple and complex tasks. The results of the first experiment indicated that CE levels quickly increased from baseline with the presentation of the WS. This was followed by a holding period in which CE was held constant until a decline in CE occurred prior to the presentation of the IS. This decline was followed by a rapid increase in CE as the movement was initiated and released. Importantly, even though levels of CE were decreasing relative to the start of the decline, participants were still in a heightened state as they prepared to release their movements. Furthermore, it is suggested that selective inhibitory control mechanisms were at least partly responsible for the decline prior to the IS. The results of the second experiment indicated that MEP amplitudes in a simple task were significantly larger compared to those in a complex task relative to both the IS and the onset of electromyography. These findings suggest that simple and complex tasks achieve differing levels of corticospinal excitability, and it is suggested that the complex requires the use of the cerebellum, which suppresses excitatory projections to the thalamus, and consequently to the motor cortex.

Chapter I: Literature Review

In the 1970s, Merton and Morton were the first researchers to electrically stimulate the brain without opening the skull, by using electrodes in order to penetrate it (Merton & Morton, 1980). In the early 1980s, transcranial magnetic stimulation (TMS) of the brain appeared and promised to be a major advancement in brain stimulation. Instead of using electrodes to generate electrical currents in the brain, TMS stimulates the brain using the principles of electromagnetic induction to generate electrical currents non-invasively (Barker, Freeston, Jalinous, Merton, & Morton, 1985). The first magnetic stimulation experiments were conducted by Polson and colleagues (1982) who used a prototype time-varying magnetic field machine designed to induce an electrical current in the vicinity of a nerve, thereby stimulating it. TMS was touted as the natural progression from transcranial electrical stimulation (TES) due in large part to three benefits: The first benefit was its ability to penetrate bony structures such as the skull, which has between 8 and 15 times the resistance to electricity compared to soft tissue (Adrian & Yamagiwa, 1935), with relative ease (Barker & Jalinous, 1985). Second was its ability to stimulate the brain with very little discomfort to the participant, which is reflective of the greater stimulation intensity need to achieve neural stimulation with TES. The third benefit is that TMS does not require any kind of physical or electrical contact with the body; stimulation can be achieved with the coil tens of millimetres from the body (Barker, Freeston, Jalinous, & Jarratt, 1987).

TMS has the ability to evoke descending corticospinal activity in response to stimulation of the motor cortex. Magnetic stimulation is thought to actively initiate an action potential by depolarizing cortical neurons and/or altering the level of neural excitability. Furthermore, TMS may also manifest its effects through the induced modification of membrane resting potentials and thresholds (Wagner, Rushmore, Eden, & Valero-Cabre, 2009).

1. Corticospinal Activation

Experiments using both electrical and magnetic stimulation have led to a greatly improved knowledge of the human motor system (Di Lazzaro, Ziemann, & Lemon, 2008). For example, experiments on monkeys from the early 1950s demonstrated that a single electric pulse on the surface of the brain recruited corticospinal (output) neurons by activating the axons of the pyramidal tract neurons directly or indirectly. Output from directly activated axons are known as D-waves, while that from indirectly activated axons are known as I-waves, which are activated transsynaptically via delay chains of interneurons. These waves can be distinguished from one another based on their latencies. The D-wave represents the earliest descending volley, while the I-wave represents the later volley (Patton & Amassian, 1954). The D-wave evoked by a posterior to anterior TMS coil orientation has the shortest latency. It is therefore believed to originate from the activation of corticospinal axons in the subcortical white matter at some distance from the cell body. Lower intensity TMS stimulation with a posterior to anterior induced current evokes a single descending wave that is thought to originate from monosynaptic corticospinal connections projecting onto corticospinal neurons. This descending wave, indirectly activated via corticospinal cells, is termed the I_1-wave. At higher intensities, later I-wave (I_2,I_3, etc.) volleys appear and are thought to originate from a more complex circuit whose activation produces a repetitive discharge of pyramidal tract neurons (Di Lazzaro, et al., 2008).

2. Motor Evoked Potentials

TMS can induce a muscular response, measured using electromyography (EMG), called a motor-evoked potential (MEP). A variety of parameters relating to the MEP can be examined, such as latency, size, threshold, and silent period, amongst others. There are three basic physiological mechanisms that may influence the size of the MEP: the number of recruited motor neurons in the spinal cord, the number of motor neurons discharging more than one stimulus, and the synchronization of the TMS-induced motor neuron discharges. A MEP increases with an increasing stimulus intensity, which suggests that stronger stimuli may also recruit more corticospinal neurons, more spinal motor neurons, or both (Rosler & Magistris, 2008).

3. TMS Techniques

A variety of TMS techniques exist, including single-pulse as well as paired or multi-pulse protocols. Single-pulse TMS provides a measure of corticospinal excitability (CE) and can be used to map muscle representation in the motor cortex. Techniques include stimulation at motor threshold, subthreshold, and suprathreshold stimulation methods for single-pulse TMS (explained in more detail below). In contrast, paired or multi-pulse protocols involve methods known as short-interval intracortical inhibition (SICI), long-interval intracortical facilitation, short-interval cortical facilitation, intracortical facilitation, and repetitive TMS (rTMS). For the purposes of this proposal, only the single-pulse techniques will be discussed.

3.1. Motor threshold

Motor threshold is a measure of the TMS intensity necessary to evoke a peripheral motor response. It is generally lower for distal compared to proximal muscles (Rossini et al., 1994), highly variable across individuals, but remarkably constant for a given individual (Ziemann & Hallett, 2000). Because of this variability, most TMS researchers attempt to correct for this by dosing TMS intensity as a proportion of each individual's motor threshold (McConnell et al., 2001). Motor threshold can be measured while the participant is at rest, known as the resting motor threshold (RMT), or when the participant is slightly contracting the target muscle, known as the active motor threshold (AMT). RMT is typically ~10% of maximum stimulator output higher than AMT, produces lower amplitude MEPs, and is typically used as a reference to set the stimulation intensity during subsequent MEP recordings. AMT is less commonly used, used as an alternative measure, or used as an additional value. Furthermore, AMT can be useful if the participants are unable to remain at rest, or if attempting to measure MEPs in lower extremity muscles (Sandbrink, 2008). Motor threshold is found by determining the minimum TMS intensity that is sufficient to elicit an MEP in 50% of trials; known as the Rossini-Rothwell method (Rossini et al., 1999). Furthermore, it is suggested that 50% of trials represents a total of 5 out of 10 trials with a minimal EMG response amplitude of 50μV while measuring RMT (Ziemann, Corwell, & Cohen, 1998) and ~200-300 μV while measuring AMT since distinguishing a MEP from background muscle activity is difficult (Rossini, et al., 1994).

3.2 Subthreshold and Suprathreshold TMS

Two TMS techniques that are used in research are subthreshold and suprathreshold TMS, commonly measured as an intensity that is a relative percentage of the participant's resting or active motor threshold. Subthreshold TMS refers to a stimulus intensity that is lower than the participants previously measured RMT and does not produce a MEP at rest, while suprathreshold TMS refers to a stimulus intensity that is higher than the measured

RMT and does produce a MEP. A subthreshold TMS pulse could potentially behave very differently in relation to the RMT or AMT. For example, a TMS pulse whose intensity would be considered “subthreshold” with respect to RMT might be considered “suprathreshold” with respect to AMT. Due to the fact that the current proposed experiments will require the participant to begin each trial at rest, suprathreshold TMS intensities will be used that are a percentage of each participant’s RMT.

4. Corticospinal excitability in simple reaction time tasks

4.1 Foreperiod excitability

Studies that have previously been performed on monkeys have shown that instructions regarding an upcoming movement results in anticipatory activity in the motor cortex. These changes in activity persist for several seconds as the monkey awaits the cue to begin the movement. During the interval between the warning stimulus and the IS (i.e. the foreperiod), 61% of pyramidal tract neurons modulated their discharge patterns according to the nature of the instruction. For example, if the upcoming movement was a push movement, activity in the neurons that usually discharge during push movements increased. Conversely, activity in the neurons that usually discharge during pull movements decreased (Tanji & Evarts, 1976).

4.2 Post-movement excitability

Chen and colleagues (1998) used TMS to examine the specific time course of CE before and after the IS in a simple RT task as well as a self-paced movement task. In the study, participants were instructed to abduct their right thumbs as fast as possible following the IS. TMS was delivered at both subthreshold (90% RMT) and suprathreshold (110%

RMT) levels for simple RT as well as self-paced movement conditions at pseudorandomized time points between 30 and 600ms following the IS. The subthreshold stimulus was adjusted such that it did not normally produce a MEP; however a minimal increase in the excitability of the motor cortex would lead to a recordable MEP. EMG data were collected from the right abductor pollicis brevis and MEPs were identified within the EMG. Results indicated that CE increased before EMG onset for simple RT and self-paced movements in response to subthreshold TMS. There was an increase in CE 80ms before EMG onset, which continued for 160ms following EMG offset in the simple RT task. CE for self-paced movements increased 100ms before EMG onset and is therefore increased for 20ms longer than the simple RT task. Post-hoc analysis showed a significant increase in CE beginning at 50ms before EMG onset, and a trend of increased CE 150ms after EMG offset in response to suprathreshold TMS; similar to that of subthreshold TMS.

5. Inhibition during the process of movement execution

First described by Kujirai and colleagues (1993), SICI is a paired-pulse TMS technique that uses a subthreshold conditioning stimulus, followed by a suprathreshold test stimulus, which suppresses MEP amplitudes. Reynolds and Ashby (1999) used SICI in order to probe inhibition in the motor cortex during the generation of voluntary movements. Results indicated that inhibition of the agonist muscle preceded the onset of movement. Similarly, Ridding and colleagues (1995) showed a reduction of inhibition during the maintenance of a tonic muscular contraction, which suggested that voluntary drive reduced the excitability of inhibitory circuits in the active muscle, increasing the excitability of the cortex, and therefore facilitating movement. Taken together, the above mentioned inhibition studies demonstrate that prior to voluntary movement, inhibition within the motor cortex is reduced and excitability is increased. Furthermore, these changes are specific to the muscles required and are sustained during their activation (Stinear, Coxon, & Byblow, 2009).

6. Task Complexity

In their seminal experiment, Henry and Rogers (1960) sought to understand how the complexity of a movement affected RT. The experiment consisted of three simple RT tasks, each with a varying number of movement components. The first movement (A) involved the participant lifting his or her finger from a button only a few millimeters. The second movement (B) involved the participant lifting his or her finger from the button and then grasping a hanging tennis ball approximately 30cm ahead of the starting position. The third movement (C) made use of a second hanging tennis ball mounted 30cm lateral to the first tennis ball. The participant lifted his or her finger, reached forward and upward to strike the second tennis ball with the back of his or her hand, then reversed direction to touch a second button, before striking the first tennis ball. Results demonstrated that RT for the least complex of the three movements, movement (A), was faster than the second most complex movement, movement B, by 36ms. Furthermore, movement B was faster than the most complex movement, (movement C), by an additional 13ms.

Klapp and colleagues (1974) further investigated these differences in RT by manipulating the complexity of the movements, as opposed to altering the number of movements, using Morse code “dit” and “dah,” button presses, where “dit” was a short (press-release) button press and “dah” was a long (short-hold-release) button press. The simple RT task was designed with an emphasis on the participants attending to and utilizing advance information and they were instructed repeatedly to plan ahead in order to shorten their RTs. Results revealed that RTs were longer for the more complex “dah” response.

In a follow up experiment, Klapp and colleagues (1995) compared single and multiple element movements, There were four possible response patterns; a short “dit,” a long “dah,” or a combination of the short and long sequences (e.g. dit-dah-dah-dit or dah-dit-dit-dah). Results indicated that on day one of the simple RT experiment, single element movements had faster RTs than four element movements. Interestingly, after 8 days of practice, the RT of the four element movements decreased dramatically. Klapp and colleagues (1995) suggested that with practice, the four element sequences became recoded into a single “chunk.” Early in practice, the four element movement was programmed as four separate chunks, and by consequence had a slower RT. However, later in practice, both one and four component movements were programmed as single chunks, resulting in similar RTs. Following these findings, Klapp (2003) added the notion of “INT” and “SEQ”, where INT was related to internal programming of a chunk, and can be thought of as the single “dit” or “dah” movements. SEQ relates to a sequence of chunks, and could be thought of as a series of individual “dit” or “dah” movements. Single-chunk responses require process INT but not require process SEQ due to the fact that there is no sequence. INT is assumed to occur prior to the IS in a simple RT paradigm because the response is preprogrammed and simple RT does not include the time needed to complete INT; however since the response cannot be preprogrammed in a choice RT paradigm, INT is assumed to occur following the IS. Therefore, if INT required more time for a complex response, then choice RT will depend on response complexity, whereas simple RT will not. This RT pattern is known as the *choice-up-simple-flat* pattern. Multiple-chunk movements are more difficult to interpret because both INT and SEQ process are involved. In these movements, simple RT increases as a function of the number of chunks, but choice RT does not. This is opposite to what is seen in the choice-up-simple-flat pattern, and is thus termed the *simple-up-choice-flat* pattern. Multiple-chunk movements cannot be preprogrammed and both INT and SEQ processes occur following the IS. The simple-up-choice-flat pattern can be demonstrated in a manual button press task when the number of chunks (dits and dahs) increases. This task results in an increase in simple RT, but no increase in choice RT. Therefore, simple RT is assumed to depend on the number of chunks, and not on the chunk's internal complexities.

6.1. TMS and task complexity

Flament and colleagues (1993) investigated the effects of an isolated index finger abduction (simple) versus a variety of static gripping (complex) tasks on CE. For each static gripping task, the grip was held at 5% of MVC while TMS was delivered pseudo-randomly at intervals ranging between 3.5-7s in blocks of 20-25 stimuli over the optimal spot for eliciting MEPs in the tested muscles (at intensities ranging from 35-80% maximum stimulator output). Furthermore, TES was utilised in 3 participants at intensities ranging from 40-65% of maximum stimulator output. Results indicated that in every subject, MEPs were greater in at least one of the complex tasks compared to that of the simple finger abduction. Task-related variations in EMG responses were also seen during TES; however, the response amplitudes were smaller than with TMS.

Abbruzzese and colleagues (1996) used sequential finger movements with varying complexity levels in a non-RT task to detect if changes in excitability of the motor cortex are under the influence of other cortical areas involved in motor planning. Participants executed a variety of tasks in which they were required to execute a simple repetitive or complex sequential movement, or mentally simulate the same simple or complex movement. MEPs were induced in the bilateral flexor digitorum superficialis (FDS) and opponens policis muscles using TMS intensities ranging from 42-65% of maximum stimulator output, which corresponded to 1.2-1.3 times RMT. Results indicated that the size of MEPs increased during real sequential movements as well as imagined sequential movements. These changes were seen in both increased size and decreased latency of MEPs, which demonstrates an enhanced excitatory state of corticospinal cells. Gerloff and colleagues (1998) used rTMS to transiently disturb the motor cortex. Using an electronic piano, participants played three finger sequences of differing complexities with the right hand. In Experiment 1, participants either repeatedly pressed a single key with their index finger (simple sequence) or played a scale sequence which was comprised of four finger movements (complex sequence). In Experiment 2, participants either played the same scale sequence as in Experiment 1 or a more complicated sequence consisting of a four finger non-consecutive, non-repetitive movement. Results indicated that the motor cortex plays a greater role in the performance of complex movement sequences. The authors suggest that the motor cortex may not only be an executive motor area; but also a region which functions as a movement sequence organizer.

7. Safety, application and ethical considerations of TMS and rTMS

The most severe potential side effect associated with TMS is the risk of seizure; however, this risk is very low and has only occurred in a few rare cases. The introduction of TMS safety guidelines in 1998 established “safe” stimulation parameters for rTMS, which were based on pulse intensity, frequency, and train duration administered to healthy controls (see Wassermann, 1998 for details). Since the advent of these guidelines, only eight seizures have been reported. Four of these (2 rTMS, 2 single-pulse TMS) were induced by “safe” stimulation parameters; however three of the four occurred in patients taking pro-epileptogenic medications, and two of the four were potential non-epileptic events. Four more seizures were reported using parameters that were outside the “safe” stimulation parameters (Rossi, Hallett, Rossini, Pascual-Leone, & Safety, 2009). Furthermore, the magnetic field generated by TMS produces attractive forces on ferromagnetic objects and repulsive forces on non-ferromagnetic conductors. TMS has the potential to result in forces on particular head implants that could displace these objects (Rotenberg et al., 2007). Jewellery, glasses, and other potential conductors on the head should be removed prior to TMS to prevent interactions with the magnetic field (Rossi, et al., 2009).

Chapter II: Research Article I

Time Course of Corticospinal Excitability

Michael Kennefick, Anthony N. Carlsen

School of Human Kinetics, University of Ottawa, Canada

Abstract. The process of movement execution can be separated into two sections; the foreperiod and the response time. The foreperiod represents the time between the warning signal and the presentation of the imperative "go" signal, and the response time incorporates both the reaction time (RT) and the movement time (Schmidt & Lee, 2011). Transcranial magnetic stimulation (TMS) was used to probe corticospinal excitability (CE) which has been measured in a variety of RT tasks (e.g. simple RT and choice RT) during both the foreperiod and the response time periods. In general, CE decreases as the imperative stimulus (IS) approaches and increases once the IS has been presented. Even though these two separate time frames are well described in the literature, CE during the entirety of the process of movement execution (e.g. foreperiod and response time) has not been described. The purpose of the present study was to measure when and at what rate changes in CE occur in relation to a true baseline level. Participants performed a single button press movement in response to a visual warning signal (WS). CE was measured at 21 time points following the warning signal, encompassing both a 1500ms fixed foreperiod and the response time. Furthermore, a baseline measure was taken when the participant was at rest, prior to the WS. The results of the present study indicate that CE levels quickly increase from baseline with the presentation of the warning signal. This is followed by a holding period in which CE is held constant until a decline in CE occurs prior to the presentation of the IS. This decline is followed by a rapid increase in CE as the movement is initiated and released. Importantly, even though levels of CE are decreasing relative to the start of the decline, participants are still in a heightened state as they prepare to release their movements. Furthermore, it is suggested that selective inhibitory control mechanisms are at least partly responsible for the decline prior to the IS.

Keywords. Transcranial Magnetic Stimulation, Corticospinal Excitability, Motor Preparation, Time Course, Simple Reaction Time Task.

