Investigating differences in reaction time and preparatory activation as a result of varying accuracy requirements

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Abstract

The preparation and initiation of movement has previously been described using a neural accumulation model; this model involves an increase of neural activation in the motor cortex (M1) from baseline to a subthreshold level following a warning signal, which is maintained until presentation of an imperative stimulus (IS). Activity then increases until reaching movement initiation threshold. This model predicts that variability in activation during preparation may influence reaction time (RT) and its variability. The purpose of this thesis project was to determine whether differences in RT/variability of RT during the completion of tasks with varying levels of complexity may be attributable to differences in neural excitability in M1. To test this prediction, transcranial magnetic stimulation (TMS) delivered concurrently with an IS was used to determine neural excitability for movements with different accuracy demands. It was hypothesized that higher accuracy demands would result in lowered amplitude and/or greater variability of neural activation, and consequently slower/more variable RT. Fifteen healthy participants completed a simple RT task involving a targeted wrist extension movement under three different accuracy conditions (easy, moderate, difficult). TMS was delivered concurrently with the IS on 50% of trials during each condition. While pilot testing showed RT differences between accuracy conditions (Appendix A), the data presented here failed to detect significant differences in RT latency (F(2, 28) = .074, p = .929) or variability (F(1.432, 20.053) = .633, p = .538) between conditions . Similarly, no difference in MEP amplitude was observed between difficulty conditions (F(2, 28) = 2.439, p = .106). However, a subset of participants (n = 7) did show significant RT increases between easy and hard conditions (t(6) = 2.531, p = .045), but this subset still failed to show differences in MEP amplitude (t(6) = 1.157, p = .291) or variability (t(6) = 1.545, p = .173), suggesting that preparatory levels at the IS may be similar for movements involving both high and low accuracy demands.

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Glossary of Terms

ANOVA – Analysis of variance

CSP – Cortical silent period

dB – Decibel

ECRL – Extensor carpi radialis longus

EMG – Electromyography

FCR – Flexor carpi radialis

ID – Index of difficulty

IS – Imperative stimulus

M1 – Motor cortex

MEP – Motor evoked potential

MSO – Minimum stimulator output

MT – Movement time

OOc – Orbicularis oculi

PMRF – Pontomedullary reticular formation

RMS – Root mean square

RMT – Resting motor threshold

RT – Reaction time

SAS – Startling acoustic stimulus

SCM – Sternocleidomastoid

TES – Transcranial electrical stimulation

TMS – Transcranial magnetic stimulation

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Statement of Contribution

I, Alexandra R. Leguerrier, declare that I am the sole author of this Master's thesis. The conception and design of these experiments was completed by myself in collaboration with my thesis supervisor Dr. Anthony N. Carlsen as well as Dr. Dana Maslovat, and with input from my thesis committee consisting of Dr. Erin Cressman and Dr. François Tremblay. Data collection and analysis, as well as statistical analyses, were completed by myself under the guidance of Dr. Anthony N. Carlsen, who also provided editorial corrections.

Chapter I: Literature Review

1. Introduction

On a daily basis, humans interact with their environment with little to no effort; however, these deceptively easy interactions can mask the complex neural mechanisms that underlie the control of movement. The study of motor control aims to provide insight into these mechanisms. One of the more common models used to describe these processes is the information-processing model, where humans are likened to computers, processing information from stimuli to produce an appropriate response (Schmidt & Lee, 2011). During this processing, a stimulus must be identified, an appropriate response selected, and this response programmed. Once these preparatory steps have occurred, the movement may be initiated if it is deemed appropriate for the situation at hand.

The level of cortical activation during these processes can be indirectly measured using non-invasive neurostimulatory techniques such as transcranial magnetic stimulation (TMS), which uses one or more magnetic pulses to elicit muscular responses (Barker et al., 1985). By determining the magnitude of the muscular response to TMS under varying experimental conditions, changes in neural excitability levels can be extrapolated (Rossini et al., 2015; Ziemann et al., 2008). Another indirect method of studying cortical excitability during movement preparation and initiation involves the use of a startling acoustic stimulus (SAS). When the response to be produced is known in advance and can therefore be pre-planned, the unexpected presentation of a SAS can involuntarily trigger the movement much faster than is possible through voluntary initiation (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004a; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999). This is thought to occur because of additional activation provided by the startle to neural structures (Carlsen, Maslovat & Franks, 2012; Valls-

Solé, Kumru, & Kofler, 2008). By presenting a SAS while the response is being prepared or initiated, the degree to which the movement has *already* been prepared can be inferred, based both on the length of time between SAS presentation and movement initiation as well as the overall proportion of trials where a startle response is triggered by the SAS.

Although it is important to note that the previously described techniques only allow for the indirect study of cortical excitability during movement preparation, they are nevertheless thought to be representative of processes that are actually occurring at the neural level. Motor preparation can be modelled as resulting from an increase in neuronal activation levels from some baseline level in the motor cortex (M1) up until a specific threshold, at which point movement initiation can begin (Hanes & Schall, 1996; Carpenter & Williams, 1995). This "neural accumulator" was posited following direct measurement of neural activity in saccadic eye movements (Hanes & Schall, 1996). It has since been adapted into a more general neural accumulator model for voluntary movement (Carlsen, Maslovat, & Franks, 2012), which is well supported by both the behavioural and neurostimulatory research (Maslovat, Carter, Kennefick, & Carlsen, 2014; Kennefick, Maslovat, & Carlsen, 2014; Heitz & Schall, 2012; Brown et al., 2008; Wickens, Hyland, & Anson, 1994; Starr, Caramia, Zarola, & Rossini, 1988).

While there is considerable evidence for the neural accumulator model as a basis for movement preparation, many features of the model have yet to be investigated. One such feature is the influence that the level and variability of neural activity in M1 may have on reaction time (RT) and its variability (Carlsen, Maslovat, & Franks, 2012). As a result of the inherent stochastic noise that is present in neural circuits, preparation within M1 is subject to variations in activation levels over time, and this variability has been hypothesized to in turn affect the distribution of RTs produced for a given response (Carlsen, Maslovat, & Franks, 2012; Faisal, Selen, & Wolpert, 2008).

The following literature review will first focus on describing relevant background regarding movement preparation and the use of TMS and SAS as they relate to its study. These techniques will then be discussed within the context of studying the neural accumulation model in particular. Finally, the investigation of neural variability using these techniques will be considered within the framework of this model.

2. Information Processing

During interactions with the environment, individuals process internal and external stimuli in order to produce appropriate movements. The information-processing model describes the stages of processing that must theoretically occur between the input of a stimulus and the potential output of a motor response (Schmidt & Lee, 2011). This model involves three distinct steps in the preparation of a movement. The first step, *stimulus detection*, involves the discernment and discrimination of a sensory stimulus. The second is *response selection*, where an appropriate movement is selected based on the identified stimulus. The last step, *response preparation*, involves the preparation of said movement (Donders, 1969; Sternberg, 1969). A common way of studying these stages is by considering the total length of time required to produce a given response. Since these stages are thought to primarily occur during the RT interval (measured from the time of stimulus onset to the time of movement initiation), changes in RT are often linked to changes in processing. The RT interval is separated into two components, namely premotor and motor RT (Weiss, 1965). Premotor RT is defined as the time between the go-stimulus and the time of muscle activation detected via electromyography (EMG), while motor RT is defined as the time between initial EMG activity and movement initiation. Changes in premotor RT are of particular interest in the study of movement, as this interval is thought to involve the central processing required (Weiss, 1965).

Different movement paradigms can affect the amount of processing required during the RT interval. Donders' original research involved different RT task paradigms, each with different processing requirements, to determine the length of time required to complete each stage of information processing (1969). In a simple RT task, the most basic paradigm used, a single stimulus is coupled with a single response. With advance knowledge of the movement provided, the stimulus discrimination and response selection stages are presumably not necessary, meaning movements can be produced at shorter latency in simple RT tasks compared to paradigms where no advance knowledge of the response is provided (Donders, 1969). An increase in RT is observed in the two other research paradigms used, namely the go/no-go RT task and the choice RT task (Schmidt & Lee, 2011). In a go/no-go task, a single correct response is signalled by one stimulus, while a second stimulus indicates that the movement should be withheld; subjects must therefore correctly identify the stimulus, but response selection is not necessary. In a choice RT task there are two or more possible stimuli, each with a distinct response to be produced; this requires both the discrimination of stimulus and the selection of the commensurate response. The longest RTs are seen in experiments involving the choice RT task (Donders, 1969; Sternberg, 1969).

Donders' seminal work used the subtractive method, where each stage of processing was studied by comparing paradigms that differed only in a single processing stage, with the difference in RT between the two corresponding to the duration of that stage (1969). This method required two assumptions; first, that these processing stages occurred in sequence, with the initiation of each stage requiring the completion of the previous one, and second, that the insertion of a new stage did not influence the existing ones (Sternberg, 1969). While subsequent research has since questioned these assumptions, the basic findings of these early experiments still influence experimental design for studying movement production (Haith, Pakpoor, & Krakauer, 2016; Danek & Mordkoff, 2011; Schmidt & Lee, 2011; Brebner & Welford, 1980; Woodworth, 1938).

2.1. Motor programs. Early research into response preparation posited the existence of a pre-selected set of muscle commands, termed a motor program, which can be prepared and then released upon presentation of an imperative stimulus (IS) (Keele, 1968). This was an attractive theoretical framework as it accounted for the execution of ballistic movements, which are produced too rapidly to benefit from peripheral feedback and therefore must theoretically be prepared entirely in advance (Keele, 1968). However, the idea was widely criticized, primarily due to the infeasibility of storing such a large number of motor programs (Morris, Summers, & Iansek, 1994; Mulder & Hulstijn, 1984). Nevertheless, the idea has persisted, and is now widely used metaphorically to refer to a pre-programmed response (Summers & Anson, 2009).

More recently, a possible neural substrate for the metaphorical “motor program” was proposed by Wickens, Hyland, and Anson in the form of cortical cell assemblies (1994). A cortical cell assembly is a subset of neurons whose synaptic connections have been strengthened beyond that of the average connection through repeated firing (Palm, 1990; Hebb, 1949). It has been proposed that preparation for a given movement may occur through the increase in synaptic activation within a given cell assembly, with activation reaching a steady state until an IS presented (Wickens, Hyland, & Anson, 1994). Upon IS presentation, the cell assembly is “ignited”, thereby activating corticospinal connections and ultimately resulting in movement. Importantly, the highly branched nature of cortical pyramidal neurons in M1 allow the same neuron to be part of many different cell assemblies; a movement could therefore be selectively initiated only when its specific subset of neurons have been activated beyond a certain threshold (Gatter & Powell, 1978). This particular feature of the cortical cell assembly increases its viability as the hypothetical mechanism of “motor programs”, as it would greatly reduce the amount of cortical area required for storage (Wickens, Anson, & Hyland, 1994).

3. Transcranial Magnetic Stimulation (TMS)

In order to study these cortical processes more closely, the use of TMS has become common as it allows for the indirect measurement of cortical excitability. TMS involves the delivery of one or more magnetic pulses over the scalp, which are created when a large electrical current passes through a figure-8 copper coil for approximately 1 ms (Epstein, Wassermann, & Ziemann, 2008). This current flow creates a magnetic field in an orientation perpendicular to the coil, which can in turn be directed towards the brain when the coil is placed onto the scalp. The magnetic pulse can be triggered by a computer or discharged by the experimenter using a manual switch, and is able to induce an electrical current parallel to the direction of the coil in the neural tissue directly below the site of delivery. This can result in neuronal depolarization when TMS intensity is set above an individualized response threshold (Rothwell, Thompson, Day, Boyd, & Marsden, 1991). In the case of M1 stimulation, delivery of a single TMS pulse can under certain conditions elicit descending activity within the corticospinal tract, which ultimately causes a muscular twitch in the targeted muscle. This TMS-induced twitch is referred to as a motor evoked potential (MEP) (Rossini et al., 2015; Rothwell et al., 1999). Studying these MEPs has allowed researchers to draw conclusions about the state of cortical activation at the time of stimulation. In order to elicit an MEP, TMS must be applied at a supra-threshold intensity, which is defined as any intensity above the minimum required to reliably produce an MEP in the muscle of interest (Rossini et al., 2015; Rossini et al., 1994).