Introduction. The process of movement execution can be separated into two sections; the foreperiod and the response time. The foreperiod represents the time in which movement preparation processes occur, between the WS and the presentation of the IS. The response time represents the time in which movement initiation processes occur and incorporates both the reaction time (RT) and the movement time (Schmidt & Lee, 2011). As a human prepares to perform a voluntary movement, brain activity measured using electroencephalography (EEG) can be recorded preceding the movement onset, which reflects the preparation for the execution of that movement (Deecke, 1996). Several models of activation (Carpenter & Williams, 1995; Hanes & Schall, 1996; Nazir & Jacobs, 1991) describing the preparation and initiation processes occurring during the production of movement have been proposed, one of which was the “cell assembly model”. This model, proposed by Wickens and colleagues (1994), described how the activation level of a group of cortical motor neurons related to the performance of a specific action (known as a “cell assembly”) is increased to an initial steady state which is held at a level below the threshold level for motor response production. Following the IS, the eventual triggering of the response results from additional input of activity causing “ignition” of the cell assembly, a spread of neural activity that excites movement-related corticospinal neurons. Corticospinal excitability (CE) has been measured in a variety of RT tasks (e.g. simple RT and choice RT) and these RT tasks can help reveal specific activation differences associated with the different paradigms. A relatively small body of literature exists in which CE was examined prior to the imperative stimulus (IS). Conversely, many studies have examined excitability following the IS. Interestingly however, while CE has been examined separately in both stages of movement execution, no studies have examined the time course of excitability throughout the entirety of the process of movement execution. This time course of excitability can thus also be compared to existing models of activation.

Foreperiod excitability. Touge and colleagues (1998) used transcranial magnetic stimulation (TMS) to demonstrate that the excitability of motor cortical projections to agonist muscles may decrease during the foreperiod. Results showed that in a simple RT task, the size of motor evoked potentials (MEPs) elicited in the flexor muscles of the wrist in the flexion task decreased compared to control MEPs (which were recorded in trials in which no warning signal was presented), as early as 100ms after the warning stimulus and continued until the presentation of the IS. It was postulated that this decrease in excitability levels was necessary to prevent the response from being released too early. In a follow-up experiment, the foreperiod was changed to a fixed 2000ms time interval and the results yielded no change in MEP amplitudes, possibly due to the fact that this time interval is more difficult to estimate. In this study, the depression of corticospinal excitability was seen only in simple RT tasks, and not in the choice RT task.

Similarly, Hasbroucq and colleagues (1997) also found that MEPs were greater when TMS was delivered simultaneously with the warning signal, compared to when TMS was delivered simultaneously with the response signal in the short (500ms) foreperiod of a 2-button press choice RT task. Their study did not reveal any differences in MEP amplitude during a long (2500ms) foreperiod. A second experiment in the same study revealed that CE decreased progressively, and possibly linearly, during the first 333ms of a short (500ms) foreperiod, at which time it remained stable until the IS was presented. It appears that the decrease in MEP amplitude could be an adaptive mechanism in which the sensitivity of the corticospinal tract to the forthcoming command is increased. A further explanation is that inhibition within the motor cortex may act to prevent the premature release of a response during the foreperiod of a RT task (Stinear, et al., 2009). Indeed, using single cell recordings in two monkeys, Prut and Fetz (1999) suggested that inhibitory modulations may reflect a general "braking" mechanism in which the tendency to initiate a movement during the foreperiod is suppressed and that the "brake" is released once the IS appears and movement is initiated.

Reaction time interval excitability. Contrary to the decrease in MEP amplitude during the foreperiod, MEPs elicited during the period between the IS and EMG onset (i.e. RT interval) have been shown to increase in amplitude. Using TES and TMS, Rossini and colleagues (1988) investigated motor unit potential recruitment patterns 150ms prior to EMG onset to 50ms after EMG onset. Results indicated that MEPs were evoked 100ms prior to the onset of EMG, and that the probability of eliciting MEPs gradually increased as the onset of EMG approached. Starr and colleagues (1988) used TES to examine the time course of MEP facilitation during a thumb opposition movement, in which TES was preceded by an auditory click. Similar to Rossini and colleagues (1988), results indicated that anodal scalp stimulation elicited MEPs beginning 80ms prior to EMG onset. Leocani and colleagues (2000) exclusively used TMS to evaluate CE during various auditory RT paradigms (Simple RT, Choice RT, and go/no-go), and demonstrated a gradual facilitation of the agonist muscle occurred around 100-120ms before EMG onset in all RT paradigms. Chen and colleagues (1998) also exclusively used TMS in various experiments and found that when suprathreshold TMS was used in a simple RT paradigm (similar to the current study), CE began to increase 50ms prior to EMG onset.

In summary, while the excitability of the motor cortex during various periods preceding movement onset is relatively well defined (e.g., CE decreases in the ~500ms prior to the IS), key pieces of information are missing. Previous studies examining CE levels during the foreperiod have typically compared activation to the first measured CE value in the foreperiod, or to trials without a WS, rather than to a baseline (i.e., resting) value. This comparison may not accurately capture the time course of CE activation relative to a resting level as response-related preparatory activity may have already occurred at the first measurement point.. As no single study has investigated how CE changes throughout the entire process of movement execution (e.g. the foreperiod and the RT interval), the first experiment of this thesis will attempt to characterize motor cortex excitability related to the prime mover for the duration of the time before movement onset in a simple RT task, and to provide a cohesive picture of the entire time course of corticospinal excitability. Specifically, the primary purpose was to describe when and at what rate changes in CE occur, allowing for a greater understanding of cortical activation related to movement preparation and initiation, and how this activation matches with existing models.

Methods

Participants

Nineteen healthy volunteers (10F, 9M; 24 ± 5 years) with normal or corrected to normal vision, and with no history of neurological, sensory, or motor disorders participated in this study. The data from one participant had to be excluded due to technical issues. All participants were classified as right-handed or ambidextrous participants based on the Edinburgh Handedness Inventory (Oldfield, 1971). Testing of each participant took place in a single session, and required approximately 1.5 hours to complete. All participants provided informed consent, and the experiment was conducted in accordance with ethical guidelines approved by the University of Ottawa's Research Ethics Board.

Experimental set-up and task

Participants sat comfortably facing a 17 inch LCD computer monitor with their right arm pronated and resting on a flat surface. They completed a simple RT task in which they performed a single button-press movement, in response to a visual imperative stimulus (IS). This movement required the pressing of a telegraph key (Ameco AM-K4B) and was performed by flexing the second finger using the flexor digitorum superficialis (FDS) muscle. Participants were instructed to execute this movement as quickly as possible following the IS. The presentation of the visual warning signal (WS), which was a blank, black outlined box (6cm x 5cm), indicated the start of the trial, followed by a fixed foreperiod (1500ms). A fixed foreperiod was utilized to ensure that MEP data collected during the foreperiod were time-locked to the same interval across all participants. The visual IS consisted of the same blank box located in the center of the screen which turned green, prompting the participant to initiate their movement. The presentation of the visual IS followed the foreperiod, at which time the participant initiated their movement. Feedback was provided on the computer monitor after each trial, consisting of RT. Prior to the start of the testing session, each participant performed a practice block consisting of 10 trials. The practice trials were identical to that of the testing trials, with the exception that there was no TMS.

Following the practice session, participants performed 8 blocks of 24 trials each, consisting of 22 RT trials and 2 catch trials per block, for a total of 192 testing trials. TMS stimulation (see below for details) was applied over the motor cortex at 22 time points with respect to the IS. TMS occurred once at -2500ms, which was the baseline measure, 5 different time points between -1500ms and -500ms, 3 different time points between -400ms to -200ms, and 13 different time points between -100ms to +125ms (see figure 1a for visual representation). MEPs collected at the first stimulation point (-2500ms) were considered as baseline since these occurred during the inter-trial interval, prior to the WS and thus it is presumed that the participant was fully at rest. The inter-trial interval was randomly varied between 6s and 8s to avoid anticipation of trials onsets by the participant. Catch trials consisted of the WS followed by TMS, but without the IS. This ensured that the participant reacted only when he or she was meant to. In order to encourage advance preparation, a points reward structure was provided to the participant based on RT whereby when participants produced a RT below a predetermined criterion, points were awarded. Alternatively, executing a movement during catch trials resulted in a loss of points. A visual representation of the task can be seen in figure 1b.

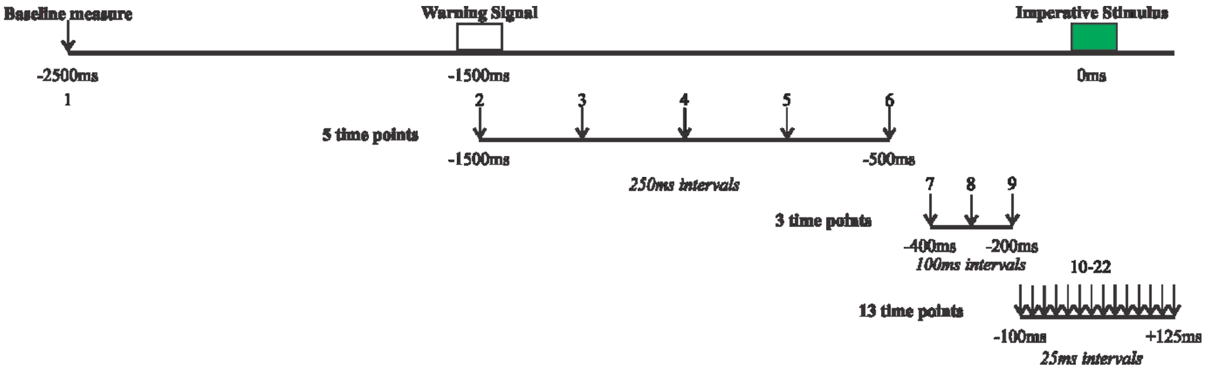


Figure 1a. Visual representation of the TMS stimulation points, represented by downwards pointing arrows.

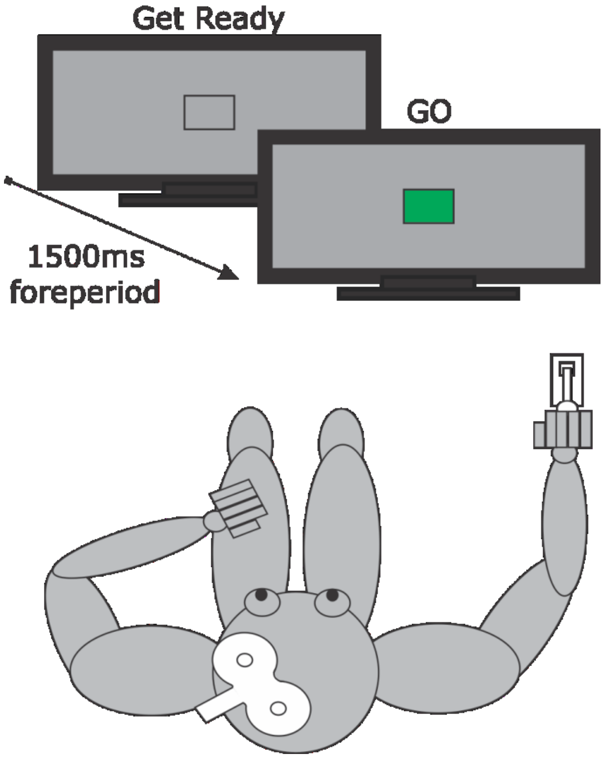


Figure 1b. Visual representation of a single trial in experiment 1.

Transcranial magnetic stimulation

TMS was applied using a figure-8 magnetic coil (70mm; Magstim 200^2, Magstim Company Ltd, UK). Prior to testing, the coil was placed over the optimal location for eliciting MEPs from the right flexor digitorum superficialis (FDS) muscle, with the handle of the coil pointing backwards at a 45° angle. The optimal location was found by first finding the midpoint between the nasion and inion, and the left and right preauricular notches. From this midpoint, a location 5cm lateral and 1cm posterior was marked on the participant's scalp using a red grease crayon. The optimal location was then found by delivering test pulses at various scalp locations around this mark and determining the location that resulted in consistently large MEPs. Resting motor threshold (RMT) was determined at rest to the nearest 1% of stimulator output using the Rossini-Rothwell method (defined as the minimum intensity required to evoke MEPs above 50 μV in at least 5 out of 10 trials). The magnetic coil was held stationary over the optimal location by the experimenter and the position was maintained by holding the coil in the reference position on the head with the assistance of neuronavigation hardware and software (ANT Neuro Visor 2, Madison, WI). Stimulus intensity was adjusted to 110% RMT. Similar intensities have been used previously to probe changes in CE (Chen, et al., 1998; MacKinnon & Rothwell, 2000).

Recording equipment

Surface EMG data was collected from the muscle belly of the right FDS muscle using bipolar preamplified (gain=10) surface electrode (Delsys Bagnoli DE-2.1) connected via shielded cabling to an external amplifier (Delsys Bagnoli-8). The electrode was placed parallel to the muscle fibres, and attached to the skin using double sided adhesive strips. A grounding electrode (Dermatrode HE-R) was placed on the participant's right lateral epicondyle. The site of each electrode was prepared and cleaned using abrasive skin prepping gel to ensure minimal electrical impedance. Unfiltered EMG and button data was digitally sampled at 1 kHz (National Instruments PCI-6024E) beginning 500ms prior to the first stimulation point for a total duration of 3500ms using a custom made program written in LabVIEW (National Instrument Inc.) and stored for offline analysis.

Data reduction

MEP amplitudes, the time between TMS pulse and EMG onset, and the time of movement onset were calculated. Trials in which TMS was delivered during the EMG burst were rejected due to the fact that it is inherently difficult to distinguish the MEP from EMG activity associated with the actual movement. MEP amplitudes were quantified by calculating the peak-to-peak voltages of the evoked responses, expressed as a percentage of the baseline MEP amplitudes. EMG burst onsets were defined as the point at which EMG activity reached a value of 2 standard deviations above baseline levels and remained at that level for more than 20ms. EMG offset was defined as the point at which EMG activity fell below a value of 80% of its maximal amplitude (Hodges & Bui, 1996). EMG markers were manually adjusted to compensate for any errors due to the strictness of the algorithm used by the custom LabVIEW (National Instrument Inc.) program. Movement related EMG activity in the FDS muscle was marked for each trial. Peak EMG amplitudes were defined as the largest EMG amplitude recorded within an interval of 100ms following EMG burst onset. In order to analyze the data relative to EMG onset, the difference in time between the onset of agonist EMG and the presentation of TMS was calculated.

Statistical Analysis

Dependent variables were analyzed using a one-way repeated measures analysis of variance to determine if differences exist between presentation times of the TMS (e.g. the 22 time points between -2500ms prior to the IS and +125ms following the IS). Data was normalized to baseline values measured at time point 1 in order to account for the variability in individuals' resting CE levels. Preplanned comparisons using uncorrected t-tests were administered to determine the exact locus of any significant differences in the overall CE time course. Uncorrected t-tests were used due to the large number of comparisons, and correcting for all these possible comparisons may lead to an increased probability of committing a type II error. Differences with a probability of less than 0.05 were considered significant. Secondary post-hoc analyses are described below.

Results

Stimulation parameters.

Across participants, RMT was 47 ± 10% of maximal stimulator output, and the mean test stimulus was 52 ± 11% of stimulator output.

MEP amplitude.

Analysis of MEP amplitude revealed a significant main effect of time ($F(21,357) = 4.580$, $p < 0.001$, $\eta_p^2 = 0.212$). T-tests comparing each time point to baseline revealed a significant difference between the baseline (time point 1) and every other time point in the current study, with the exception of time point 2 (-1500ms) (p=0.1) and time point 18 (+25ms) (p=0.066). This indicates that there was a significant increase in MEP amplitudes after time point 2, but also there appeared to be a decrease in MEP amplitudes occurring around the time of the presentation of the IS (figure 2).

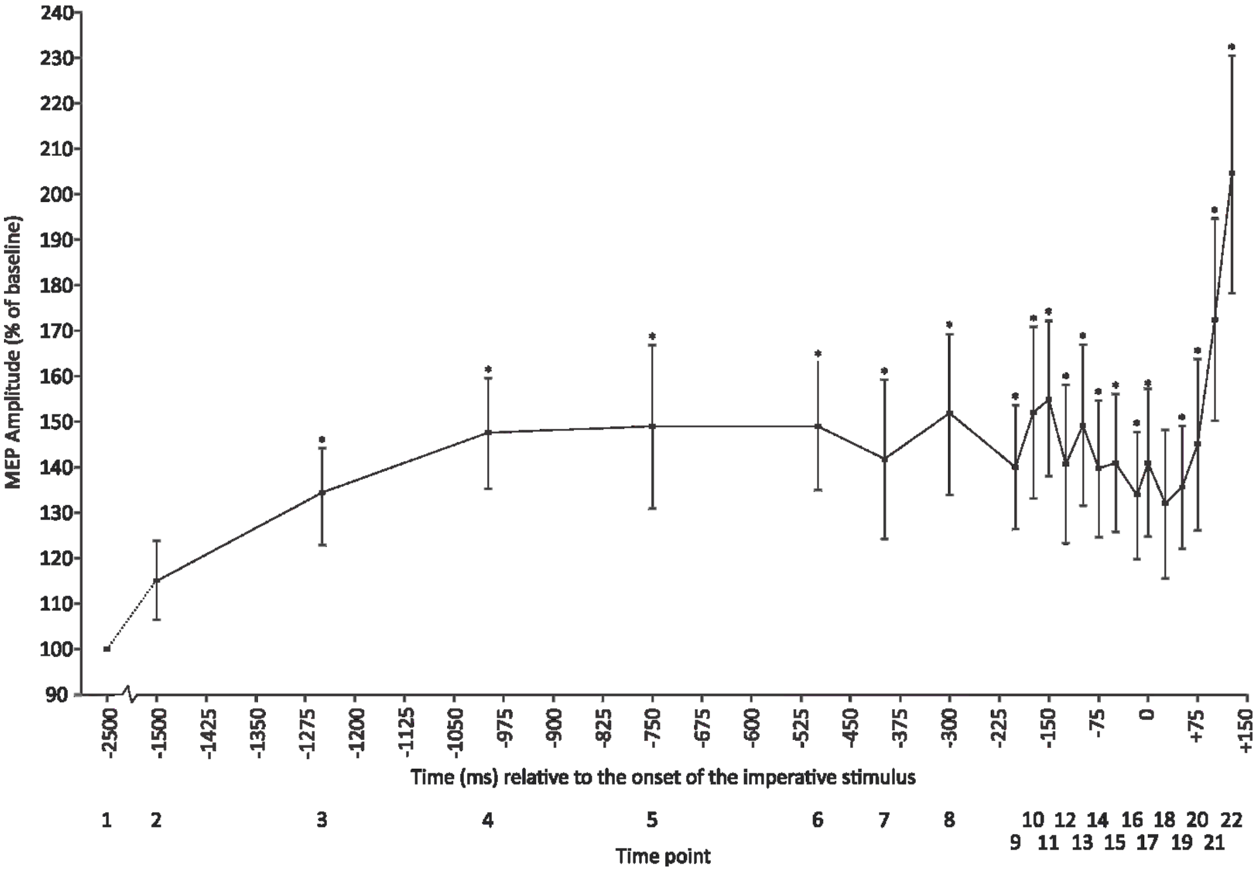


Figure 2. Motor-evoked potential (MEP) amplitude relative to the onset of the imperative stimulus expressed as a percentage of baseline. Each time point is represented with standard error.

In order to further characterize the time course of CE, a second post-hoc analysis was conducted using the MEP amplitudes measured at the time of the presentation of the WS (time point 2) as a baseline. This was undertaken due to the fact that prior studies that have used long foreperiods characterized changes in MEP amplitudes with respect to the presentation of the WS. MEP amplitudes across these time points (as a percentage of baseline measured at time point 2) was analyzed using a one-way repeated measures ANOVA. Analysis revealed a significant main effect of time ($F(20,340) = 3.670$, $p < 0.001$, $\eta_p^2 = 0.178$). Uncorrected t-tests between time point 2 and all other time points revealed a more detailed view of the time course of events (figure 3). A significant difference was found between time point 2 and all other time points up to and including time point 11, with the exception of time point 7 ($p=0.065$). However, there was no difference in MEP amplitude between time point 2 and time point 12, or between time point 2 and time points 16 - 20 (all p values >0.061). Finally, a significant difference was found between time point 2 and time point 21($p=0.006$) and time point 22 ($p < 0.001$). These results are presented in figure 3.

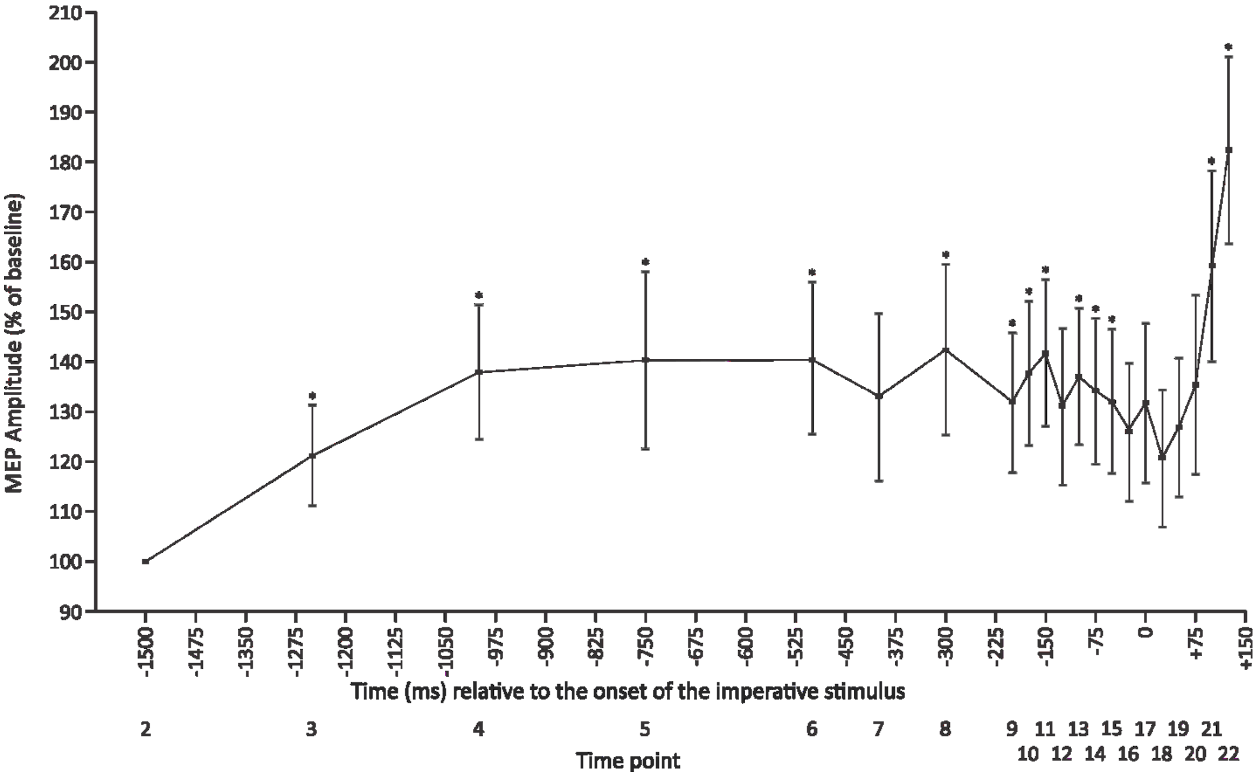


Figure 3. Motor-evoked potential (MEP) amplitude relative to the onset of the imperative stimulus expressed as a percentage of baseline (measure as the WS). Each time point is represented with standard error.

***MEP amplitude breakdown*.**

MEP amplitude data shown in figure 2 appears to show different CE "periods" throughout the time course. Specifically, there appear to be 3 separate time periods in the foreperiod leading up to the IS, and a separate time period following the IS. The first period is characterized by a steady increase from baseline levels for approximately 1500ms (from -2500ms to -1000ms). The end of the first period was chosen to be -1000ms based on inspection of the data presented in figure 2. The second phase (which begins at -1000ms) is characterized by a 500ms period in which levels of CE are stable. The third time frame (-500ms to the IS) has been characterized previously in the literature, and typically shows a decline in CE. These time periods were analyzed separately from the overall time course to protect against making errors associated with a large number of comparisons.

MEP amplitude during 1

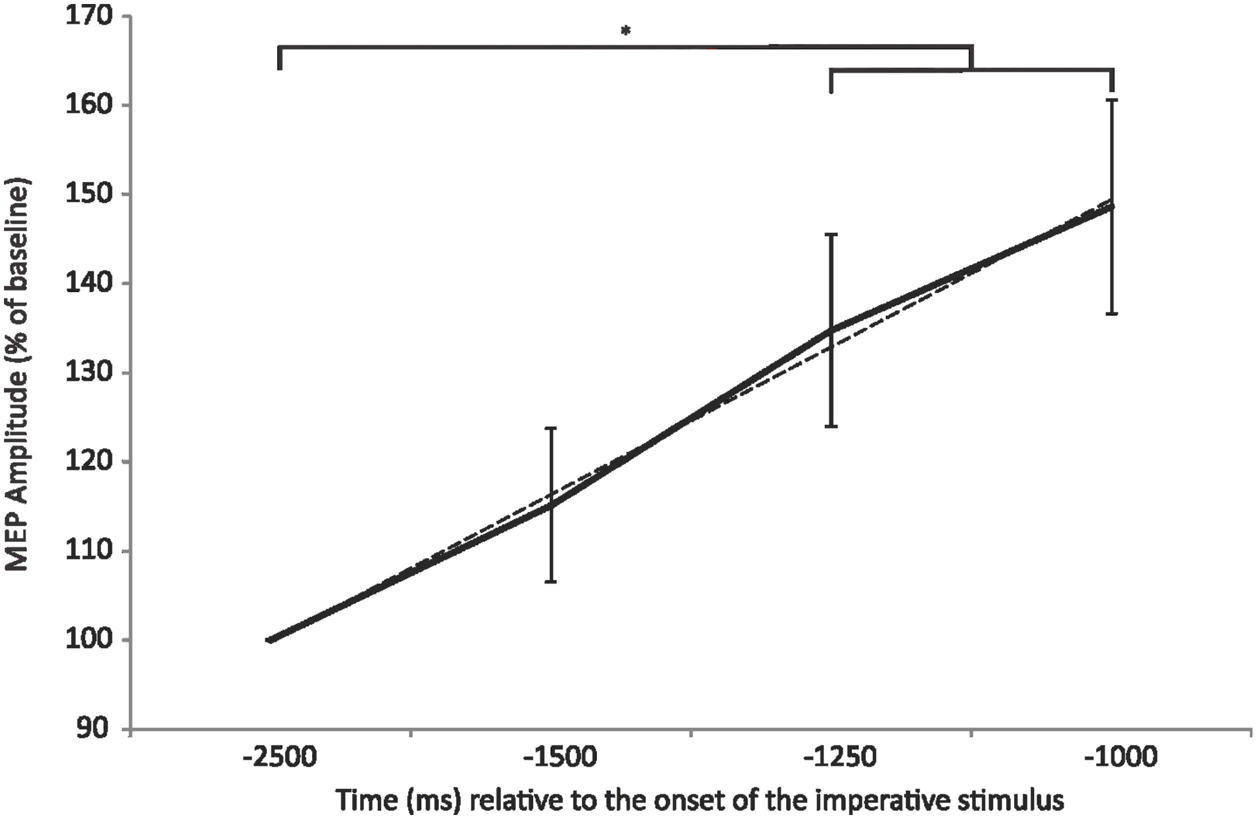


Figure 4. Motor-evoked potential (MEP) amplitude relative to the onset of the imperative stimulus expressed as a percentage of baseline for the time encompassing -2500ms to -1000ms. Each time point is represented with standard error. Dashed line represents the linear relationship (*p*=0.001) between all points.

MEP amplitude during 2nd period. In order to analyse the second phase, a subsequent one-way (3 time) repeated measures ANOVA was undertaken to characterize the "hold" or stable phase of CE between -1000ms and -500ms. The results indicated a non-significant main effect of time ($F(2,34) = 0.14$, $p = 0.986$, $\eta_p^2 = 0.001$) as well as a non-significant linear function ($F(1,17) = 0.045$, $p = 0.835$, $\eta_p^2 = 0.003$) indicating that levels of CE did not change. The results are shown in figure 5.

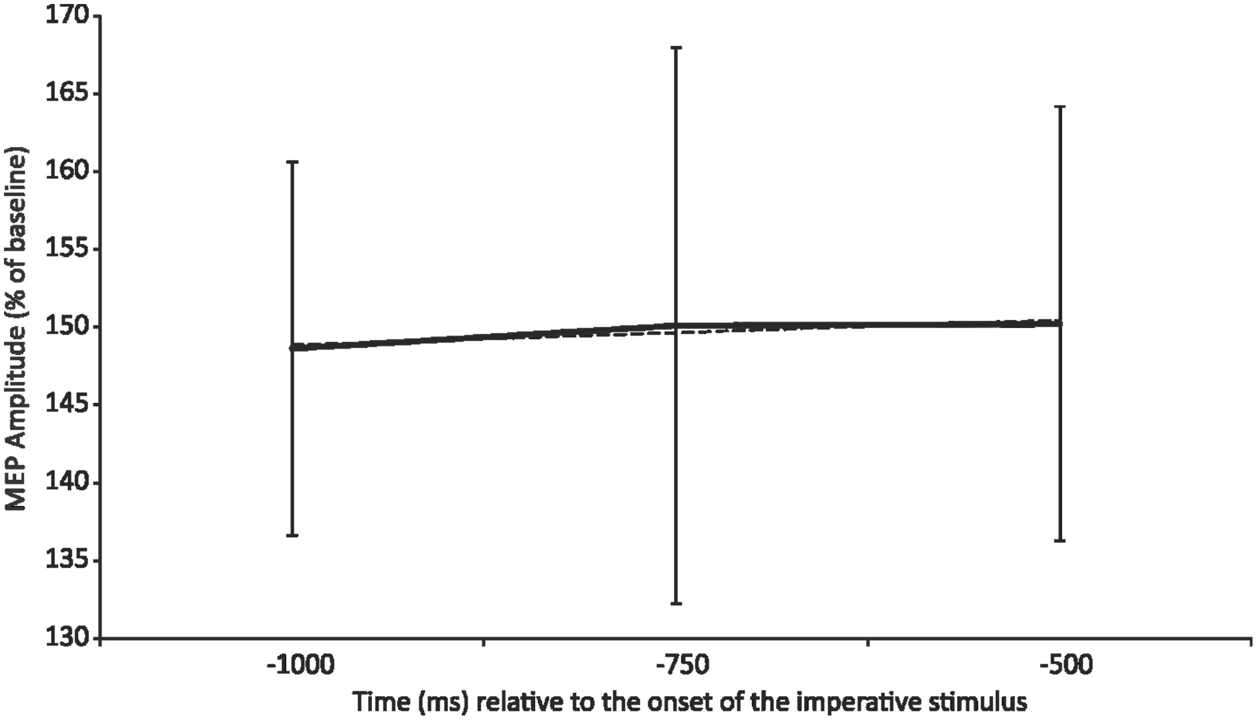


Figure 5. Motor-evoked potential (MEP) amplitude relative to the onset of the imperative stimulus expressed as a percentage of baseline for the time encompassing -1000ms to -500ms. Each time point is represented with standard error. Dashed line represents the linear relationship (*p*=0.835) between all points.

MEP amplitude during the 3rd period. In order to analyze the third period and understand how levels of CE changed as the IS approached, a one-way (12 time) repeated measures ANOVA was undertaken to examine the 500ms preceding the IS. This time frame was chosen because previous studies (Hasbroucq, et al., 1997; Touge, et al., 1998) have examined foreperiod CE during this time frame. The analysis did not show a significant main effect of time ($F(11,187) = 0.828$, $p = 0.612$, $\eta_p^2 = 0.046$), and although a linear trend was evident ($F(1,17) = 3.589$, $p = 0.075$, $\eta_p^2 = 0.174$), it was not significant. The same previous studies that described a decrease in CE levels in the 500ms prior to the IS, however, used raw data as opposed to normalized data, and thus a follow-up ANOVA was conducted in order to replicate these studies. The results indicated a non-significant main effect of time ($F(11,187) = 1.444$, $p = 0.157$, $\eta_p^2 = 0.078$), however a significant linear trend was found ($F(1,17) = 7.006$, $p = 0.017$, $\eta_p^2 = 0.292$) suggesting a decrease in CE as the IS approached, which is shown in figure 6.

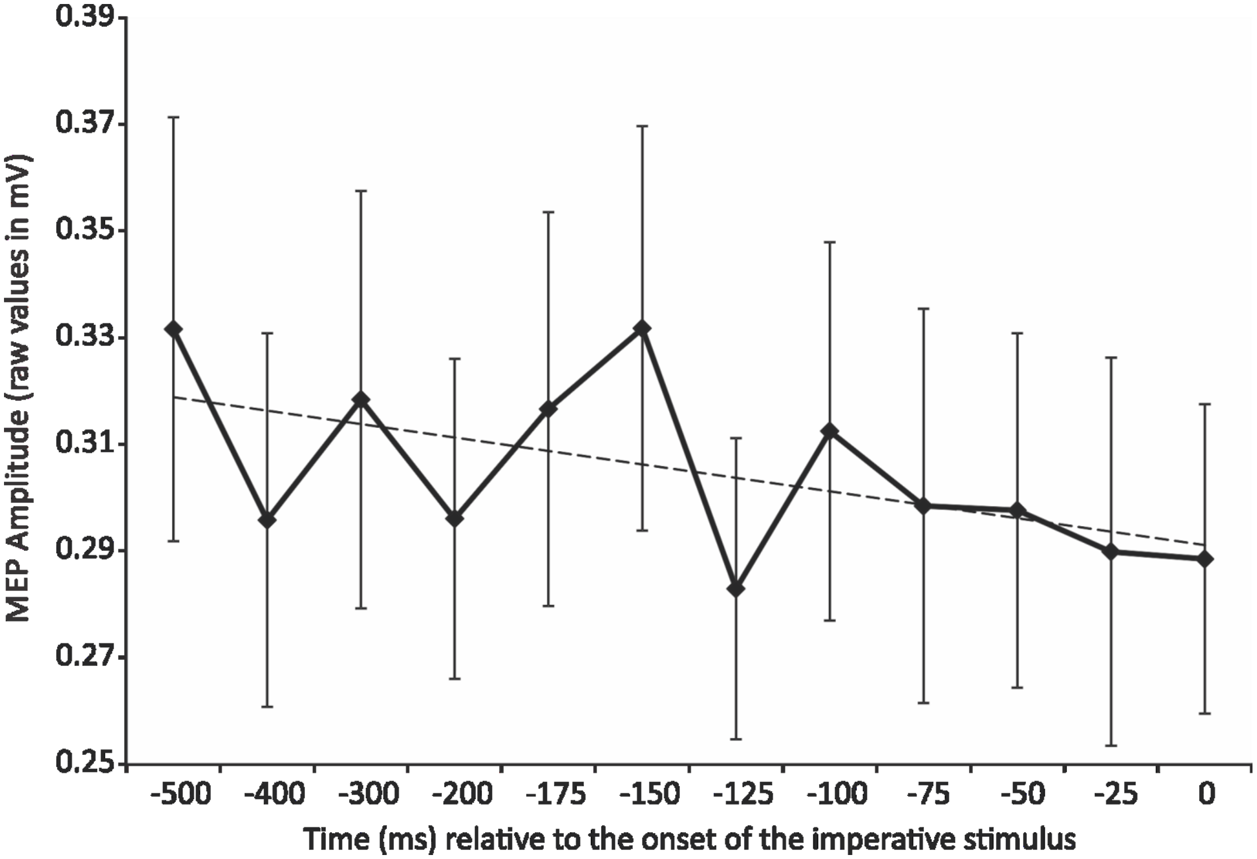


Figure 6. Raw motor-evoked potential (MEP) amplitudes (mV) relative to the onset of the imperative stimulus. Each time point is represented with standard error. Grey dashed line represents the linear relationship (*p*=0.017) between all points.

MEP amplitude during the 4th period. Figure 1 and 2 both demonstrate a substantial increase in CE following the IS as the movement was executed. In order to characterize this increase, a one-way repeated measures ANOVA was conducted between all time points between the IS and the end of the trials. The results indicated a significant main effect of time ($F(5,85) = 9.783$, $p < 0.001$, $\eta_p^2 = 0.385$). A significant linear trend ($F(1,17) = 12.887$, $p = 0.002$, $\eta_p^2 = 0.431$) and a significant quadratic trend ($F(1,17) = 19.574$, $p < 0.001$, $\eta_p^2 = 0.535$) were also noted. Post-hoc analyses using Bonferonni corrected student's t-tests were used to determine the locus of the differences. The results indicated a significant difference between time point 6 (125ms) and time point 4 (75ms), time point 3 (50ms) and time point 2 (25ms), as well as a significant difference between time point 5 (100ms) and time point 2(25ms) confirming the increase in CE following the presentation of the IS (figure 7).