3.1. Single pulse TMS. Single pulse TMS was originally developed as a more comfortable alternative to transcranial electrical stimulation (TES), an earlier form of non-invasive neurostimulation that often results in pain and local contraction of scalp muscles (Barker et al., 1985). Early studies of single pulse TMS over M1 in humans aimed to characterize the neural mechanisms that underlie the observed outward effects (such as MEPs). Research with TES in primates and in humans led to the identification of an early direct wave (D-wave) resulting from direct axonal stimulation of corticospinal neurons, and a later indirect wave (I-wave) resulting from indirect monosynaptic stimulation of these same corticospinal neurons (Di Lazzaro et al., 2004a; Ziemann & Rothwell, 2000; Patton & Amassian, 1954). Epidural spinal recordings of the descending corticospinal activity following TMS originally revealed recruitment of the I-wave only, which suggested that MEPs were solely the result of indirect, transynaptic activation of corticospinal neurons (Di Lazzaro et al., 2004; Day et al., 1989). However, later studies in humans revealed that at high intensities (180%-200% of active motor threshold), TMS could elicit earlier D-waves as well (Di Lazzaro et al., 1998; Thompson et al., 1991). In the study of cortical motor processes, single pulse TMS is typically applied at a 45° angle to the midline such that there is induction of current in the brain in a posterior-anterior direction (Di Lazzaro et al., 2012). With this coil orientation, TMS has been shown to activate descending I-waves using relatively low intensities compared to all other orientations (Rossini et al., 2015; Patton & Amassian, 1954).

Whereas the delivery of repetitive TMS pulses is used to alter the excitability of the brain area of interest, single-pulse TMS is frequently used in order to assess the state level of excitability of the targeted brain area at a given point in time. Although single pulse TMS can itself have a small facilitatory effect on motor excitability, this effect is very slight and only apparent at short intervals between pulses (~4 sec) and only after several hundred TMS pulses (Pellicciari et al., 2016). As such, the cumulative effects of single-pulse TMS on neural excitability are thought to be negligible in most experimental designs. A change in MEP amplitude following a given manipulation is therefore indicative of a corresponding change in excitability when TMS intensity is held constant between measurements, with increased MEP amplitude indicating an increase in corticospinal excitability and decreased amplitude indicating a decrease in excitability (Rossini et al., 2015). This technique has been used to assess the effectiveness of neuromodulation via repetitive TMS and other non-invasive brain stimulation protocols, as well as to investigate the state of the motor system at various times during movement preparation and initiation for a variety of tasks (Ziemman, 2011).

3.2. Safety. Given the potential risks of using neurostimulation in human subjects, the safety of this technique has been extensively studied. Overall, TMS has been established as a safe and painless form of neurostimulation, although some minor risks do exist. While extremely rare, seizures are one possible side effect of TMS (Rossi et al., 2009). Seizures have been reported in studies using supra-threshold repetitive TMS, as well as in subjects who are taking medications that increase the risk of seizures. A TMS safety questionnaire has been developed by Rossi and colleagues in order to mitigate these risks (see Appendix A), and eliminates participants on the basis of certain medications, a history of seizures or head injuries, implanted metal objects, and pregnancy (2011). Using these exclusion criteria, TMS can be used with little to no risk of any neurological side effects (Rossi, Hallett, Rossini, & Pascual-Leone, 2011; Rossi et al., 2009). When TMS is delivered for an extended period of time, headaches and neck pain have been reported; however, these side effects have been linked to the sustained pressure applied by the coil on the head throughout the experiment as opposed to the stimulation itself (Rossi et al., 2009). These symptoms are frequently mild and can be mitigated by providing participants with breaks when necessary. As long as these guidelines are followed, TMS can be used in a safe and painless manner.

4. Startle

In recent years, the use of a startling stimulus has also become increasingly popular as a tool for the study of motor preparation and initiation. The startle reflex is an evolutionarily protective response to an unexpected intense stimulus, and is characterized by involuntary generalized flexion of the muscles (Landis, Hunt, and Strauss, 1939). Other characteristics include a startle blink response and contractions in the sternocleidomastoid (SCM) muscles (Brown et al., 1991). The startle response is the result of descending activation from the pontomedullary reticular formation (PMRF) activating motor neurons in the spinal cord by way of the reticulospinal tract, ultimately resulting in whole body flexion (Yeomans & Frankland, 1995). When a SAS is used, the PMRF is stimulated by direct projections from the cochlear nucleus, thereby bypassing typical processing in the auditory cortex and allowing for the production of the startle response with exceptionally fast latency compared to a voluntary response to an auditory stimulus (Yeomans & Frankland, 1995).

4.1. The StartReact Effect. An interesting feature of the startle response is its ability to trigger a prepared movement under the right conditions, termed the StartReact effect. When sufficient information has been provided regarding the movement to be produced, such as in a simple RT task paradigm, the unexpected presentation of a SAS can result in the involuntary initiation of this pre-planned movement (Carlsen et al., 2003; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999). Movements triggered by the StartReact effect can be produced at significantly reduced latency (~70 ms) compared to voluntarily-initiated ones. Additionally, when comparing the EMG profiles of voluntarily initiated movements and movements completed following the presentation of a SAS, there were no differences in the triphasic burst patterns produced (Carlsen et al., 2004b). The early elicitation of an otherwise unaltered response led to the conclusion that the SAS may be acting as a trigger for a pre-planned, subcortically stored motor program, effectively bypassing the traditional cortical route of movement production (Carlsen et al., 2012; Carlsen et al., 2004a; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999).

The early conclusion that the StartReact effect must bypass cortical control, possibly through the release of a subcortically stored motor program, has been recently called into question (Alibiglou & MacKinnon, 2012). Research using TMS has suggested that the cortex may in fact play a role in the StartReact effect. Alibiglou and MacKinnon used supra-threshold TMS to induce a cortical silent period (CSP) in order to disrupt cortical processing when either a control tone or a SAS were presented during a simple RT task (2012). If the StartReact effect in fact occurred in the complete absence of cortical processing, then it was expected that a significant increase in control but not in startle RTs would result from this disruption. However, their results demonstrated that temporarily knocking out activity in M1 slowed RT in both conditions, suggesting that the CSP interfered with cortical processing related to the StartReact effect (2012). Further evidence for cortical processing during the StartReact effect is provided by Stevenson and colleagues (2014), who used a vocalization task paired with a SAS in a simple RT paradigm. Given the largely cortical nature of vocalization movements, the early triggering of these responses by the SAS further supports some degree of cortical involvement (Stevenson et al., 2014). While the exact pathway by which the StartReact effect triggers movement is still unclear, it appears that both cortical and subcortical structures play a role.

Importantly, it has been shown that there is dishabituation of the startle reflex when subjects are engaged in preparatory processes, such as during a simple RT task. Habituation is the reduction of a behavioural response following repeated presentation of a given stimulus, and this typically occurs quite rapidly in the case of the startle response (between 2-6 presentations) (Brown et al., 1991). However, when subjects are preparing a motor response in anticipation of an IS, this habituation is not seen after as many as 20 SAS trials (Carlsen et al., 2003). Even when participants were given advance knowledge regarding the impending SAS, there was no significant change in the incidence of the startle response and RT facilitation was not significantly different between warned and unwarned conditions, although the magnitude of the elicited response was slightly attenuated in the warned condition (Drummond, Leguerrier, & Carlsen, 2016). Furthermore, it has been suggested that this dishabituation of the startle response may result from increased activity within the response pathway as a consequence of the preparatory processes taking place prior to the SAS presentation (Carlsen et al., 2003).

4.1.1. Startle indicators. In order to establish that a startle has occurred and that a movement has consequently been triggered via the StartReact effect, various indicators have been used. The most common are EMG activity in the orbicularis oculi (OOc) and in the SCM, as these muscles are the most consistently activated and the last to habituate during the startle response (Brown et al., 1991). However, SCM activity is typically considered to be a more reliable indicator of a startle response because OOc activation has been shown to occur following presentation of a loud acoustic stimulus even in the absence of a startle response (Maslovat, Franks, Leguerrier, & Carlsen, 2015; Carlsen, Dakin, Chua, & Franks, 2007; Brown et al., 1991).

Even following startle habituation, OOc activity can still sometimes be detected, suggesting that it may not be a reliable indicator of startle (Brown et al., 1991). Additionally, when comparing between trials where SCM was or was not detected, the EMG of the blink response was significantly extended only when SCM activity was present; this provides further evidence for a distinction between a startle blink response and an auditory blink response that reduces the suitability of OOc activity as a startle indicator (Carlsen, Dakin, Chua, & Franks, 2007).

4.1.2. Alternate hypotheses. Following the seminal work regarding the StartReact effect by Valls-Solé and colleagues (1999), alternate explanations to the involuntary triggering of a pre-planned movement were proposed to account for the significant reductions in RT seen in the StartReact effect. It was first proposed that instead of a separate triggering mechanism, this effect might in fact be the result of a larger-than-normal stimulus intensity effect (Carlsen et al., 2004a). A well-known phenomenon, the stimulus intensity effect is the reduction in RT that is seen when the intensity of an IS is increased (Woodworth, 1938). To investigate this hypothesis, Carlsen and colleagues (2007) used several SAS of five different intensities (83-123 dB in 10 dB increments) and found that, irrespective of stimulus intensity, RT was not significantly different across intensities for trials where a startle reaction was actually elicited. It should be noted that, as discussed above, this consistent pattern of significantly reduced RT (~75ms) was only observed in trials where EMG activity was detected in the SCM (Carlsen, Dakin, Chua, & Franks, 2007).

A second alternate hypothesis put forth to explain the StartReact effect attributed the significant RT speeding to intersensory facilitation, whereby the presentation of an IS and an accessory stimulus (in this case the SAS) of different stimulus modalities could result in reduced RT compared to either stimulus alone (Nickerson, 1973). This was originally suggested due to the pairing of a visual IS with a SAS in much of the early research into the StartReact effect (Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999). While this possibility cannot be completely discounted, the StartReact effect has since been studied in simple RT paradigms using an auditory IS (Carlsen et al., 2004a), suggesting that the contribution of intersensory facilitation to the significant RT reduction seen following SAS presentation is minimal. Additionally, the magnitude of the reduction in RT seen in the case of intersensory facilitation (20-50ms; Nickerson, 1973) is substantially smaller than that seen in the case of the StartReact effect (~70ms; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999), further reducing the likely impact of intersensory facilitation in the RT speeding induced by a SAS.

5. Neural Accumulation Model

Following the selection and programming of an appropriate response, movement initiation processes can begin. For initiation to occur, the overall level of neural activation in M1 involved in the given movement is believed to increase from a resting subthreshold level until an arbitrary threshold level of activity is reached (often referred to as 100% of preparatory activation). This was first proposed by Hanes & Schall following the recording of single neurons during saccadic eye movements in macaque monkeys (1996). Irrespective of saccadic RT, the firing frequency (spikes/s) of the neurons at the time of movement initiation was not significantly different between trials, suggesting that a consistent threshold level of activity was reached at time of movement onset. Observed variability in saccadic RT was therefore likely not linked to the magnitude of firing frequency at the time of movement initiation, but instead appeared to be the result of variability in the rate of increase of firing frequency, whereby a steeper activation slope resulted in faster saccade initiation (Hanes & Schall, 1996).

Based on these findings in the saccade literature, Carlsen and colleagues proposed a neural accumulation model of voluntary movement preparation and initiation (Carlsen, Maslovat, & Franks, 2012). They proposed that upon presentation of a warning signal, neural activity in M1 increases from baseline to some subthreshold level, then plateauing until presentation of an IS at which point activity would again increase at a constant rate until the movement initiation threshold was reached. Furthermore, they suggested that this process might occur within cortical cell assemblies, which have been put forward as the neural substrate for motor program storage (see above; Wickens, Hyland, & Anson, 1994).

Within this framework, the inherent stochasticity, or "noise", resulting from electrical activity and synaptic transmission in these neural networks must also be accounted for (Faisal, Selen, & Wolpert, 2008). Carlsen and colleagues proposed that the amplitude of the noise may directly influence the degree to which neural activation can be increased during movement preparation (2012). In order to prevent early release of the prepared movement, activation would need to be held at a subthreshold level low enough that random noise alone could not raise it beyond threshold. This would be offset by the RT advantage to be gained by increasing activation levels as close to threshold as possible in anticipation of the go-signal. Preparatory activation levels for a given movement would therefore be constrained by these two competing factors (Carlsen, Maslovat, & Franks, 2012).

5.1. Evidence from TMS. Much of the support for the neural accumulation model comes from the neurostimulation literature (Kennefick, Maslovat, & Carlsen, 2014; Leocani et al., 2000; Touge, Taylor, & Rothwell, 1998; Starr, Caramia, Zarola, & Rossini, 1988). When TMS was used to investigate movement preparation leading up to presentation of a tactile IS, significant suppression of MEPs was seen ~350 ms prior to the IS; however, this effect was only seen when a warning signal was presented 500 ms prior to the IS (Touge, Taylor, & Rothwell, 1998). The authors suggested that when temporal uncertainty is reduced by the warning signal, cortical excitability is depressed immediately before the IS to prevent premature triggering of the response. When the warning signal was presented 2 seconds before the IS, this reduction in MEP amplitude was not seen; given that this longer foreperiod is more difficult to estimate and therefore provided less temporal information, this supports the authors' explanation (Touge, Taylor, & Rothwell, 1998). A similar study by Kennefick and colleagues using TMS to study cortical excitability during both the preparatory and initiation periods in a simple RT task provided additional evidence to support this model (Kennefick, Maslovat, & Carlsen, 2014). The amplitude of the elicited MEPs reached a plateau prior to the presentation of an IS (i.e., during movement preparation), followed by a sharp increase after the IS presentation, thereby providing additional support for the presence of a subthreshold plateau in activation levels during preparation of the movement, as was suggested by Carlsen and colleagues (2012) within their framework of neural accumulation.