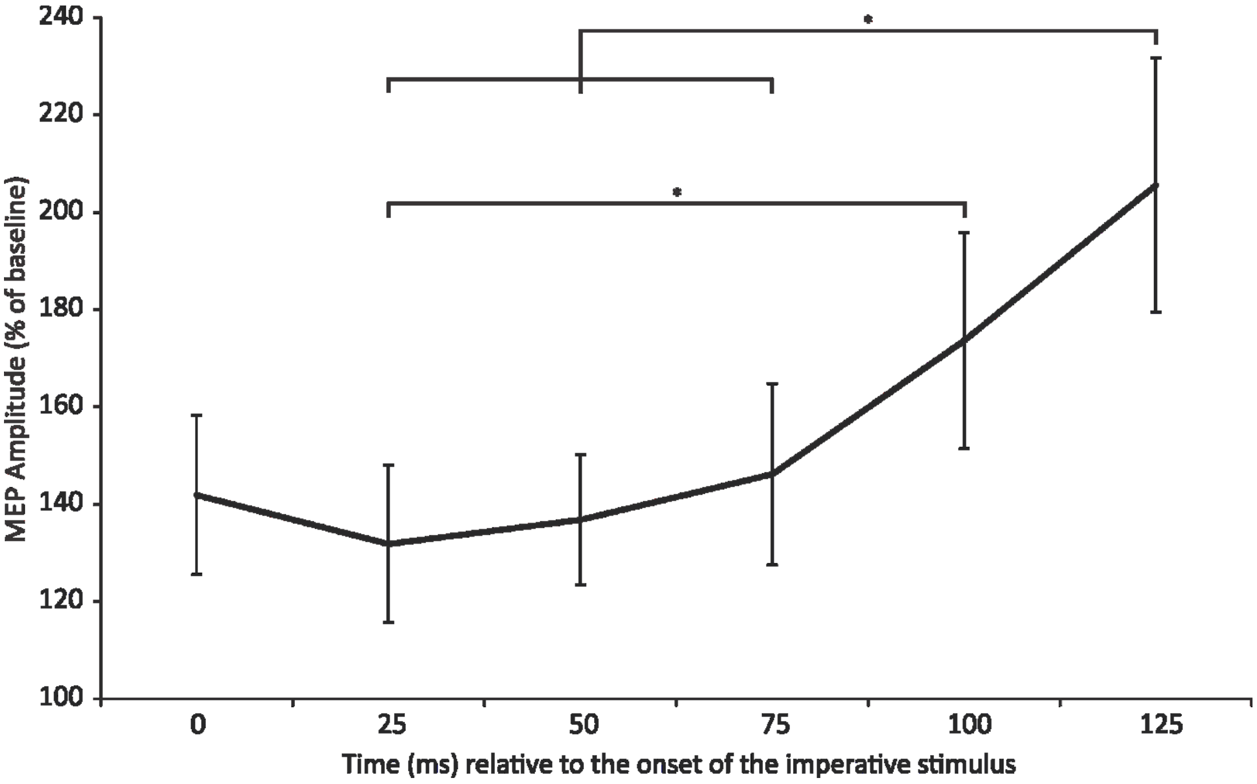


Figure 7. Motor-evoked potential (MEP) amplitude relative to the onset of the imperative stimulus expressed as a percentage of baseline for the time encompassing 0ms to +125ms. Each time point is represented with standard error.

MEP amplitude relative to the onset of EMG. Due to the fact that RTs varied among participants, a final data analysis investigated the MEP amplitude data time-locked to the onset of EMG. For this analysis, similar data reduction techniques from prior studies (Chen, et al., 1998) were used. Specifically, MEP amplitudes were expressed as a percentage of each participant's maximal MEP amplitude, and organized by onset time into 7 time bins relative to EMG onset by calculating the time difference between the onset of EMG and the MEP. These time bins included MEP onsets that occurred either <60 ms prior to EMG onset or 10 ms time bins at increasing intervals up to 110 ms prior to EMG onset (see figure 8). Participants who did not react within 60ms following the TMS pulse were excluded from this analysis, due to the fact that there would be a large number of missing values. This left 11 of the original 18 participants. Some participants did not have any values for specific 10ms time bins, and thus missing values (4 out of 77) were filled using a linear-based multiple imputations procedure in SPSS (IBM Inc.) in order to perform a full factorial analysis. A one-way repeated measures ANOVA was conducted between 7 time bins (<60ms, 60-70ms, 70-80ms, 80-90ms, 90-100ms, 100-110ms, and >110ms) relative to the onset of EMG at 10ms intervals. The results indicated a significant main effect of time ($F(6,60) = 2.898$, $p = 0.015$, $\eta_p^2 = 0.225$). Post-hoc analyses using Bonferonni corrected student's t-tests were used to determine the locus of the differences. These results indicated that the only significant difference was between the <60ms time bin and the 70-80ms time bin, indicating that CE increased approximately 70ms prior to EMG onset, in accordance with previous studies. These results are shown in figure 8.

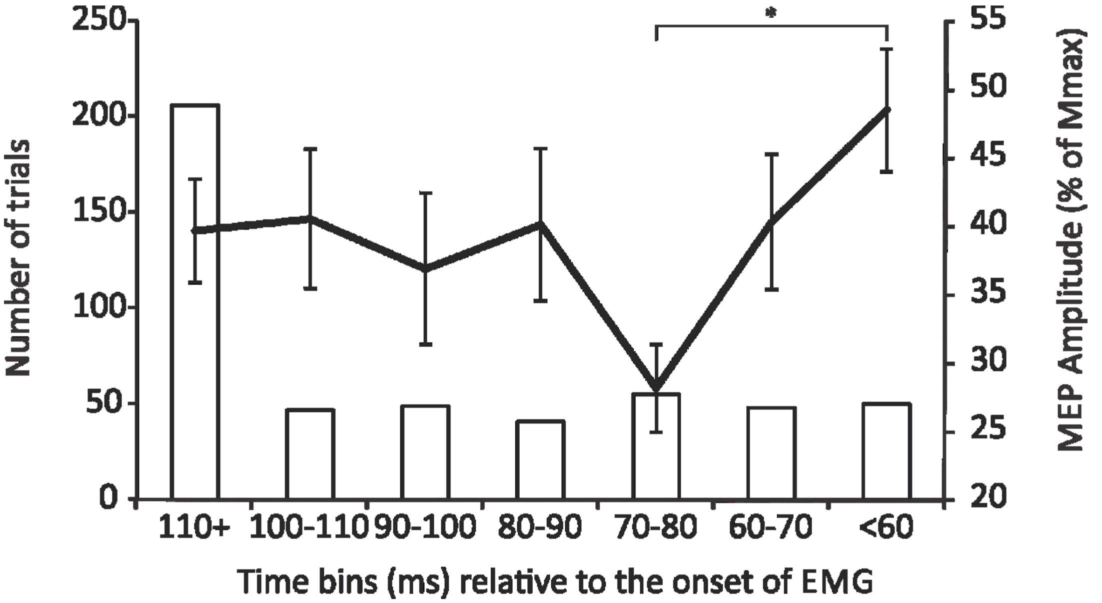


Figure 8. Motor-evoked potential (MEP) amplitude relative to the onset of EMG expressed as a percentage of Mmax. Each time point is represented with standard error. The number of trials making up the mean for each of the time points is represented by columns.

Discussion

The purpose of this study was to examine the time course of CE encompassing the entirety of the process of movement execution. Previous work examined CE in two separate sections; the foreperiod and the reaction-time interval, and CE has been shown to decrease with the presentation of a warning signal in short foreperiods, while an opposing increase occurs during the reaction-time interval, following the IS. A major issue with trying to describe the time course of CE is that one cannot simply take the results from two separate time intervals and create a time course. Past studies have described increases and decreases in CE in relation to CE measured at previous time points, without comparing these to a "true" baseline level. For example, some studies used trials in which no warning signal was presented as their control or baseline trials, as opposed to the current study which measured CE levels 1000ms prior to the presentation of the warning signal. The current study examined CE throughout the entirety of the process of movement execution, comparing changes in CE throughout the process to baseline values measured while participants were at rest.

The major finding of the study is that CE increases from a resting, baseline level to a variety of other levels throughout the entirety of the process of movement execution; importantly however, once a heightened level of CE is reached, it never decreased to a level lower than 132% (time point 18) of baseline (figure 2). The results of the current study (figures 2 and 3) demonstrate a trend in which CE can be described in four periods. Based on visual inspection of the data, the first period was arbitrarily set between -2500 and -1000ms. The second period begins at -1000ms and ends at -500ms, which is the beginning of a well-established time frame in the literature between -500ms and the IS (the third period). The first three periods relate to the foreperiod, and the fourth period relates to the reaction time interval. In the current study, the fourth period is set between 0ms and +125ms.

In general, CE increases from a baseline level for the first 1500ms (between -2500ms and 1500ms) in the first period that can be described as the “initial increase”, represented in figure 4. This increase can be thought of as the time frame in which the participant begins to prepare for the upcoming movement. CE levels appear to stabilize for the next 500ms (between -1000ms and -500ms) in the period that can be described as the “hold”, represented in figure 5. For the next 500ms (between -500ms and 0ms), there is a decrease in excitability leading up to the presentation of the IS, in a period than can be described as the “decline”, represented in figure 6. As the participant reacts to the IS, excitability increases dramatically, in a period than can be considered to be the “final increase”, in figures 7 and 8.

The results of the current study appear to generally follow those of Touge and colleagues (1998) and Hasbroucq and colleagues (1997) in the sense that MEP amplitude decreased in the 500ms prior to the IS; however important distinctions must be made. Contrary to previous studies, the current study found that there was a decrease in MEP amplitude during a long foreperiod, even in a paradigm in which it would be difficult for a participant to accurately estimate the timing of the IS. The decrease seen in the decline portion of the time course is indeed a suppression of MEP amplitude; however, the MEP amplitude at the beginning of this decline is at 150% of baseline values and decreases to 132% of baseline values at its lowest. Importantly, this indicates that even though levels of CE are decreasing relative to the start of the decline, participants are still in a heightened state as they prepare to release their movements. In addition, the results of the current study also closely follow those of previous studies (Chen, et al., 1998; Rossini, et al., 1988; Starr, et al., 1988) in which MEP amplitude increases roughly 60ms prior to the onset of EMG. The differences between the current study and previous studies hinge on the fact that the current study examined CE throughout the entirety of the process of movement execution, as opposed to measuring CE in snippets either prior to or after the IS, and demonstrates the importance of understanding processes in their entirety.

Previous studies that have used EEG to investigate self-initiated voluntary movements have shown increasing activity over motor areas that can be measured 2000ms to 1000ms preceding movement onset. This so-called Bereitschaftspotential (BP) or "readiness potential" is an indication that the brain is preparing to execute a movement (Deecke, 1996). These preparatory properties at the neural level are indicative of an increasing level of neural activation related to the process of motor preparation, which in a simple RT paradigm can be completed in its entirety prior to the IS, if there is enough time and information available between the warning signal and the IS (Carlsen, Maslovat, & Franks, 2012). In order to precisely control or modulate the initiation of a response, there must exist some sort of mechanism that regulates neural activation to a desired level for a particular motor command. First introduced by Hebb (1949), the cell assembly model proposed a neural explanation to the much debated concept of a motor program. The underlying assumption of Hebb's work is that when a hypothetical cell *A* is close enough to a hypothetical cell *B* and is able to excite it repeatedly, a growth or metabolic process occurs in which one or both cells, such that cell *A*'s efficiency, as one of the cells firing *B*, is increased. Therefore, any systems of cells that are repeatedly active at the time become associated to the point where activity in one facilitates activity in another. This original concept quickly evolved into what is now referred to as long-term potentiation (Summers & Anson, 2009). Some fifty years later, Wickens and colleagues (1994) proposed a model derived from Hebb's original work. In their model, a motor program is stored within the connections between cortical pyramidal cells, and the "retrieval" of a motor program occurs with the activation of a cell assembly. Preparation for a specific action involves the activation of a particular cell assembly to an initial "steady state" which is held to a level below the threshold level, or the "ignition point". The final stage of preparation is the eventual triggering of the response due to additional activity of a population of cells that represent the IS to begin movement. The eventual "ignition" of the cell assembly results in a spread of neural activity that excites movement-related corticospinal neurons. The "retrieval" of a motor program requires a specific temporal sequence of corticospinal activity which is determined by the dynamics of the cell assembly ignition. Therefore, ideal motor programming raises the level of activation of the cell assembly as close as possible to the "ignition point" so that a minimal amount of additional input would cause the "ignition" of the cell assembly. However, keeping the level of activation below threshold is inherently difficult due to the variability of "noise" within the system caused by sensory noise, cellular noise and motor noise (Faisal, Selen, & Wolpert, 2008). Inevitably, a certain amount of noise is present within the cell assembly which results in the restriction of the level of activation within the cell assembly to ensure random noise could not raise the activation level above threshold and cause the premature release of a movement (Carlsen, et al., 2012). Carlsen and colleagues (2012) further proposed that motor programming can be thought of as the noisy activation of neural networks to a threshold level below that required to cause a cascading output. The results of the current study appear to fit the cell assembly model as the results indicate that the level of corticospinal excitability rises from a resting state to a fluctuating level below the threshold level of the "ignition point". The "ignition point" is reached after the IS is presented which adds sufficient activation to the cell assembly. This results in a steep increase in the level of corticospinal excitability as the movement is released.

As the results of the current study have indicated, the decline in the level of CE occurs prior to the IS, which followed a period of relatively stable CE. It is firstly important to note that the current study used a relatively long, 1500ms foreperiod. The length of the foreperiod affects the way humans behave, because a longer foreperiod makes it more difficult to anticipate the upcoming IS (Requin, Brener, & Ring, 1991). Hasbroucq and colleagues (1997) suggested that the decrease in CE prior to the IS could be an adaptive mechanism in which the sensitivity of the corticospinal tract to the forthcoming command is increased in such a way that afferents unrelated to the motor structures could be filtered out, thus reducing signal-to-noise ratio. Prut and Fetz (1999) attribute this decline in the motor cortex to inhibition, or a general braking mechanism that suppresses the tendency to initiate a response during the foreperiod. A review by Stinear and colleagues (2009) has shown that the prevention of prepared voluntary movements involve cortical and subcortical networks which include regions of the prefrontal cortex, the basal ganglia, the SMA, and the pre-SMA. However, the primary motor cortex appears to be the most likely site of convergence of mechanisms relating to the selection, initiation and inhibition of movements.

Undoubtedly, “inhibition” is a rather large concept, and can be described using concepts of neuroscience and psychology (Aron, 2007). Aron (2011) sought to disentangle the concept of inhibition by attempting to classify the concept into categories and subcategories; the first category being global inhibition and the second being selective inhibition. Global inhibition can be thought of as the stopping or cancellation of *all* actions (Aron & Verbruggen, 2008), or as an “emergency” shut down (Aron, 2011). Selective inhibition, as the name implies, can be thought of as a mechanism that allows the suppression of specific tendencies, rather than all current tendencies (Aron & Verbruggen, 2008). These two stopping mechanisms can be broken down further, into subcategories or types of control. The first type of control is reactive, which implies the outright stopping of a response once instructed by a signal. The second type of control is proactive, which implies the preparation to stop an upcoming response. The difficulty in describing these types of inhibition in relation to the current study lies in the fact that most studies use some variation of a selective stop signal task, go/no-go or choice RT task, whereas the current study uses a pure simple RT task. Even though the current task does not require the participant to stop, the current study best reflects a proactive type of control due to the fact that the participant must hold their response until the presentation of the IS to ensure they do not prematurely release the movement. Since participants are attempting to inhibit a specific response tendency as opposed to attempting to shut down all movements, the current study appears to best reflect selective proactive inhibition.

The best evidence describing selective inhibition in relation to the current task comes from studies that have looked at inhibition during the foreperiod or preparatory phase of a reaction time task. Cai and colleagues (2011) used a cued selective stopping task in which the participants were instructed that they might have to “stop left” or “stop right” at the beginning of each trial. This is followed by an IS which requires a simultaneous bimanual response. Occasionally, a stop signal occurs which requires the participant to stop one response, while continuing the other. In their study, TMS was delivered over the left motor cortex prior to the IS. Results indicated a suppression of MEP amplitudes when participants were presented with the “maybe stop right” cue, but importantly no suppression of MEPs when participants were presented with the “maybe stop left” cue, indicating a degree of selectivity at the level of the selected respondent, the first dorsal interosseous muscle. The absence of a global stopping or cancellation mechanism can be observed further downstream, at the level of the leg. Greenhouse and colleagues (2012) used a conditional stop task to see if global suppressive mechanisms were occurring in which MEP amplitudes at the level of the tibialis anterior muscle were suppressed as participants prepared for and anticipated a movement with either their right index finger or their right 5th digit. Results indicated that there was no suppression of MEP amplitudes at the level of the leg during the anticipation phase; however there was inhibition of the leg when participants were forced to stop their movements. This furthermore demonstrates the absence of a global inhibitory control mechanism as a participant prepares to stop a specific upcoming response, but rather demonstrates the presence of a selective inhibitory control mechanism. Duque and colleagues (2010) used a choice RT task to examine levels of excitability during response preparation and movement initiation, in which participants had to make a speeded response with one or two fingers, either from the same hand or different hands. Results indicated that MEP amplitudes were inhibited only when the target muscle was a potential respondent for the forthcoming movement. This mechanism was termed “impulse control”, and is said to have a specific effect on selected respondents and that this mechanism ensures that movements are not prematurely released. Furthermore, this selective effect fits with previous studies that have suggested that the decrease in CE prior to the IS is an adaptive mechanism that filters out unrelated afferents to motor structures (Hasbroucq, et al., 1997; Hasbroucq, Kaneko, Akamatsu, & Possamai, 1999). The decrease in CE seen prior to the IS in the current study fits these description as MEP amplitudes are seen to decrease in the FDS muscle prior to the IS in order to prevent the premature release of the movement.

The mechanism of action of selective proactive inhibitory control is beyond the scope of the current study, however in broad terms, this selective mechanism is postulated to make use of the frontostriatal circuit that engages the indirect pathway of the basal ganglia. In this circuit, the dorsolateral prefrontal cortex (dlPFC) signals the striatum to inhibit the globus pallidus pars externa (GPe), which removes inhibition from the globus pallidus pars interna (GPi) directly or via the subthalamic nucleus (STN), which increases the level of inhibition of a particular cortical response representation, for example within the motor cortex (Aron, 2011). Furthermore, Smittenaar and colleagues (2013) have implicated the dlPFC and striatum in selective inhibition, as suggested by the Aron model, but also speculate that the putamen plays a pivotal role in selective inhibition, and that the putamen and dlPFC are more active when speed of inhibition is prioritized over selectivity. The authors have also suggested that the STN is more involved in global rather than selective inhibition.

Overall, the current study has demonstrated that CE quickly increases from baseline values, coinciding with the presentation of the warning signal. This is followed by a holding period in which CE is held constant until the “decline” that occurs prior to the presentation of the IS. This “decline” is followed by a rapid increase in CE as the movement is initiated and released. It is speculated that levels of CE decrease in order to prevent the premature release of the programmed movement. Prior to the “ignition point”, the neural system undergoes selective proactive inhibition directed at the motor representation of the FDS muscle within the motor cortex, which decreases the level of CE until the critical moment where sufficient neural input within the system is reached, setting off the “ignition point”. These results are in contrast to previous studies that demonstrated an overall decrease or no change in CE during the foreperiod of reaction time tasks, however previous studies have failed to look at the entirety of the process of movement execution and did not take into account baseline MEP values.