Further support for this type of model comes from the use of TES. Starr and colleagues found that the likelihood of eliciting an MEP in the primary effector increased as the electrical stimulus was delivered closer to the time of movement production following IS presentation (1988). This provided some of the first evidence for a seemingly linear increase in neural activation from a constant subthreshold level to a threshold one, at which point movement can be initiated. This finding was again supported when TMS was used instead of TES. Leocani and colleagues (2000) found that when TMS was presented following the IS, there was a progressive increase in MEP amplitude during the 80-120 ms window leading up to EMG initiation, a finding which was later replicated by Kennefick and colleagues (2014).

5.2. Evidence from startle. Evidence for an accumulation model of movement preparation and initiation also comes from literature involving the startle reflex and the StartReact effect. As previously mentioned, the presentation of a SAS can be used as a tool to indicate levels of preparation prior to and following the presentation of an IS by determining the degree to which the presentation of a SAS reduces RTs (Maslovat, Franks, Leguerrier, & Carlsen, 2015; Maslovat, Carter, Kennefick, & Carlsen, 2014; Carlsen & MacKinnon, 2010; MacKinnon et al., 2007). MacKinnon and colleagues presented a SAS prior to an IS signalling the initiation of a stepping movement and found that there was involuntary triggering of the associated anticipatory postural adjustment, suggesting that some degree of preparation had already occurred as early as 1400 ms prior to the go (2007). Extending these results, Carlsen and MacKinnon used a SAS to study preparatory levels as early as 1500 ms prior to IS presentation in a wrist extension movement (2010). Their results showed that while a startle response was elicited on ~60% of SAS trials 1500 ms before the IS, this increased to >90% 500 ms before the IS and remained high until IS presentation. Additionally, premotor RT was significantly reduced compared to control as early as 1500 ms prior to the IS and this RT speeding was observed for all later SAS presentation times (Carlsen & MacKinnon, 2010). Based on these results, it was concluded that movement preparation could occur as early as 1500 ms before the IS, and that this preparation may increase until a plateau is reached ~500 ms prior to the go-signal.

A later study used a similar method to determine the levels of preparatory activation immediately before and after IS presentation. Maslovat and colleagues presented a loud acoustic stimulus at one of five time points prior to, concurrent with, or following an auditory IS (2015). Their results show no differences in startle RT across all five time points, suggesting no significant change in cortical activation levels immediately before (65 ms before the IS) or immediately after (15 ms after the IS) an auditory go (Maslovat, Franks, Leguerrier, & Carlsen, 2015). Taken together, these results support an increase in activation during the preparatory foreperiod from baseline to a subthreshold level before plateauing.

5.3. Variability of neural activation. As previously stated, while the current body of literature supports the neural accumulator model overall, there are various features of the model that have yet to be investigated. The effect of variability of neural activation in particular is of interest, as it is an inherent property of the neural signals involved in the production of movement. Carlsen and colleagues proposed that this variability might have an effect on the latency with which each movement is produced (2012). Increased noise in the neural network, resulting from an increase in neuronal inputs into the system, may constrain the level of initial activation that can be reached in order to prevent early accidental initiation of the movement and therefore result in slower RT. If the level of activation at the time of movement initiation varies from trial to trial, this may in turn lead to differences in RTs for each trial if a constant rate of activation is assumed post-initiation (see Figure 1). Additionally, the amplitude of the noise may increase the width of the RT distribution, whereby the RTs produced in movement conditions that involve noisier signals may be more variable than those involving “quieter” signals (see Figure 2).