Chapter II: Research Article II

Corticospinal Excitability is Modulated During Complex Tasks

Michael Kennefick, Anthony N. Carlsen

School of Human Kinetics, University of Ottawa, Canada

Abstract. Increasing the complexity of a movement by adding response components has been shown to increase reaction time (RT) (Henry and Rogers, 1960; Klapp, 1995). It has been suggested that these differences are due to the requirement to sequence the additional components following the imperative stimulus (IS) (Klapp, 1995). However, a portion of the RT differences may also be due to differences in corticospinal excitability. For example, previous studies (Flament et al., 1993; Abbruzzese et al., 1996) using simple and complex non-RT tasks have shown an enhanced excitatory state of corticospinal cells in complex tasks. The purpose of the present study was to probe the excitability of the motor system following the "go" signal in a simple RT task with differing levels of complexity. Participants performed either a single button press response (simple) or a multiple component button press sequence (complex) on a telegraph key in response to an IS. Suprathreshold (110% of resting motor threshold) transcranial magnetic stimulation (TMS) was delivered at 6 time points (0ms, 25ms, 50ms, 75ms, 100ms, 125ms) following the IS over the motor representation of the flexor digitorum superficialis muscle. Reaction time data indicated that participants responded significantly faster (28.1ms) on average in the simple task compared to the complex task. This difference in RT confirmed that adding response components was sufficient to alter the complexity of the task. Analysis of the corticospinal excitability data indicated that MEP amplitudes in the simple task were significantly larger compared to those in the complex task relative to both the IS and the onset of EMG. These findings suggest that simple and complex tasks achieve differing levels of corticospinal excitability, which may partially explain differences in RT. Furthermore, it is suggested that the complex, timing task requires the use of the cerebellum, which suppresses excitatory projections to the thalamus, and consequently to the motor cortex.

Keywords. Transcranial Magnetic Stimulation, Corticospinal Excitability, Motor Preparation, Complexity, Simple Reaction Time Task.

Introduction

In their seminal experiment, Henry and Rogers (1960) sought to understand how the complexity of a movement affected reaction time (RT). They found that increasing the complexity of a movement by adding movement components resulted in increased RTs, and that the increased RT was due to an increased amount of time required to program the movement (Schmidt & Lee, 2011, p. 78). Klapp and colleagues (1974) investigated the differences in RT for both simple RT and choice RT tasks in which the button press corresponded to the Morse code “dit” and “dah,” where “dit” was a short (press-release) button press and “dah” was a long (short-hold-release) button press. Klapp (1995) performed a series of experiments that were based on the assumption that responses are composed of a sequence of one or more “chunks” (e.g. one or more Morse code key presses). In these experiments, Morse code sequences consisted of either a one element movement (single “dit” or “dah”) or a four element movement (string of “dit” and “dah” presses). Results indicated that simple RT depends primarily on the number of chunks, rather than the complexity or duration within a single chunk. In contrast, choice RT appeared to depend primarily on the duration or complexity within a single chunk, and less strongly or not at all on the number of chunks. The differences between the two paradigms appears to lie in the fact that in a simple RT task the participant is able to pre-plan certain aspects of the response prior to the imperative signal (IS), while choice RT cannot be pre-planned and can only be initiated once the IS has been presented. More specifically, the internal complexity or parameters of a single chunk can be programmed prior to the IS, and therefore does not affect RT. However, it is presumed that the sequencing of “chunks” is programmed following the presentation of the IS, resulting in longer RTs.

A variety of neuroimaging techniques have been used to describe the role of the motor cortex in the execution of simple and complex finger movements, such as positron emission tomography (PET) and electroencephalography (EEG). Shibasaki and colleagues (1993) used PET during simple and complex motor tasks, and showed that the motor cortex played an important role in the planning and/or execution of complex sequential movements. Kitamura and colleagues (1993) used EEG measured scalp-recorded movement-related cortical potentials during simple and complex sequential finger movements in a non-RT paradigm to determine if there was an effect due to movement complexity. Results indicated that both the simple and complex tasks showed increases in movement-related cortical potentials, but that there was no difference between the conditions.

In contrast, transcranial magnetic stimulation (TMS) has not been used to examine the effects of complexity in RT tasks; however, the effect of finger movement complexity on corticospinal excitability (CE) in static, non-RT tasks have been examined. Flament and colleagues (1993) investigated the effects of an isolated index finger abduction (simple) versus a variety of static gripping (complex) tasks and demonstrated that MEPs were greater in at least one of the complex tasks compared to that of the simple finger abduction. Abbruzzese and colleagues (1996) used sequential finger movements with varying complexity levels in a non-RT task to detect changes in excitability of the motor cortex. Results indicated that the size of motor-evoked potentials (MEPs) increased during real sequential movements as well as imagined sequential movements. Furthermore, Roosink and Zijdewind (2010) noted that during a complex finger sequence, MEP amplitudes were significantly greater compared to the simple finger sequence.

In summary, it is clear that MEP amplitude increases as a result of increasing the complexity of a static, non-RT based task. However, Klapp and colleagues (1995) have shown that complexity in a simple RT based task is determined by the number of chunks or movements, and not on the complexity or duration within a single chunk. Thus, manipulating the number of chunks in a simple RT paradigm should result in differing levels of CE. To date, no studies have looked at CE following the IS in a simple RT task with varying complexities. As such it is unclear whether CE increases more slowly following the IS or must reach a higher level prior to movement output. Therefore this study was conducted to examine if differences in CE exist when complexity is manipulated by using a complex button press movement sequence as compared to a simple single button press movement in a simple RT task. It was hypothesized that CE would increase prior to the initiation of both simple and complex movements; however it was also predicted that CE would need to reach a higher level in order to trigger a more complex movement. Differences in MEPs were measured and compared to each other in order to determine whether the execution of a chunked complex movement results in changes in CE.

Methods

Participants.

Sixteen healthy volunteers (11M, 5F; mean age 25 ± 5 years), with normal or corrected to normal vision, and with no history of neurological, sensory, or motor disorders participated in this study. All participants were classified as right-handed or ambidextrous participants based on the Edinburgh Handedness Inventory (Oldfield, 1971). Testing of each participant took place in a single session, and required approximately 1.5 hours to complete.

All participants provided informed consent, and the study was conducted in accordance with ethical guidelines approved by the University of Ottawa's Research Ethics Board.

Experimental set-up and task.

Participants sat comfortably facing a 17 inch LCD computer monitor with their right arm pronated and resting on a flat surface. They were informed that the upcoming task was a simple RT task consisting of a button press movement. The presentation of a visual "Ready" along with an auditory template WS (described below) indicated the start of the trial, which was followed by a random foreperiod (2500-3500ms). The presentation of an auditory IS (82dB, 1000Hz) followed the foreperiod, at which time the participant initiated their movement. Auditory stimuli were generated using digital to analog hardware (National Instruments PCI-6024E) and the signal was amplified and presented via a loudspeaker (MG Electronics Model M58-H) located in front the participant.

Participants completed a simple RT task in which they performed either a simple, single button-press movement, or a complex sequence of button presses in response to an auditory IS. The movement consisted of pressing a telegraph key (Ameco AM-K4B) and was performed by the flexor digitorum superficialis (FDS) muscle by flexing the second finger. Similar to Klapp (1974), the simple movement was a single "dit" button press movement (150ms), while the complex movement was a sequence of three quick "dit" button press movements with the third "dit" separated by a 450ms pause from the first two (dit-dit-pause-dit).

Both the simple and complex tasks were accompanied by an on-screen prompt as well as an auditory template of the upcoming task, which acted as the WS. The auditory template represented the movement pattern including the amount of time each telegraph key should be pressed and time between presses. In the simple task, the on-screen prompt showed “dit”, while a 150ms auditory tone was initiated at the same time. In the complex task, the on-screen prompt showed “dit dit ___ dit”, while a series of three 150ms auditory tones, separated by a 450ms break between the 2nd and 3rd tones was initiated at the same time. Participants were instructed to execute this movement as quickly and as accurately as possible in response to the IS. Feedback was provided on the computer monitor after each trial, consisting of RT and task accuracy. Task accuracy represented the participant’s ability to replicate the auditory template played prior to the IS. If the participant pressed the telegraph key for an incorrect amount of time, the on-screen feedback would be red, and consequently green for a correct telegraph key press. A point system rewarded participants for fast RTs as well as successfully completing the task. The amount of points earned by the participant was only an incentive, and was not analyzed. Each participant began by completing a practice session prior to the testing session, which consisted of 10 simple and 10 complex movements. The practice trials were identical to that of the testing session, with the exception that there was no TMS. Following the practice session, participants performed 180 trials separated into 5 blocks of 36 trials each, which included 3 trials of each of 12 trial types; 2 movements (simple and complex) and 6 TMS timing points (0ms, 25ms, 50ms, 75ms, 100ms, and 125ms) following the IS. The order of the trials within each block was randomized. The task is visually represented in figure 9a and 9b.

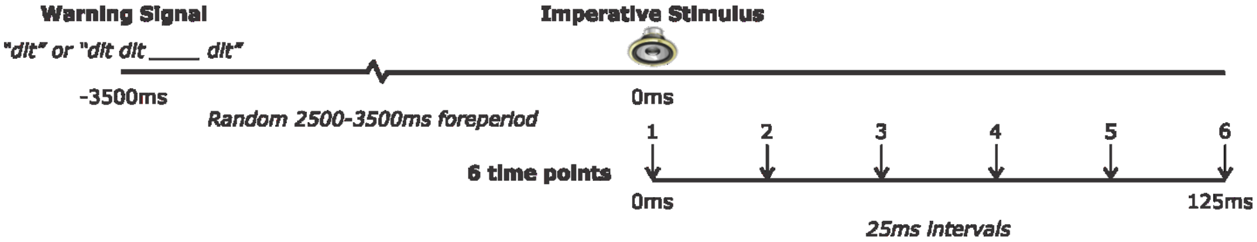


Figure 9a. Visual representation of the TMS stimulation points, represented by downwards pointing arrows.

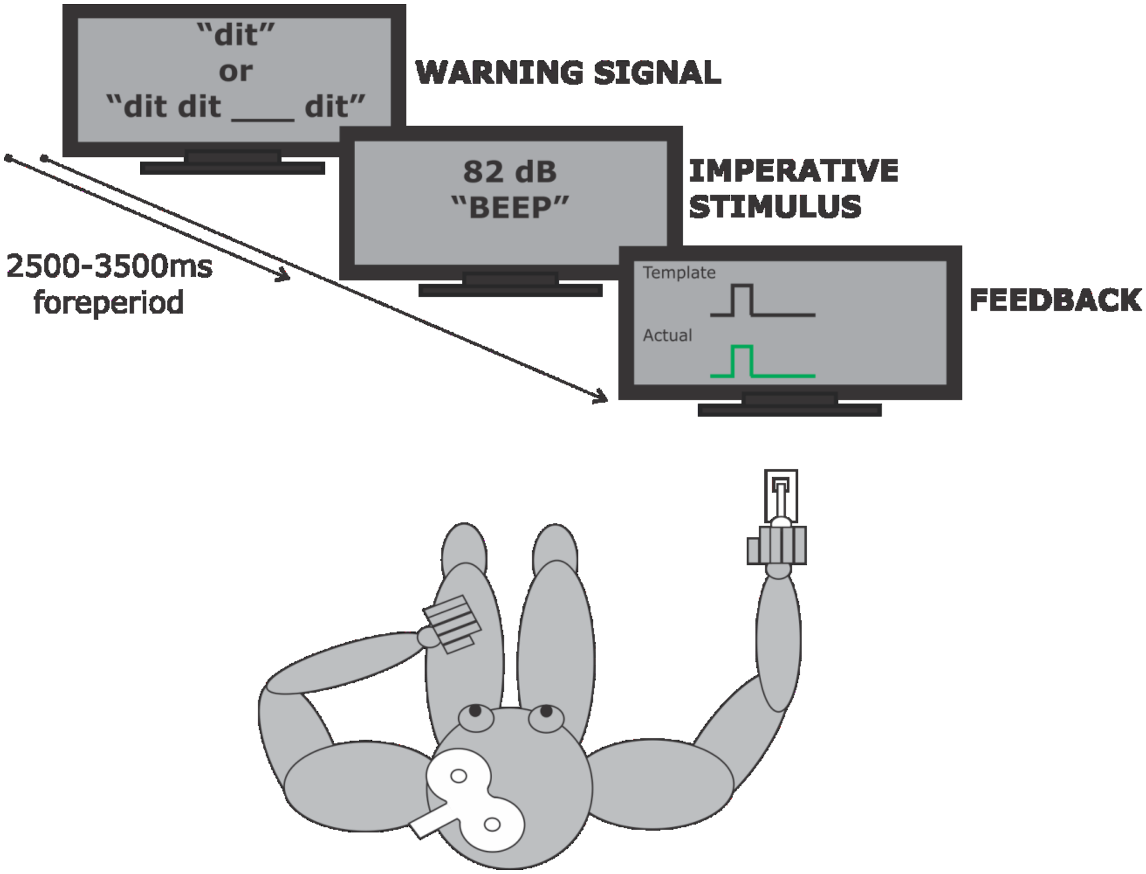


Figure 9b. Visual representation of a single trial in experiment 2.

Transcranial magnetic stimulation

TMS was applied using a figure-8 magnetic coil (70mm; Magstim 200^2, Magstim Company Ltd, UK). Prior to testing, the optimal location for stimulation of the right flexor digitorum superficialis (FDS) was found by first finding the midpoint between the nasion and inion, and the left and right preauriculars. From this midpoint, a location 5cm lateral and 1cm posterior was marked on the participant's scalp using a red grease crayon. The optimal location was then found by delivering test pulses a various scalp locations around this mark and determining the location that consistently produced the largest MEPs. Resting motor threshold (RMT) was determined at rest to the nearest 1% of stimulator output using the Rossini-Rothwell method (defined as the minimum intensity required to evoke MEPs above 50 µV in at least 5 out of 10 trials). The magnetic coil was positioned pointing backwards at a 45˚ angle and held stationary by the experimenter while the position was maintained by holding the coil in the reference position on the head with the assistance of neuronavigation hardware and software (ANT Neuro Visor 2, Madison, WI). Stimulus intensity during testing was adjusted to 110% RMT. Similar intensities have been used previously to probe changes in CE (Chen, et al., 1998; MacKinnon & Rothwell, 2000).

Recording equipment

Surface EMG data was collected from the muscle belly of the right FDS muscle using bipolar preamplified (gain=10) surface electrode (Delsys Bagnoli DE-2.1) connected via shielded cabling to an external amplifier (Delsys Bagnoli-8). The electrode was placed parallel to the muscle fibres, and attached to the skin using double sided adhesive strips. A grounding electrode (Dermatrode HE-R) was placed on the participant's right lateral epicondyle. The site of the electrode was cleaned using abrasive skin prepping gel to ensure minimal electrical impedance. Data collection for each trial was initiated by the computer 500ms prior to the WS and continued for 2000ms. Unfiltered EMG and button data was digitally sampled at 1kHz (National Instruments PCI-6024E) using a custom made program written in LabVIEW (National Instruments Inc.) and stored for offline analysis.

Data reduction

MEP amplitudes, time between TMS pulse and EMG onset, and the beginning and end of movements were calculated. Trials in which TMS was delivered during the EMG burst were rejected because it is inherently difficult to distinguish the MEP from movement related EMG. This resulted in some participants not having a value for certain time points, and missing values were filled using a linear-based multiple imputations procedure in SPSS (IBM Inc.) in order to perform a full factorial analysis. Six out of 96 cases had to be filled using multiple imputations for the simple condition, while 2 out of 96 cases had to be filled for the complex condition. MEP amplitudes were quantified by calculating the peak-to-peak voltages of the evoked responses and were expressed as a percentage of baseline values measured at time point 1 (0ms) for each condition. Agonist EMG burst onsets associated with the voluntary movement were defined as the point at which EMG activity reached a value of 2 standard deviations above baseline levels and remained at that level for more than 20ms. EMG offset was defined as the point at which EMG activity fell below a value of 80% of its maximal amplitude (Hodges & Bui, 1996). EMG markers were manually adjusted to compensate for any errors due to the strictness of the LabVIEW algorithm. Movement related EMG activity in the FDS muscle was marked for each trial. Peak EMG amplitudes were defined as the largest EMG amplitude recorded within an interval of 100ms following

EMG burst onset. In order to analyze the data relative to EMG onset, the difference in time between the onset of agonist EMG and the presentation of TMS was calculated. Reaction times that were greater than 350ms were excluded from the analysis (Klapp, 2003), as these were considered to be bad trials since the participants reacted too slowly to have been properly preparing for the task.

Statistical Analysis

Dependent variables were analyzed using Repeated Measures Analysis of Variance to determine if differences existed between presentation times of the TMS, and between the two different conditions (simple vs. complex). Differences with a probability of less than 0.05 were considered significant. Greenhouse-Geisser corrected values were used if violations in sphericity occurred. Post-hoc tests using Bonferonni corrected student's t-tests were conducted to determine the locus of any differences.

Results

MEP amplitude

Peak-to-peak MEP amplitudes measured as a percentage of baseline for both the simple and complex conditions were analyzed using a 2 condition (simple and complex) x 6 time point (0ms, 25ms, 50ms, 75ms, 100ms, 125ms) repeated measures ANOVA. A significant main effect was found for both condition ($F(1,15) = 5.613$, $p = 0.032$, $\eta_p^2 = 0.272$), indicating that MEPs in the simple task were larger than those in the complex task. A significant main effect was also found for time ($F(5,75) = 7.822$, $p = 0.002$, $\eta_p^2 = 0.343$). Post-hoc analysis of the time main effect revealed significant differences in MEP amplitude between time point 4(75ms) and time point 1(0ms), 2(25ms), and 3(50ms), as well as between time point 3(50ms) and time point 2(25ms), shown in figure 10. The interaction between the factors was found to be non-significant ($F(5,75) = 2.631$, $p = 0.066$, $\eta_p^2 = 0.149$), however, since a trend can be seen to be emerging, the raw MEP amplitude values at baseline (IS onset, 0ms) were compared using a paired samples t-test. Results showed that MEP amplitudes for both the simple and complex conditions were not different (0.39mV). Finally, the data was also analyzed relative to the onset of EMG – as has been done in past studies, (Chen, et al., 1998). The data was binned in 25ms intervals relative to EMG onset by calculating the time difference between the onset of EMG and the MEP. These time bins included MEP onsets that occurred either <75 ms prior to EMG onset or 25 ms time bins at increasing intervals up to >150 ms prior to EMG onset (see figure 11). Bonferonni corrected student's t-tests were used to determine if any differences existed between MEP amplitudes at each of the time bins. This analysis revealed a significant ($p=0.002$) difference between the simple and the complex condition only at 75ms prior to EMG onset (figure 11).