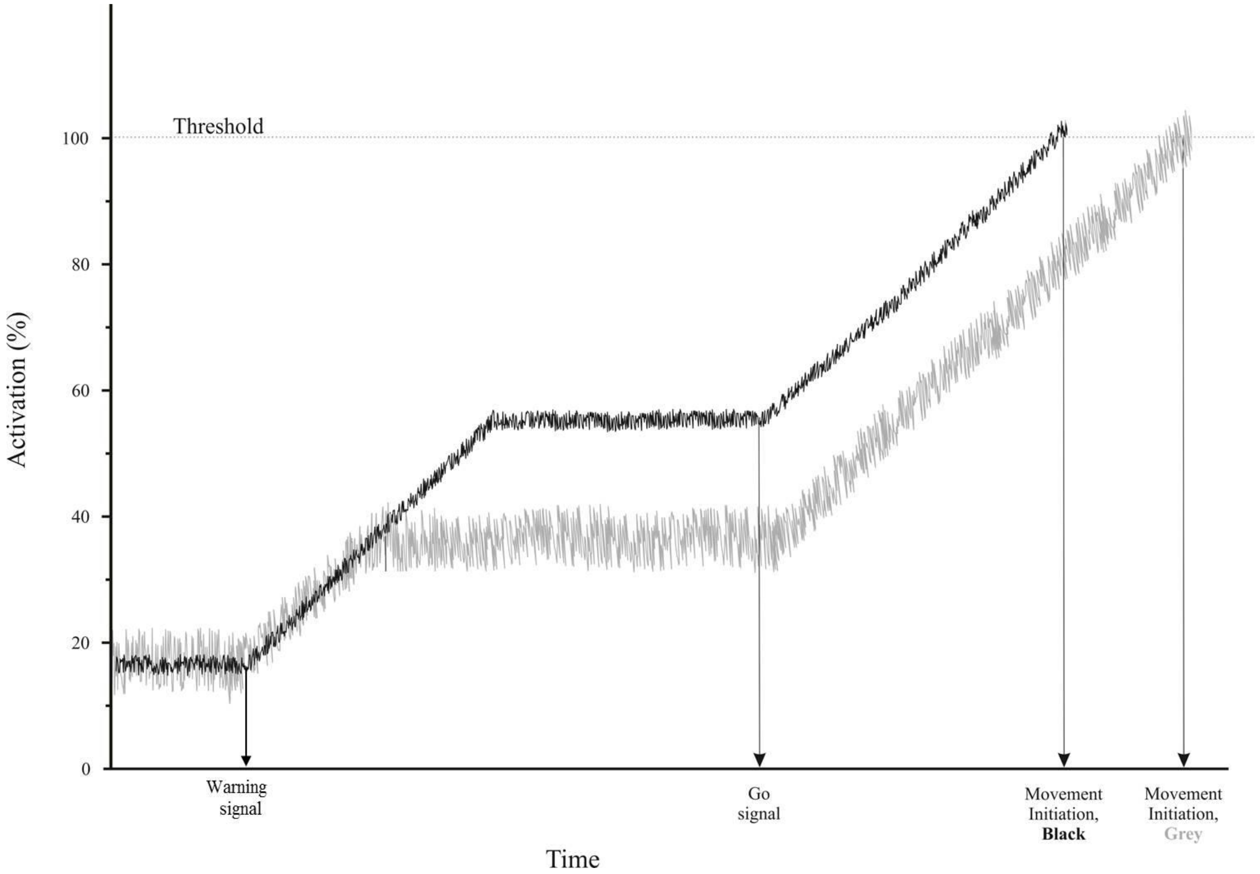

Figure 1. Visual representation of a neural accumulation model during movement preparation and initiation. Following a warning signal, preparatory activation level increases until a plateau, after which activation is held constant until the presentation of a go. Once a go-signal is presented, activation again increases until a ‘threshold’ level, at which point the movement is initiated. If a higher level of preparatory activation is reached (**grey**), then threshold is reached earlier than if activation is lower (**black**).

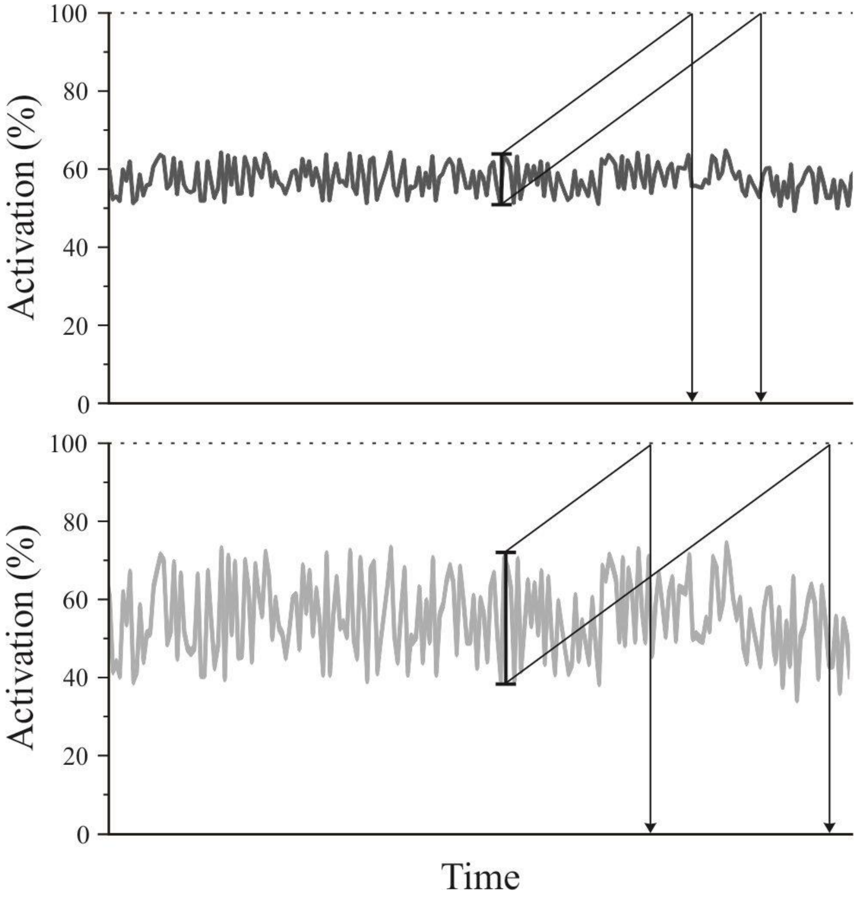

Figure 2. Visual representation of neural activation resulting from movement preparation. In the top panel (**black**), stochastic noise is low and the possible predicted distribution of time at which threshold is reached is smaller than that of the bottom panel, where neural noise is higher (bottom, **grey**).

To test this particular prediction of the neural accumulation model, the use of a simple RT task with different accuracy requirements has been proposed as a way to manipulate stochastic noise, and potentially RT by extension (Carlsen, Maslovat, & Franks, 2012). The manipulation of accuracy is a common alteration of movement parameters used to increase the complexity of a task without changing the nature of the movement itself from one condition to another. This type of manipulation was first used in 1954 with the seminal Fitts task (Fitts, 1954). This task required participants to tap back and forth between two targets as rapidly as possible; both the amplitude of and the distance between the targets could be manipulated. It was found that as the difficulty of the task increased, participants sacrificed speed in order to remain accurate in their movements, a phenomenon that is commonly referred to as the speed-accuracy trade-off.

The applicability of the speed-accuracy trade-off to discrete movements instead of cyclical ones was first investigated by Fitts and Peterson, who performed a series of experiments using a single, ballistic movement to a target whose amplitude and distance could be manipulated (1964). In this experiment, the correct target was known in advance, but the go-signal was only presented 50% of the time, while the other 50% served as “catch” trials (no movement). The required movement could therefore be pre-planned, but uncertainty introduced by the large proportion of catch trials likely resulted in the inhibition of preparatory levels in order to prevent incorrect movement initiation. RT was significantly slowed by increases in difficulty, although this effect was small (Fitts & Peterson, 1964).

More recently, Lajoie and Franks reported similar results when subjects performed a discrete reaching task to a target of varying sizes. Results showed that there was a significant effect of target size on RT, whereby slower RTs were produced when a smaller target was presented (1997). Although the variability of RT was not statistically analyzed in this experiment, there also appears to be a slight effect of target size on the standard deviation of RT, whereby increased target size led to less variable RT. Similarly, a study by Castellote and Valls-Solé reported increased RTs with increased accuracy requirements during a reaching task (2015). Additionally, during this study a SAS was presented on a subset of trials; while the interaction between stimulus type and RT was not significant, there was a trend towards greater RT speeding in SAS trials when the subject was presented with a larger target (Castellote & Valls-Solé, 2015). Combined, these results suggest that manipulating the accuracy requirement of a discrete movement can slow RT, and this may in turn be indicative of lower preparation levels. Additionally, it is possible that SAS presentation and consequent triggering of the response may allow conclusions to be drawn regarding the preparatory state at the time of movement initiation.

6. Summary

While both behavioural and neurophysiological studies have supported the neural accumulator model as a basis for movement, there are still many features of the model that have yet to be investigated. TMS has previously been used to study preparatory and initiation processes by measuring MEPs during the foreperiod and RT intervals, while the use of a SAS can elucidate activation levels at the time of presentation. These tools can therefore be used to investigate the possible effects of movement accuracy requirements on the level and variability (i.e., noise) of neural activation, as well as the potential downstream effects of these on RT and RT variability. A simple RT task that manipulates the required level of task accuracy to be achieved may be an appropriate paradigm with which to study this feature of the proposed model, as it has previously been shown to produce slower RTs when accuracy requirements are increased, potentially pointing to differences in neural activation.

7. Research Question

The objective of this experiment was to study changes in the level and variability of neural activation as a function of task accuracy requirements in order to infer the level of preparation at the time that a given movement is initiated. Specifically of interest was the potential effect of varying accuracy requirements on the level of neural preparedness that can be achieved, and how this potential alteration of neural noise may in turn have affected the variability of RTs produced. The use of both TMS and startle provided insight into the levels of preparatory-related neural activation under different conditions.

It was hypothesized that TMS applied concurrently with the IS would reveal a decrease in MEP amplitude and an increase in MEP amplitude variability as accuracy requirements increased. Additionally, it was hypothesized that a similar pattern would be seen when a SAS was presented, whereby conditions with greater accuracy requirements would result in fewer responses being involuntarily initiated as a result of lower preparation levels at the time of SAS presentation. Finally, it was hypothesized that movements requiring greater accuracy would result in slower RTs, and that the variability of RTs produced would be greater in a high accuracy condition compared to a lower accuracy condition.

Chapter II: Research Article

Reaction time differences resulting from varying movement accuracy requirements may not be attributable to differences in movement preparation

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1. Introduction

One of the primary ways in which humans interact with their environment is through the production of movement. In order to produce appropriate movements in any given context, relevant stimuli must be detected, and this information is subsequently used to prepare one's actions. Classically, the information processing model has been used to describe the central processing that is required to produce movements (Schmidt & Lee, 2011). When a subject is presented with a stimulus, this must first be detected; once the stimulus has been detected, this information is used to inform the selection of the movement to be produced and subsequently prepare the selected movement. Once this preparation has occurred, the motor command can be sent to the effector muscles and the movement is executed (Schmidt & Lee, 2011).

Under certain conditions, it is thought that some of these processing steps can be bypassed. One example of this is the simple RT task. In this instance, the subject is aware of the movement to be executed and is therefore theoretically able to prepare it in advance. When they receive a go stimulus, they are then able to rapidly initiate the appropriate action without first having to select it. Early research into response preparation proposed that a pre-selected set of muscle commands, or a "motor program", can be prepared and released upon presentation of an IS (Keele, 1968). A possible neural mechanism for the motor program was proposed by Wickens, Hyland, and Anson (1994) in the form of cortical cell assemblies, which are subsets of neurons whose synaptic connections have been strengthened beyond that of the average connection through repeated firing (Palm, 1990; Hebb, 1949). Within the framework of cortical cell assemblies, preparation for a given movement may occur through an increase in synaptic activity within the assembly, with this activity reaching a steady state until IS presentation

(Wickens, Hyland, & Anson, 1994). Once the IS is presented, the cell assembly is “ignited”, thereby activating corticospinal connections and ultimately resulting in movement.

In order to better describe the neural processes that are occurring in the motor cortex in instances where the movement to be produced is known in advance, Carlsen and colleagues (2012) built on this proposal by Wickens et al. (1994), as well as findings from both neurostimulatory literature and startle research (Maslovat, Franks, Leguerrier, & Carlsen, 2015; Kennefick, Maslovat, & Carlsen, 2014; Carlsen & MacKinnon, 2010; MacKinnon et al., 2007; Leocani et al., 2000; Touge, Taylor, & Rothwell, 1998), to develop a neural accumulator model of movement preparation. In this model, excitatory activation increases following a warning signal until it reaches a plateau, at which point it remains approximately constant until the presentation of a go-signal. Following the go, activation again increases up to a movement threshold, at which point movement initiation processes occur. While the general progression of this model is fairly well supported by the literature, the effect of varying movement characteristics on neural activation have yet to be investigated within this framework.

To that end, the purpose of this experiment was to study the level and variability of neural activation, and the effects that these may have on RT and RT variability, during preparation when movement accuracy requirements were varied. This led to two hypotheses. First, that in cases of higher accuracy demands, lower levels of neural activation would be observed, and that this may result in subsequently slower reaction times. Second, it was hypothesized that higher accuracy demands would result in increased variability in neural excitability, consequently resulting in increased reaction time variability.

2. Methods

2.1. Participants. A power analysis using G*Power 3 (Faul, Erdfelder, Lang, & Buchner, 2007) indicated that for our design and given a desired power of 0.8, a probability of Type 1 error of .05, and a small expected effect size (.1) on RT, a sample size of 11 would be required. In order to remain conservative, several extra participants above this number were recruited, for a total of 15 healthy participants (age 24.6 ± 2.3 yrs.: 3 male, 12 female). All participants had normal or corrected to normal vision and no history of neurological or motor impairments, and all were right-handed in order to control for potential differences in the RT of right-handed responses as a result of handedness. Participants also completed a written informed consent and a TMS safety questionnaire prior to testing to detect and mitigate potential neurological risk factors (see Appendices E, F; Rossi et al., 2011). This study was approved by and conducted in accordance with the ethical guidelines set by the Behavioural Research Ethics Board at the University of Ottawa, and conforms to the most recent revision of the Declaration of Helsinki.

2.2. Apparatus and Task. Participants were seated comfortably in a height adjustable chair approximately 1 m away from a 24” LCD computer monitor and completed three blocks of a simple RT task. The right forearm was placed in a custom manipulandum such that it was positioned parallel to the floor with the palm facing towards the body midline, with the elbow flexed at 90° and the shoulder abducted at ~30°. Two Velcro straps placed proximal to the wrist and distal to the elbow were used to secure the forearm and restrict movement to extension and flexion of the wrist. The task itself consisted of a ballistic 32° wrist extension movement made from a “home position” 17° to the left of centre to a target 15° to the right of centre. The size of the target varied per block, as described below. A linear potentiometer attached to the central axis of the manipulandum was used to collect wrist position (angle) data. For a graphical representation of the experimental setup, see Figure 3.