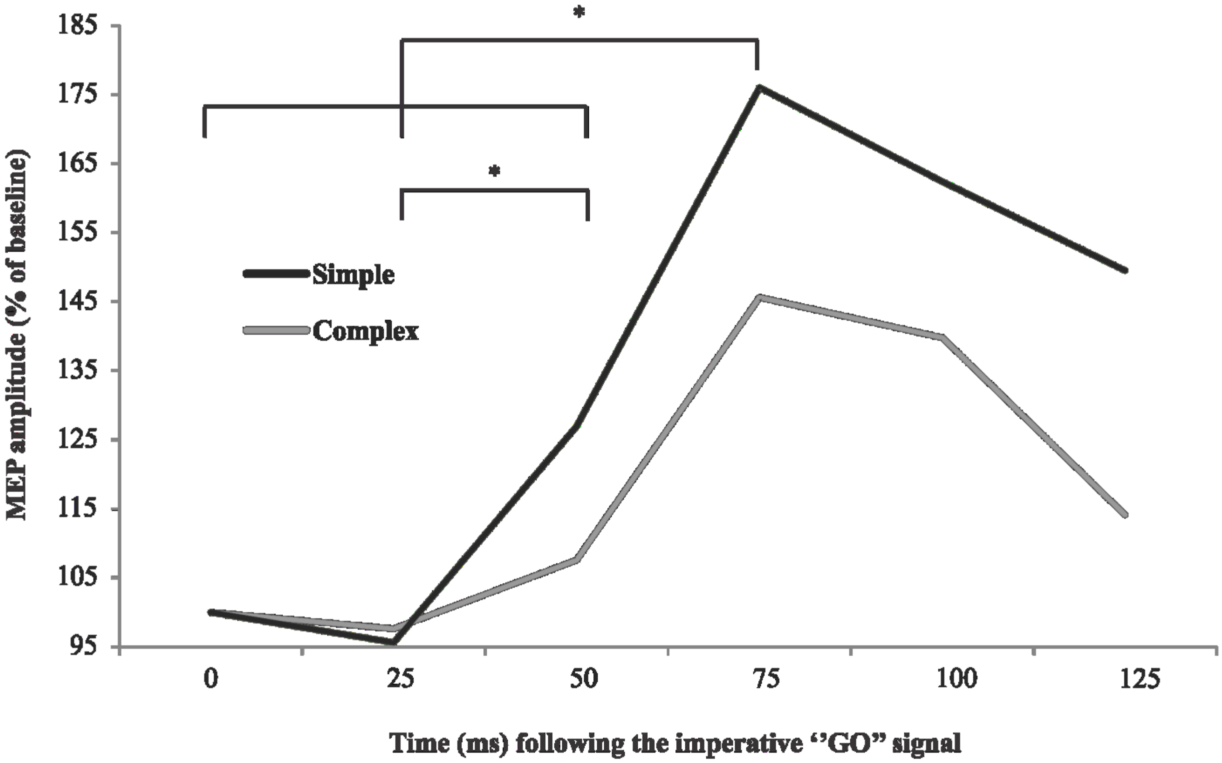


Figure 10. MEP amplitudes as a percentage of baseline following the imperative signal for both the simple and the complex task. Significant main effects were found for both condition (black vs. grey) and time point (significant comparisons indicated with *). A non-significant condition x time interaction (p=0.066) was found.

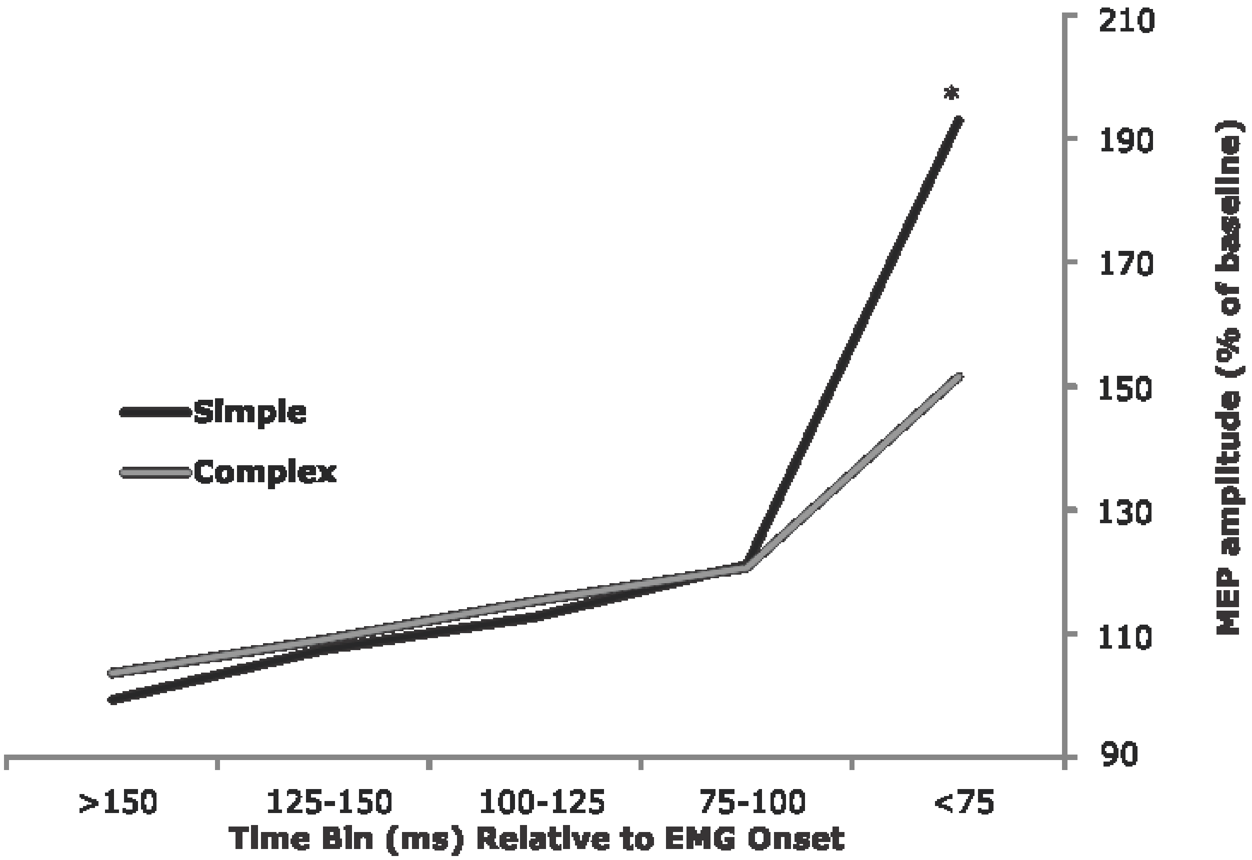


Figure 11. MEP amplitudes as a percentage of baseline relative to EMG onset for both the simple and the complex task. Bonferonni corrected student's t-tests revealed a significant (*p*=0.002) difference at 75ms prior to EMG onset.

Reaction Time

Displacement reaction time (RT) was analyzed using a paired-samples student's t-test. The analysis confirmed that RT in the complex task was significantly different than the simple task ($T(15) = 5.579$, $p = 0.01$), whereby RTs were 28.1ms longer on average in the complex task. Figure 12 depicts this difference using box plots, in which the box represents the 25th to 75th percentile range of the data, the horizontal line represents the median, and the small square box within the 25th-75th percentile range box represents the mean.

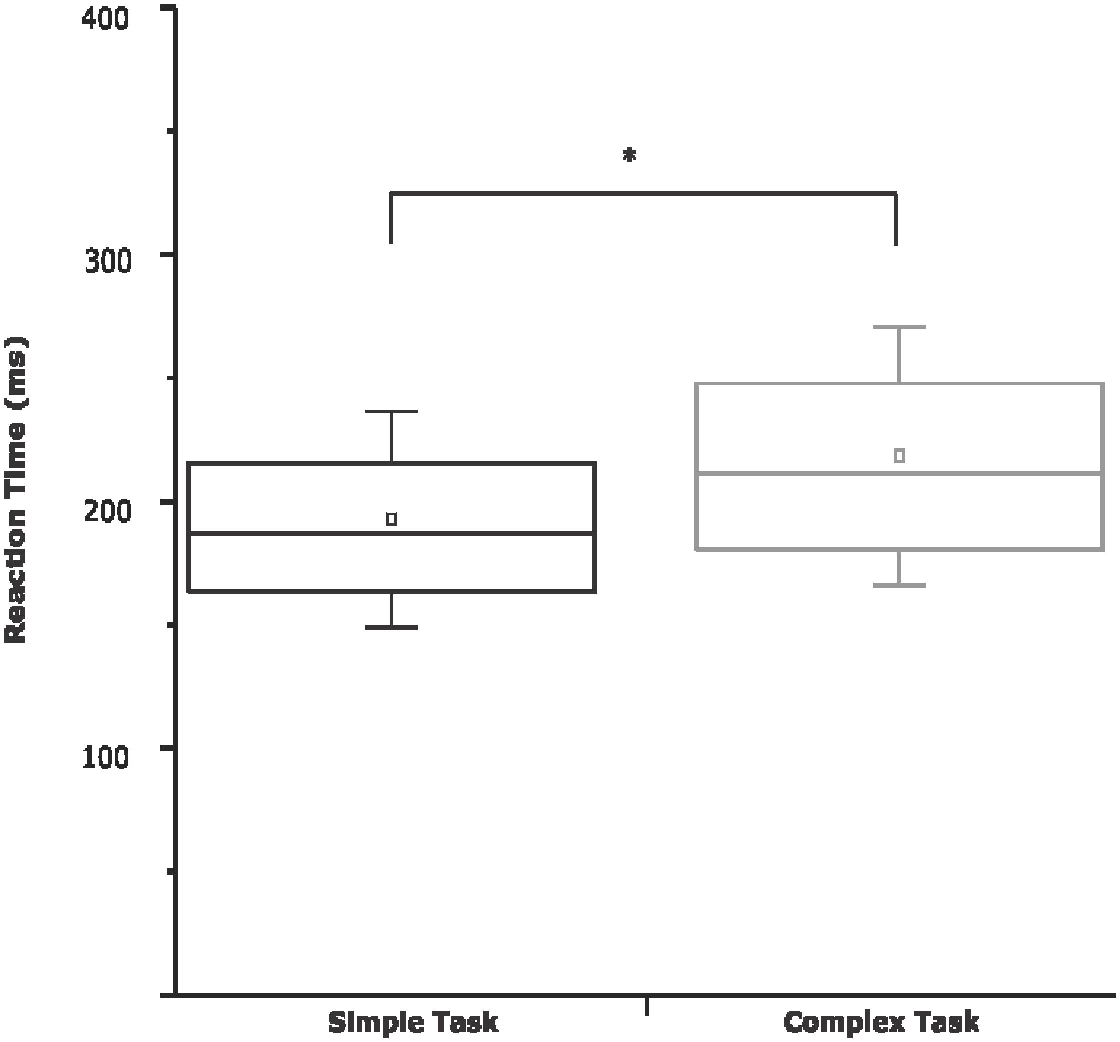


Figure 12. Box plots representing the reaction times for the simple and the complex tasks. For each data point, the small square indicates the mean, the horizontal line within the rectangular box indicates the median, boundaries of the rectangular box indicate the 25th- and 75th -percentile, and the whiskers indicate one standard deviation from the mean.

Discussion

The purpose of this study was to probe the excitability of the motor system following the imperative “go” signal in a simple RT task with differing levels of complexity. Previous work has shown that RTs in a complex task are typically significantly longer than those in a simple task (Henry & Rogers, 1960), and that these differences are presumably due to the requirement to sequence the additional components following the IS (Klapp, 1995). Data from the current study demonstrated similar results, with RTs in the complex task being 28.1ms longer on average than in the simple task. This difference in RT confirmed that adding response components was sufficient to alter the complexity of the task and represented the classic simple-up-choice-flat pattern (Klapp, 2003). Of particular interest in this study was how differences in CE contribute to these differences in RT.

One of the fundamental concepts that revolves around the idea of complexity is that longer, or more complex movements are organized into chunks (Klapp, 1995). These chunks are programmed in a hierarchical fashion, whereby the programming of the internal components within a single chunk are programmed separately to the programming of an overall sequence formed by chunks. Therefore, response complexity can be classified as either the internal complexities of an individual chunk (INT), or the complexity of the entire sequence, determined by the number of chunks (SEQ). Bearing this in mind, the SEQ classification of complexity is specifically concerned with multiple component movements (Klapp, 2010). In his seminal work, Klapp used a button-press task to demonstrate that simple RT does not depend on the complexity of a single chunk, such as a single button-press (INT), suggesting that single chunk actions can be prepared in advance. However, simple RT is affected by the number of chunks that make up a sequence, as in multiple button-presses (SEQ) (Klapp, 1995). Klapp also postulated that unlike INT, SEQ cannot be programmed in advance, resulting in the need to carry out this process just prior to the start of the response, resulting in longer RTs. Furthermore, Klapp argued that the timing of chunk onset may be responsible for the relationship between the number of chunks and simple RT, and that a control structure for timing exists, if the precue of the simple RT paradigm specifies the number of chunks (Klapp, 2010).

The current study manipulated complexity by requiring multiple response components and thus, an added element of timing. The notion of timing can be broken down into two categories relating to task dynamics; event-based and emergent. Spencer and Ivry (2013) have described event-based timing as task in which the representation of temporal information is an explicit part of the task goal. An example of this would be a finger tapping task, in which the participant attempts to align movements with specific events, such as a metronome. Furthermore, they defined emergent timing as a task in which temporal regularities may arise from the dynamics of a continuous event, for example a repetitive circle drawing. Further evidence to support this dissociation was demonstrated by Spencer and colleagues (2005) in which participants with cerebellar damage performed three tasks. In one task, the participants pressed and immediately released a key, while in the other two tasks, participants had to press a key, and after a delay, release the key. Participants with cerebellar lesions were impaired on the two conditions requiring the insertion of a temporal delay. It has previously been concluded that the cerebellum has a clear role in event-based timing, and that the basal ganglia, another proposed area of timing control, appears to be involved in emergent timing (Spencer & Ivry, 2005). Therefore, we suggest that the complex, sequenced finger-pressing movement used in the current study is an example of an event-based timing task.

Previous studies (Abbruzzese, et al., 1996; Flament, et al., 1993) that have used TMS to examine the effect of complexity on CE levels in static, non-RT based tasks have shown that CE increases as a movement becomes more complex. In contrast, the current study has demonstrated a *decrease* in CE in the complex condition. These differences may due to the fact that the current study is a RT based task; however, as noted above, since the event-based timing component of the complex condition required the use of the cerebellum, we suggest that that input from the cerebellum is ultimately responsible for decreased levels of CE.

The cerebellum has been long been considered to play a major role in the planning, initiation and organization of movement (Allen & Tsukahara, 1974). The seminal work by Braitenburg (1967, 1983) examining the organization of Purkinje cells within the cerebellar cortex was instrumental for specifying the precise role of the cerebellum, and he hypothesized that the cerebellum was involved in the production of coordinated movements. The cerebellum, located dorsal to the brain stem, is connected to the brain stem by three peduncles, with the superior peduncle acting as the output pathway. Deeper within the cerebellum lies the cerebellar cortex, which uniquely connects to the deep cerebellar nuclei and to the vestibular nuclei. The neurons in the deep cerebellar nuclei are virtually all of the output from the cerebellum. The dentate nucleus, within the deep cerebellar nuclei, has connections through the superior peduncle to the ventral lateral nucleus of the thalamus, which relays to the cerebral motor cortex (Swenson, 2006). The cerebellum contains a greater number of neurons than any other part of the brain. It does however, only contain a small number of neuronal types, one of which is the Purkinje cell (Kandel, 2000). The

Purkinje cell axons are the only nerve fibres that exit the cerebellar cortex, and they synapse on the deep cerebellar nuclei neurons, inhibiting them powerfully using the gamma-Aminobutyric acid (GABA) inhibitory neurotransmitter. At a deeper level, only two types of axons enter the cerebellum; mossy fibres and climbing fibres (Swenson, 2006). Climbing fibres arise only from the inferior olivary nucleus, while mossy fibres originate from the spinal cord and brain stem, and comprise all of the other cerebellar inputs. Each mossy fibre terminal synapses on a group of granule cell dendrites, exciting the granule cell. These granule cell axons run along the axis of the cerebellar folia, exciting many Purkinje cells (Kandel, 2000). Both of the climbing and mossy fibres synapse on the deep cerebellar nucleus neurons. Therefore, the excitatory input that enters the cerebellum with the mossy fibre is responsible for exciting the deep nucleus neuron and consequently exciting granule cells that stimulate Purkinje cells, which "turn off" the deep nucleus neuron. It is critical that the correct amount of inhibition arrive at the deep cerebellar nucleus in order to produce an appropriate output, inhibiting unwanted activity in the deep cerebellar nuclei (Swenson, 2006). Thus it is proposed that due to the involvement of the olivocerebellar system in timing based tasks, the inhibitory Purkinje cells lead to a suppression of excitatory output from the deep cerebellar nuclei. In the current experiment this suppression led to the observed reduced levels of CE.

A recent, extensive review of the olivocerebellar system by Llinás (2014) concluded that this system appears to be a unique control system, in which timing appears to be the main purpose. The olivocerebellar system is part of the cerebrocerebellar functional subdivision of the cerebellum, which receives its input exclusively from the cerebral cortex, and is intimately involved in the planning of complex motor actions (Chez & Thach, 2000). Anatomical connections mediating the exchange of information between the cerebrum and the cerebellum remain unknown, as well as if these pathways are direct connections from the motor cortex or indirect connections via intermediate relays (Apps & Watson, 2013). The olivocerebellar circuit, first described by Ramon y Cajal (1911), comprises three main element within the cerebellum; the inferior olive, the Purkinje cell, and deep cerebellar nucleus. The basic circuitry of this system relies on the initial activation of Purkinje cells by the inferior olive axons via climbing fibres, which then send signals to cerebellar nuclear cells that feedback to the inferior olive as well and cerebellar nuclear cells. As mentioned prior, the dentate nucleus within the deep cerebellar nucleus relays information from the thalamus to the cerebral motor cortex (Swenson, 2006). Early studies of the olivocerebellar system sought to understand how it "controlled" movements, for example how the cerebellum restrained the nearly infinite number of possible movements, to a select few. It has previously been established that the olivary neurons fire in an oscillatory and rhythmic manner, and that membrane potentials of these neurons oscillate in the a subthreshold range for firing action potentials, which result in a clear cycle of excitability in which synaptic inputs are more or less likely to trigger an action potential. The olivocerebellar system rhythmically modulates movement and it dynamically reconfigures itself during the execution of a more complex movement. The removal of the inferior olive severely disrupts the ability to perform complex movements that require synergic activation of muscles (Welsh & Llinas, 1997). Therefore, the olivocerebellar circuit appears to mediate and restrain descending information from the cerebrum to the cerebellum before forwarding timing related information to other structures.

Interactions between the cerebellum and the cortex in humans were examined by Ugawa and colleagues (1995). Using a paired-pulse paradigm, the authors demonstrated that magnetic stimulation over the cerebellum with a double-cone coil reduced the size of the first dorsal interosseous EMG response, and that increasing cerebellar stimulation also resulted in stronger inhibition. Furthermore, the same protocol was used on participants with cerebellar dysfunction. Results indicated that there was no inhibition, suggesting that the inhibitory activity is modulated at the level of the cerebellum. Other TMS studies conducted on patients who have suffered strokes have indicated that the cerebellum has a strong influence on motor cortex excitability. Liepert and colleagues (2004) showed that patients performing the Nine-Hole-Peg Test displayed lowered excitability levels, which was said to have been induced by a disruption of facilitatory drive from the deep cerebellar nuclei, more specifically the interposited nuclei and the dentate nucleus. Furthermore, Battaglia and colleagues (2006) found that lesions to the deep cerebellar nuclei, more specifically the dentate nuclei, decreased excitability of the contralateral motor cortex, which impaired the patient's abilities to prepare and imagine sequential movements. Studies of the interaction between the cerebellum and motor cortex have also been conducted in healthy populations. Pinto and Chen (2001) used paired-pulse TMS with the conditioning stimulus over the cerebellum and the test stimulus over the motor cortex during a voluntary activation of target muscle as well as an arm extension movement and showed a reduction in excitability during the arm extension movement, which was hypothesized to be due to decreased excitability of cerebellar Purkinje cells or reduced activity of the dentatothalamocortical pathway. Daskalakis and colleagues (2004) coined this inhibition as "cerebellar inhibition" as it occurs at the level of the cerebellar cortex, and revealed a significant reduction in short-interval intracortical inhibition (SICI) in the presence of cerebellar inhibition compared to SICI alone. Therefore, the proposed model, which is controlled by inhibitory Purkinje cells, leads to a suppression of excitatory output from the deep cerebellar nuclei. The suppression of the deep cerebellar nuclei results in the suppression of excitatory output from the ventrolateral nucleus of the thalamus, which consequently leads to a decreased excitatory drive to output neurons. This would finally cause a decrease in MEP amplitude (Figure 13).

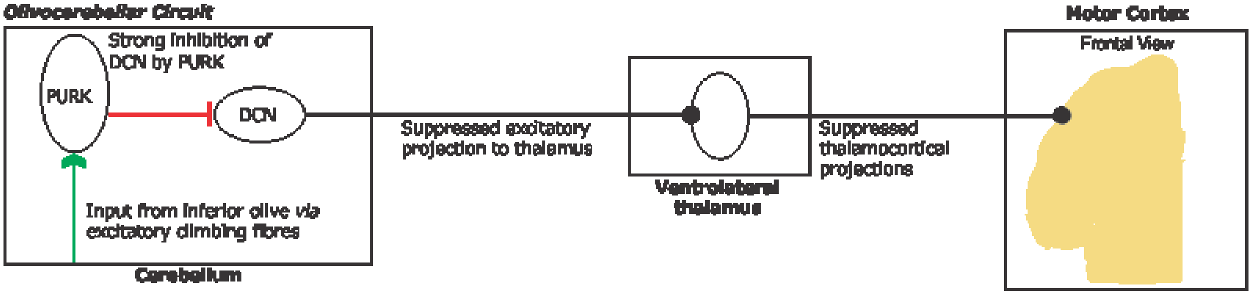


Figure 13. Visual representation of proposed model of corticospinal modulation via the cerebellum.

The results of the current experiment can alternatively be described using a neural activation model. Movement initiation can be thought of as the process of increasing neural activation of cortical motor neurons, until an "ignition" threshold level is reached (Carpenter & Williams, 1995; Hanes & Schall, 1996; Nazir & Jacobs, 1991). It has been suggested that more complex movements may either require a greater threshold (Maslovat, Hodges, Chua, & Franks, 2011), begin the activation rise from a lower level of activation (Carlsen, et al., 2012), or have differences in the rate of activation accumulation (Carpenter & Williams, 1995), thus requiring greater time to reach initiation threshold, resulting in a delayed RT. The results of the current experiment show that at baseline (IS onset, 0 ms), there were no differences in excitability levels between the simple and complex tasks. Excitability levels remained consistent for the first 25ms, and then began to diverge with excitability in the simple task rising at a quicker rate compared to the complex task (Figure 10). When CE was normalized to EMG onset, there was also evidence for decreased activation in the complex task, as shown by a lower MEP amplitude just prior to movement execution (Figure 11). Collectively, these results suggest that it is possible that movement complexity RT differences may be due to a decreased rate of neural activation, rather than threshold or starting activation levels.

In conclusion, the results of the current study demonstrated that the requirement to perform complex, sequenced movements led to a decrease in corticospinal excitability compared to simple, non-sequenced movements. These results initially appear to be in contrast to previous studies (Abbruzzese, et al., 1996; Flament, et al., 1993) that have examined corticospinal excitability in simple and complex static tasks; however the current task is inherently different. None of the previous studies required a sequencing component, nor were they tested in a RT paradigm. The sequencing in the current study reflects the increased difficulty of the complex task in a RT paradigm. It is therefore proposed that the lower level of corticospinal excitability is due to the increased inhibition at the level of the cerebellum required to control the timing of the sequence. Furthermore, due to similar levels of CE at the presentation of the go-signal, it is hypothesized that simple and complex movement are at least partly programmed in a similar manner.

Chapter III: General Discussion

The overall goal of this thesis was to gain a better understanding of the processes that occur in the brain as humans prepare to make a movement. In the two experiments contained in this thesis, transcranial magnetic stimulation (TMS) was used to assess changes in corticospinal excitability in the foreperiod as well as the reaction time (RT) interval of a simple reaction time task. The first experiment used more robust methods than prior studies in an attempt to better describe the time course of excitability in the foreperiod of a simple RT button press task, as well describe how levels of corticospinal excitability (CE) changed once an imperative stimulus (IS) is presented to participants and they initiate their movements. The second experiment sought to understand how CE is affected by task complexity in a simple RT paradigm, and how these changes in excitability might affect the preparation of complex movements and RTs.

Levels of CE have previously been shown to decrease in the 500ms prior to the presentation of an IS in simple RT tasks that use short foreperiods. Importantly, these studies have also shown that no differences exist in levels of CE in simple RT tasks that use long foreperiods. These studies have all described excitability changes in relation to previous time points in which participants were presumably already in a heightened state of preparation, but not to time points in which the participants were at rest. Experiment 1 described CE levels during the entirety of the process of movement execution (e.g. throughout the foreperiod and response time) in relation to levels measured while the participant was *at rest*, prior to the presentation of a warning signal (figure 2). The results of Experiment 1 showed that humans quickly raise their levels of preparation following the presentation of the warning signal (figure 4) in a long foreperiod to a point at which they hold and restrict this sharp increase (figure 5). In the 500ms prior to the presentation of the IS, and in agreement with prior short foreperiod studies, levels of corticospinal excitability decreased in anticipation of the IS (figure 6). It is proposed that the brain undergoes selective proactive inhibition to ensure that the prepared movement is not prematurely released and held until the IS is presented, at which point levels of corticospinal excitability quickly increase once again as the inhibition is overcome and the movement is released.

Levels of CE have previously been shown to increase as a function of increasing task complexity in static, non-RT based tasks. However, the effect of increasing complexity on corticospinal excitability levels have never been described in relation to reaction time tasks. Previous work by Klapp (1995; 2010) has suggested that response complexity can be classified as either the internal complexities of an individual movement chunk, or the complexity of the entire sequence, determined by the number of chunks. Furthermore, simple RT is not affected by the internal complexities single chunk actions, for example a single button-press movement, but rather by the number of chunks that make up a sequence seen in multiple button-press movements. Importantly, the longer RTs seen in more complex, sequenced movements are postulated to occur because the number of chunks in a sequence cannot be prepared in advance. Experiment 2 used similar button-press tasks as Klapp to show that as participants are presented with the IS, no difference is seen in corticospinal excitability levels between the simple and complex task. Whether time locked to the presentation of the IS or EMG onset, corticospinal levels in both tasks begin to diverge shortly thereafter and ultimately become greater in the simple condition (figures 10 and 11). It is postulated that in timing based tasks, the cerebellum and, more specifically, the olivocerebellar system is responsible for depressing levels of corticospinal excitability by way of inhibition at the level of the deep cerebellar neuron, which ultimately suppresses projections to the motor cortex (figure 13). Furthermore, due to the fact that levels of corticospinal excitability were no different in the 75-100ms prior to EMG onset across both conditions (figure 11), it appears that both the simple and complex task, both requiring different types of programming can at least partly be prepared in a similar manner prior to the IS, in contrast to a currently understood model of complexity.

Based on Klapp's work, the current model of complexity states that the internal parameters (INT) of a chunk can be preprogrammed during the foreperiod of a simple RT, while the sequencing (SEQ) of chunks cannot be preprogrammed, and therefore must occur following the IS, during the RT interval. Since single chunk movements do not require a sequencing component, they can be entirely preprogrammed during the foreperiod, while multiple chunk movements cannot, which leads to longer RTs. In a novel way, the current thesis has presented further evidence in support of this model. Whether time locked to the presentation of the IS (figure 10) or the onset of EMG (figure 11), there was no difference in CE between single chunk and multiple chunk movements at the beginning of the movement. This indicates that these tasks may be similarly programmed, and follow the same time course of CE (figure 2) prior to the presentation of the IS. Following the IS, the task requiring a SEQ component is modulated by the cerebellum, which depresses levels of CE. Furthermore, the longer RTs traditionally seen in complex tasks can thus be attributed to the depressed levels of CE.

References

Abbruzzese, G., Trompetto, C., & Schieppati, M. (1996). The excitability of the human motor cortex increases during execution and mental imagination of sequential but not repetitive finger movements. *Experimental Brain Research, 111*(3), 465-472.

Adrian, E. D., & Yamagiwa, K. (1935). The origin of the berger rhythm. [Article]. *Brain, 58*, 317-+.

Allen, G. I., & Tsukahara, N. (1974). Cerebrocerebellar communication systems. *Physiological Reviews, 54*(4), 957-1006.

Apps, R., & Watson, T. C. (2013). Cerebro-Cerebellar Connections. In M. Manto, J. Schmahmann, F. Rossi, D. Gruol & N. Koibuchi (Eds.), *Handbook of the Cerebellum and Cerebellar Disorders*.

Aron, A. R. (2007). The neural basis of inhibition in cognitive control. *Neuroscientist, 13*(3), 214-228.

Aron, A. R. (2011). From reactive to proactive and selective control: Developing a richer model for stopping inappropriate responses. *Biological Psychiatry, 69*(12), e55-e68.

Aron, A. R., & Verbruggen, F. (2008). Stop the Presses: Dissociating a Selective From a Global Mechanism for Stopping. *Psychological Science, 19*(11), 1146-1153. doi: 10.1111/j.1467-9280.2008.02216.x

Barker, A. T., Freeston, I. L., Jalinous, R., & Jarratt, J. A. (1987). Magnetic stimulation of the human-brain and peripheral nervous-system - an introduction and the results of an initial clinical evaluation. *Neurosurgery, 20*(1), 100-109.

Barker, A. T., Freeston, I. L., Jalinous, R., Merton, P. A., & Morton, H. B. (1985). Magnetic stimulation of the human brain. *Journal of Physiology-London, 369*(DEC), P3-P3.

Barker, A. T., & Jalinous, R. (1985). Non-invasive magnetic stimulation of the human motor cortex. *Lancet, 1*(8437), 1106-1107.

Battaglia, F., Quartarone, A., Ghilardi, M. F., Dattola, R., Bagnato, S., Rizzo, V., . . . Girlanda, P. (2006). Unilateral cerebellar stroke disrupts movement preparation and motor imagery. *Clinical Neurophysiology, 117*(5), 1009-1016.

Braitenberg, V. (1967) Is the Cerebellar Cortex a Biological Clock in the Millisecond Range? *: Vol. 25* (pp. 334-346).

Braitenberg, V. (1983). The cerebellum revisited. *Journal of Theoretical Neurobiology, 2*(3), 237-241.

Cai, W., Oldenkamp, C. L., & Aron, A. R. (2011). A proactive mechanism for selective suppression of response tendencies. *Journal of Neuroscience, 31*(16), 5965-5969.

Carlsen, A. N., Maslovat, D., & Franks, I. M. (2012). Preparation for voluntary movement in healthy and clinical populations: Evidence from startle. *Clinical Neurophysiology, 123*(1), 21-33.

Carpenter, R. H. S., & Williams, M. L. L. (1995). Neural computation of log likelihood in control of saccadic eye-movements. *Nature, 377*(6544), 59-62. doi: 10.1038/377059a0

Chen, R., Yaseen, Z., Cohen, L. G., & Hallett, M. (1998). Time course of corticospinal excitability in reaction time and self-paced movements. *Annals of Neurology, 44*(3), 317-325. doi: 10.1002/ana.410440306

Chez, C., & Thach, W. T. (2000). The Cerebellum. In E. R. Kandel, Schwartz, James H., Jessell, Thomas M. (Ed.), *Principles of Neural Science* (4 ed.): The McGraw-Hill Companies, Inc.

Daskalakis, Z. J., Paradiso, G. O., Christensen, B. K., Fitzgerald, P. B., Gunraj, C., & Chen, R. (2004). Exploring the connectivity between the cerebellum and motor cortex in humans. *Journal of Physiology, 557*(2), 689-700.

Deecke, L. (1996). Planning, preparation, execution, and imagery of volitional action. *Cognitive Brain Research, 3*(2), 59-64. doi: 10.1016/0926-6410(95)00046-1

Di Lazzaro, V., Ziemann, U., & Lemon, R. N. (2008). State of the art: Physiology of transcranial motor cortex stimulation. [Review]. *Brain Stimulation, 1*(4), 345-362. doi: 10.1016/j.brs.2008.07.004

Duque, J., Lew, D., Mazzocchio, R., Olivier, E., & Ivry, R. B. (2010). Evidence for two concurrent inhibitory mechanisms during response preparation. *Journal of Neuroscience, 30*(10), 3793-3802.

Faisal, A. A., Selen, L. P. J., & Wolpert, D. M. (2008). Noise in the nervous system. *Nature Reviews Neuroscience, 9*(4), 292-303. doi: 10.1038/nrn2258

Flament, D., Goldsmith, P., Buckley, C. J., & Lemon, R. N. (1993). Task dependence of responses in 1st dorsal interosseusmuscle to magnetic brain-stimulation in man. *Journal of Physiology-London, 464*, 361-378.

Gerloff, C., Corwell, B., Chen, R., Hallett, M., & Cohen, L. G. (1998). The role of the human motor cortex in the control of complex and simple finger movement sequences. *Brain, 121*, 1695-1709. doi: 10.1093/brain/121.9.1695

Greenhouse, I., Oldenkamp, C. L., & Aron, A. R. (2012). Stopping a response has global or nonglobal effects on the motor system depending on preparation. *Journal of Neurophysiology, 107*(1), 384-392.

Hanes, D. P., & Schall, J. D. (1996). Neural control of voluntary movement initiation. *Science, 274*(5286), 427-430. doi: 10.1126/science.274.5286.427

Hasbroucq, T., Kaneko, H., Akamatsu, M., & Possamai, C. A. (1997). Preparatory inhibition of cortico-spinal excitability: A transcranial magnetic stimulation study in man. [Article]. *Cognitive Brain Research, 5*(3), 185-192. doi: 10.1016/s0926-6410(96)00069-9

Hasbroucq, T., Kaneko, H., Akamatsu, N., & Possamai, C. A. (1999). The time-course of preparatory spinal and cortico-spinal inhibition: an H-reflex and transcranial magnetic stimulation study in man. *Experimental Brain Research, 124*(1), 33-41. doi: 10.1007/s002210050597

Hebb, D. O. (1949). *The organization of behaviour: A neuropsychological theory*. New York: Wiley.

Henry, F. M., & Rogers, D. E. (1960). Increased response latency for complicated movements and a memory drum theory of neuromotor reaction. *Research Quarterly, 31*(3), 448-458.

Hodges, P. W., & Bui, B. H. (1996). A comparison of computer-based methods for the determination of onset of muscle contraction using electromyography. *Electromyography and Motor Control-Electroencephalography and Clinical Neurophysiology, 101*(6), 511-519. doi: 10.1016/s0921-884x(96)95190-5

Kandel, E. R., Schwartz, James H., Jessell, Thomas M. (2000). *Principles of Neuroscience* (4 ed.). New York: McGraw-Hill.

Kitamura, J., Shibasaki, H., Takagi, A., Nabeshima, H., & Yamaguchi, A. (1993). Enhanced negative slope of cortical potentials before sequential as compared with simultaneous extensions of 2 fingers. *Electroencephalography and Clinical Neurophysiology, 86*(3), 176-182. doi: 10.1016/0013-4694(93)90005-g

Klapp, S. T. (1995). Motor response programming during simple and choice-reaction time - the role of practice. *Journal of Experimental Psychology-Human Perception and Performance, 21*(5), 1015-1027. doi: 10.1037//0096-1523.21.5.1015

Klapp, S. T. (2003). Reaction time analysis of two types of motor preparation for speech articulation: action as a sequence of chunks. *J Mot Behav, 35*(2), 135-150. doi: 10.1080/00222890309602129

Klapp, S. T. (2010). Comments on the classic henry and rogers (1960) paper on its 50th anniversary: Resolving the issue of simple versus choice reaction time. *Research Quarterly for Exercise and Sport, 81*(1), 108-112.

Klapp, S. T., Wyatt, E. P., & Lingo, W. M. (1974). Response programming in simeple and choice reactions. *Journal of Motor Behavior, 6*(4), 263-271.

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., . . . Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. *Journal of Physiology-London, 471*, 501-519.

Leocani, L., Cohen, L. G., Wassermann, E. M., Ikoma, K., & Hallett, M. (2000). Human corticospinal excitability evaluated with transcranial magnetic stimulation during different reaction time paradigms. *Brain, 123*, 1161-1173. doi: 10.1093/brain/123.6.1161

Liepert, J., Kucinski, T., Tüscher, O., Pawlas, F., Bäumer, T., & Weiller, C. (2004). Motor cortex excitability after cerebellar infarction. *Stroke, 35*(11), 2484-2488.

Llinás, R. R. (2014). The olivo-cerebellar system: A key to understanding the functional significance of intrinsic oscillatory brain properties. *Frontiers in Neural Circuits, 7*(JAN).

MacKinnon, C. D., & Rothwell, J. C. (2000). Time-varying changes in corticospinal excitability accompanying the triphasic EMG pattern in humans. *Journal of Physiology-London, 528*(3), 633-645. doi: 10.1111/j.1469-7793.2000.00633.x

Maslovat, D., Hodges, N. J., Chua, R., & Franks, I. M. (2011). Motor Preparation and the Effects of Practice: Evidence From Startle. *Behavioral Neuroscience, 125*(2), 226-240. doi: 10.1037/a0022567

McConnell, K. A., Nahas, Z., Shastri, A., Lorberbaum, J. P., Kozel, F. A., Bohning, D. E., & George, M. S. (2001). The transcranial magnetic stimulation motor threshold depends on the distance from coil to underlying cortex: A replication in healthy adults comparing two methods of assessing the distance to cortex. *Biological Psychiatry, 49*(5), 454-459. doi: 10.1016/s0006-3223(00)01039-8

Merton, P. A., & Morton, H. B. (1980). Stimulation of the cerebral-crotex in the intact human subject. *Nature, 285*(5762), 227-227. doi: 10.1038/285227a0

Nazir, T. A., & Jacobs, A. M. (1991). The effects of target discriminability and retinal eccentricity on saccade latencies - an analysis in terms of variable-criterion theory. *Psychological Research-Psychologische Forschung, 53*(4), 281-289. doi: 10.1007/bf00920481

Oldfield, R. C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia, 9*(1), 97-113.

Patton, H. D., & Amassian, V. E. (1954). Single-unit and multiple-unit analysis of cortical stage of pyramidal tract activation. [Article]. *Journal of Neurophysiology, 17*(4), 345-363.

Pinto, A. D., & Chen, R. (2001). Suppression of the motor cortex by magnetic stimulation of the cerebellum. *Experimental Brain Research, 140*(4), 505-510.

Polson, M. J. R., Barker, A. T., & Freeston, I. L. (1982). STIMULATION OF NERVE TRUNKS WITH TIME-VARYING MAGNETIC-FIELDS. *Medical & Biological Engineering & Computing, 20*(2), 243-244. doi: 10.1007/bf02441362

Prut, Y., & Fetz, E. E. (1999). Primate spinal interneurons show pre-movement instructed delay activity. *Nature, 401*(6753), 590-594. doi: 10.1038/44145

Ramon y Cajal, S. (1911). *Histologie du Systeme Nerveux de l'homme et des Vertebres* (Vol. II). Paris: Maloine.

Requin, J., Brener, J., & Ring, C. (1991). Preparation for action. In J. Jennings & M. Coles (Eds.), *Handbook of cognitive psychophysiology: central and autonomic nervous system approaches* (pp. 357-448). Chichester: Wiley.

Reynolds, C., & Ashby, P. (1999). Inhibition in the human motor cortex is reduced just before a voluntary contraction. *Neurology, 53*(4), 730-735.

Ridding, M. C., Taylor, J. L., & Rothwell, J. C. (1995). The effect of voluntary contraction on corticocortical inhibition in human motor cortex. *Journal of Physiology-London, 487*(2), 541-548.

Roosink, M., & Zijdewind, I. (2010). Corticospinal excitability during observation and imagery of simple and complex hand tasks: Implications for motor rehabilitation. *Behavioural Brain Research, 213*(1), 35-41. doi: 10.1016/j.bbr.2010.04.027

Rosler, K. M., & Magistris, M. R. (2008). The size of motor-evoked potentials: influencing parameters and quantification. In V. W. Eric M. Wasserman, Charles M. Epstein, Tomas Paus, Ulf Ziemann, Sarah H. Lisenby (Ed.), *The Oxford Handbook of Transcranial Stimulation*. Oxford: Oxford University Pres.

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety, T. M. S. C. G. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology, 120*(12), 2008-2039. doi: 10.1016/j.clinph.2009.08.016

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., . . . Tomberg, C. (1994). Noninvasive electrical and magnetic stimulation of the brain, spinal-cord and roots - basic principle and procedures for routine clinical-application-report of an IFCN committee. *Electroencephalography and Clinical Neurophysiology, 91*(2), 79-92. doi: 10.1016/0013-4694(94)90029-9

Rossini, P. M., Berardelli, A., Deuschl, G., Hallett, M., Maertens de Noordhout, A. M., Paulus, W., & Pauri, F. (1999). Applications of magnetic cortical stimulation. The International Federation of Clinical Neurophysiology. *Electroencephalography and clinical neurophysiology. Supplement, 52*, 171-185.

Rossini, P. M., Zarola, F., Stalberg, E., & Caramia, M. (1988). Pre-movement facilitation of motor-evoked potentials in man during transcranial stimulation of the central motor pathways. *Brain Research, 458*(1), 20-30. doi: 10.1016/0006-8993(88)90491-x

Rotenberg, A., Harrington, M. G., Birnbaum, D. S., Madsen, J. R., Glass, L. E. S., Jensen, F. E., & Pascual-Leone, A. (2007). Minimal heating of titanium skull plates during 1 Hz repetitive transcranial magnetic stimulation. *Clinical Neurophysiology, 118*(11), 2536-2538. doi: 10.1016/j.clinph.2007.08.003

Sandbrink, F. (2008). The MEP in clinical neurodiagnosis. In V. W. Eric M. Wasserman, Charles M. Epstein, Tomas Paus, Ulf Ziemann, Sarah H. Lisenby (Ed.), *The Oxford Handbook of Transcranial Stimulation*. Oxford: Oxford University Press.

Schmidt, R. A., & Lee, T. D. (2011). *Motor Control and Learning: a behavioral emphasis* (5th ed.). Champaign, IL: Human Kinetics.

Shibasaki, H., Sadato, N., Lyshkow, H., Yonekura, Y., Honda, M., Nagamine, T., . . . Konishi, J. (1993). Both primarymotor cortex and supplementary motor area play an important role in complex finger movement. *Brain, 116*, 1387-1398. doi: 10.1093/brain/116.6.1387

Smittenaar, P., Guitart-Masip, M., Lutti, A., & Dolan, R. J. (2013). Preparing for selective inhibition within frontostriatal loops. *Journal of Neuroscience, 33*(46), 18087-18097.

Spencer, R. M. C., & Ivry, R. B. (2005). Comparison of patients with Parkinson's disease or cerebellar lesions in the production of periodic movements involving event-based or emergent timing. *Brain and Cognition, 58*(1), 84-93.

Spencer, R. M. C., & Ivry, R. B. (2013). Cerebellum and Timing. In M. Manto, D. Gruol, J. K. Schmahmann, N. & F. Rossi (Eds.), *Handbook of the Cerebellum and Cerebellar Disorders*: Springer.

Spencer, R. M. C., Ivry, R. B., & Zelaznik, H. N. (2005). Role of the cerebellum in movements: Control of timing or movement transitions? *Experimental Brain Research, 161*(3), 383-396.

Starr, A., Caramia, M., Zarola, F., & Rossini, P. M. (1988). Enhancement of motor cortical excitability in human by non-invasive electrical-stimulation appears prior to voluntary movement. *Electroencephalography and Clinical Neurophysiology, 70*(1), 26-32. doi: 10.1016/0013-4694(88)90191-5

Stinear, C. M., Coxon, J. P., & Byblow, W. D. (2009). Primary motor cortex and movement prevention: Where Stop meets Go. [Review]. *Neuroscience and Biobehavioral Reviews, 33*(5), 662-673. doi: 10.1016/j.neubiorev.2008.08.013

Summers, J. J., & Anson, J. G. (2009). Current status of the motor program: Revisited. *Human Movement Science, 28*(5), 566-577.

Swenson, R. S. (2006). Review of Clinical and Functional Neuroscience. In G. Holmes (Ed.), *Educational Manual in Neurology*. New York: Castle Connolly Graduate Medical Publishing, Ltd.

Tanji, J., & Evarts, E. V. (1976). Anticipatory activity of motor cortex neurons in relation to direction of an intended movement. *Journal of Neurophysiology, 39*(5), 1062-1068.

Touge, T., Taylor, J. L., & Rothwell, J. C. (1998). Reduced excitability of the cortico-spinal system during the warning period of a reaction time task. *Electromyography and Motor Control-Electroencephalography and Clinical Neurophysiology, 109*(6), 489-495. doi: 10.1016/s0924-980x(98)00050-2

Ugawa, Y., Uesaka, Y., Terao, Y., Hanajima, R., & Kanazawa, I. (1995). Magnetic stimulation over the cerebellum in humans. *Annals of Neurology, 37*(6), 703-713.

Wagner, T., Rushmore, J., Eden, U., & Valero-Cabre, A. (2009). Biophysical foundations underlying TMS: Setting the stage for an effective use of neurostimulation in the cognitive neurosciences. *Cortex, 45*(9), 1025-1034. doi: 10.1016/j.cortex.2008.10.002

Wassermann, E. M. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the international workshop on the safety of repetitive transcranial magnetic stimulation, June 5-7, 1996. *Evoked Potentials-Electroencephalography and Clinical Neurophysiology, 108*(1), 1-16. doi: 10.1016/s0168-5597(97)00096-8

Welsh, J. P., & Llinas, R. (1997) Some organizing principles for the control of movement based on olivocerebellar physiology. *Vol. 114* (pp. 449-462).

Wickens, J., Hyland, B., & Anson, G. (1994). Cortical cell assemblies - A possible mechanism for motor programs. *Journal of Motor Behavior, 26*(2), 66-82.

Ziemann, U., Corwell, B., & Cohen, L. G. (1998). Modulation of plasticity in human motor cortex after forearm ischemic nerve block. *Journal of Neuroscience, 18*(3), 1115-1123.

Ziemann, U., & Hallett, M. (2000). Basic neurophysiological studies with TMS. In M. S. B. Gerorge, R.M. (Ed.), *Transcranial Magnetic Stimulation in Neuropsychiatry*. Washington: American Psychiatric Press.

Appendix 1. Informed Consent Form for Motor Control Research

Principal Investigator: Dr. Anthony N. Carlsen
Affiliation: School of Human Kinetics, University of Ottawa

Co- Investigator:
Erin K. Cressman, PhD. Assistant Professor, School of Human Kinetics, University of Ottawa
Research Assistants:
Neil Drummond, PhD Student, School of Human Kinetics, University of Ottawa
Michael Kennefick, MSc Student, School of Human Kinetics, University of Ottawa

Invitation to Participate: You are invited to participate in the abovementioned research study conducted by the above researchers.

Purpose of the Study: This research is concerned with the production of simple limb movements and how weak electrical brain stimulation affects the production of those movements and the excitability of the brain. It is designed to answer several questions that relate to how human subjects represent and control the execution of rapid discrete responses and ongoing coordination.

Participation: During the experiment, you will be positioned in a chair and your arms may be placed on manipulandums (simple levers) or over simple buttons. You will be asked to make button responses or limb extension and/or flexion movements between a 'home' position and a target in a single, continuous motion. You will respond by moving your limb after an auditory or visual stimulus.

Testing will consist of four phases: practice, pretest, stimulation, and post-testing. The total time for this testing session will be approximately 1.5 hours. Practice and testing trials consist of performing the movements of your elbows or wrists, with real-time graphical display of your movement on a computer screen. During the experiment, we will be recording muscular activity. In order to do this, surface electromyography (EMG) electrodes will be attached to various locations on your body including your biceps and triceps muscles, and forearm muscles. Very small patches of skin may be shaved and cleaned prior to attaching the surface electrode to your skin. Between the pretest and post-test trials a weak electrical stimulus will be applied over part of your scalp for 10 minutes.

If so informed, testing will also involve the placement of an insulated copper wire coil positioned over top of your head which delivers a magnetic pulse. The coil makes a clicking sound and sometimes produces a twitch in targeted muscles. The magnetic pulse causes a weak electrical current in your brain, in the same way that you produce a weak electric current in your brain when you voluntarily contract a muscle. Electrodes will be placed over the arm muscles used to make the movements to pick up the electric current your muscles generate when they contract or are stimulated by the magnetic coil.

Risks: The risks involved in participating in this experiment are minimal, however the use of investigational magnetic and electrical stimulation may pose risks slightly greater than those involved in everyday life. Specifically, a weak electrical stimulus will be applied over part of your scalp which may cause a slight tingling sensation for the first 30 seconds to 1 minute. Magnetic stimulation of the brain has been reported to have caused a seizure in rare instances. No lasting effects were reported in these rare cases. Some people experience a “tapping” sensation on top of the head when the magnetic stimulator is triggered. Sometimes people notice that one eye “winks”, or their jaw closes slightly when the magnetic stimulator is triggered. Some potential side effects may include fatigue, headache, nausea or dizziness. If any of these symptoms should arise the experiment will be terminated without penalty to you. Since you will be making repeated targeted movements your muscles may become slightly tired. This risk will be decreased by providing rest periods every 10 minutes. Muscle activity will be recorded using plastic sensors attached with tape to the surface of your skin. The skin beneath each sensor will be lightly scrubbed, which may cause brief minor irritation. As the electrode is sticky, you may experience some very minor discomfort when the electrode is removed (it is similar to removing a Band-Aid). Be assured that every effort will be made to minimize these risks.

Benefits: Your participation in this study will lead to a greater understanding of how the human brain prepares and controls movements, and although this research may not benefit you directly, it is possible that the knowledge gained from this study will lead to future benefits and treatments for people with movement disorders.

Confidentiality and anonymity: All information and data collected are coded to maintain confidentiality. Specifically, raw data will be stored using an alphanumeric coding system so that no one will be able to identify you as your name will not appear on these files.

The data will be analyzed on password protected computers that only the researchers directly involved in this study will have access to. Once analyzed the data will be kept in in a locked room at the University of Ottawa, in locked filing cabinets and only the researchers directly involved in this study will have access to your data.

No records bearing your name will leave the institution. You are encouraged to request and discuss the results of the experimental trials at any time.

The data collected in this study will be published in scientific journals. The data will kept for a period of 5 years post-publication and will subsequently be destroyed by the physical resources service of the University of Ottawa.

Please be aware that you are under no obligation to participate. For the entire duration of the study, you may refuse to participate or withdraw from the study at any time, without question or penalty and any data collected will be destroyed. In addition you are free to ask the researcher any question about any part of the research being conducted at any time.

Acceptance: I, ________________________ agree to participate in the above research study conducted by Dr. Anthony Carlsen of the School of Human Kinetics at the University of Ottawa.

If I have any questions, I may contact the *principal investigator*.

If I have any questions regarding the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5
Tel.: (613) 562-5387
Email: ethics@uottawa.ca

There are two copies of the consent form, one of which is yours to keep.

Participant's signature: ________________________ Date: ______________

Researcher's signature: ________________________ Date: ______________

Appendix 2. TMS Safety Questionnaire

Safety Screening Questionnaire for Transcranial Magnetic Stimulation

Please answer the following questions by putting a check mark (☐) in the appropriate YES or NO box.

1. Have you ever had an adverse reaction to transcranial magnetic stimulation? YES ☐ NO ☐

2. Had a seizure? YES ☐ NO ☐

3. Had an EEG (electroencephalogram)? YES ☐ NO ☐

4. Had a stroke? YES ☐ NO ☐

5. Had a head injury (include neurosurgery)? YES ☐ NO ☐

6. Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, or fragments from welding or metalwork? YES ☐ NO ☐

7. Do you have any implanted devices such as cardiac pacemakers, medical pumps, or intracardiac lines? YES ☐ NO ☐

8. Do you suffer from frequent or severe headaches? YES ☐ NO ☐

9. Have you ever had any other brain-related condition? YES ☐ NO ☐

10. Have you ever had illness that caused brain injury? YES ☐ NO ☐

YES ☐ NO ☐

11. Are you taking any medications?

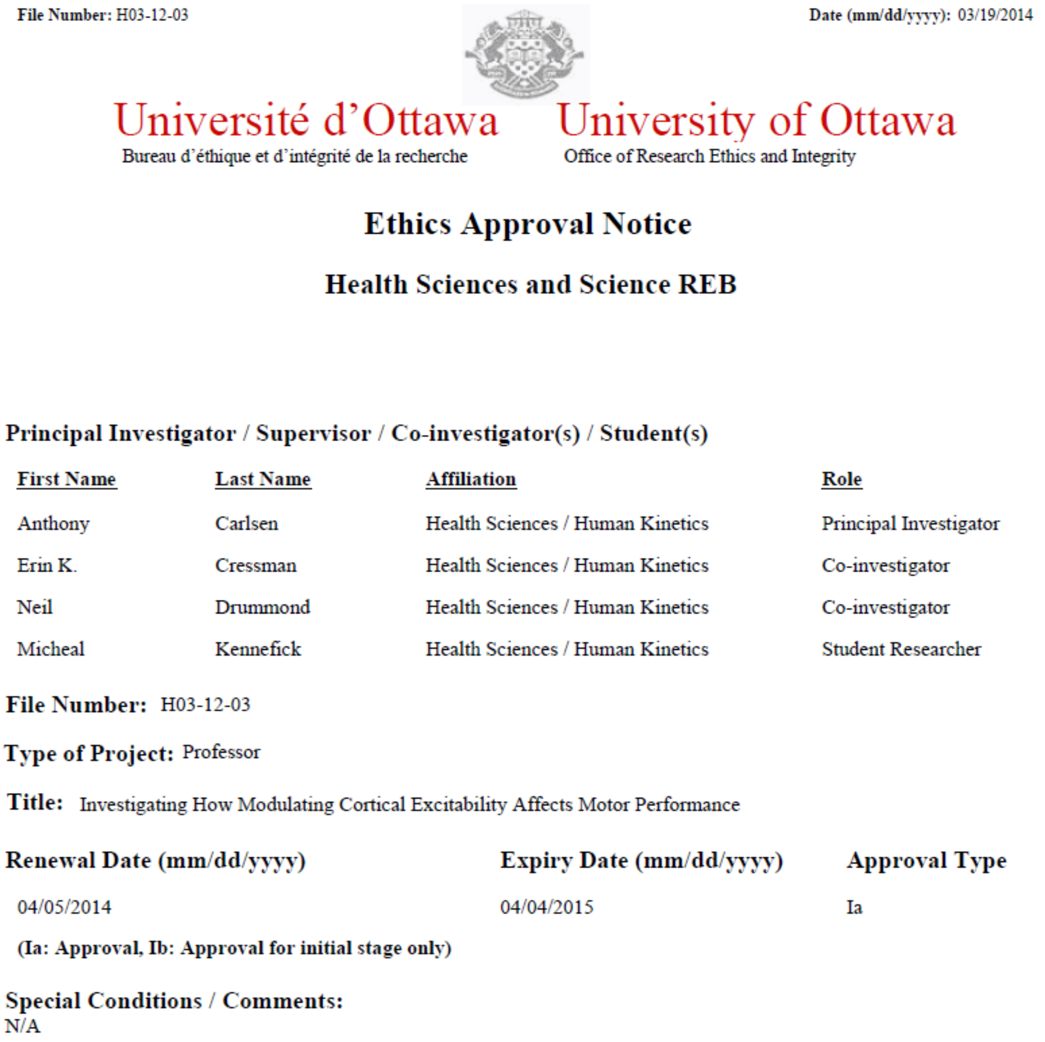
12. Does anyone in your family have epilepsy? YES ☐ NO ☐

13. Are you pregnant? YES ☐ NO ☐

PARTICIPANT NAME: ____

PARTICIPANT SIGNATURE: _________________________________ DATE: _____
OR SIGNATURE OF LEGALLY AUTHORIZED INDIVIDUAL

Appendix 3. Ethics Certificate



File Number: H03-12-03

Date (mm/dd/yyyy): 03/19/2014

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Anthony	Carlsen	Health Sciences / Human Kinetics	Principal Investigator
Erin K.	Cressman	Health Sciences / Human Kinetics	Co-investigator
Neil	Drummond	Health Sciences / Human Kinetics	Co-investigator
Micheal	Kennefick	Health Sciences / Human Kinetics	Student Researcher

File Number: H03-12-03

Type of Project: Professor

Title: Investigating How Modulating Cortical Excitability Affects Motor Performance

Renewal Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
04/05/2014	04/04/2015	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:
N/A