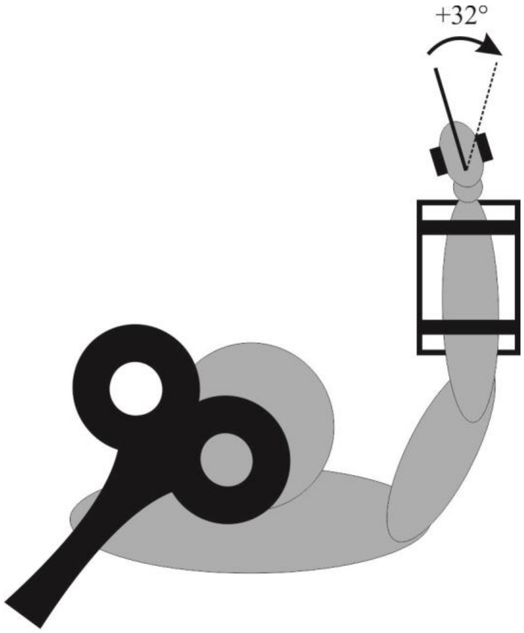

Figure 3. Participant setup during the experiment. Participants were seated comfortably with their right arm secured in a custom manipulandum by 2 straps and their right wrist flexed 17°. From this starting position, they completed a 32° wrist extension movement on each trial. TMS was delivered by the experimenter over the left M1 in order to target the neural representation of the right extensor carpi radialis longus.

2.3. Experimental Design. The experimental procedure was separated into two sections, referred to here as “Set 1” and “Set 2”. Each set consisted of 3 blocks of trials, each corresponding with three difficulty conditions (easy, moderate, and difficult). Task difficulty was manipulated by varying the width of the target presented on the screen, thereby changing the accuracy required to successfully complete a trial (Figure 4). The "easy" target measured 32 degrees wide with a calculated index of difficulty (ID) of 1. The "moderate" target measured 4 degrees with a calculated ID of 4. The "difficult" target measured 0.5 degrees with a calculated ID of 7. The centre location of each target remained consistent throughout the three blocks, and was situated at 32° of angular rotation to the right of the "home" position. Block order was counterbalanced, with participants being randomly assigned to one of the six possible block orders, and this order was conserved between Sets 1 and 2 for each participant.

Both sets of the experiment were almost identical, with the exception of 5 practice trials that were completed at the beginning of each block in Set 1 in order to familiarize participants with the task; these 5 practice trials per block were not completed in Set 2. Each block was further subdivided into two sub-blocks, namely a TMS sub-block and a SAS sub-block (Figure 5). The TMS sub-block always preceded the SAS sub-block of the same difficulty, and consisted of 20 trials. During this sub-block, single pulse TMS was delivered on every trial, for a total of 20 pulses per TMS sub-block. The SAS sub-blocks each consisted of 20 trials, with a SAS presented on 30% of trials (i.e. 6 trials). The SAS was presented pseudorandomly, such that it was never presented on two consecutive trials, and was not presented on the first trial of the sub-block. Participants were informed prior to beginning the experiment that they would hear a loud acoustic stimulus on some trials, but that this sound was task-irrelevant.

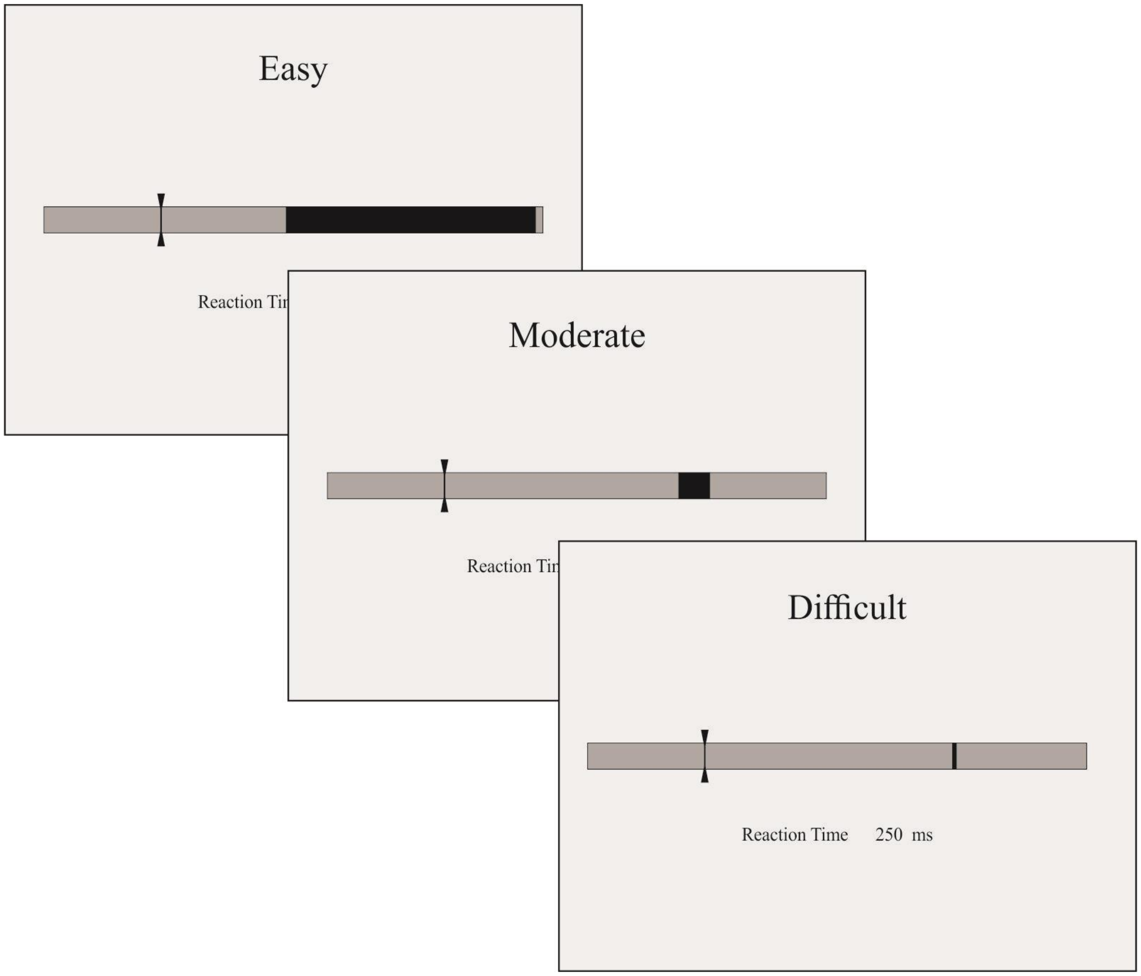

Figure 4. Representation of the visual display that was shown to subjects during each condition. The “easy” target was 32° wide, the “moderate” target was 4° wide, and the “difficult” target was 0.5° wide. Reaction time and position feedback was provided.

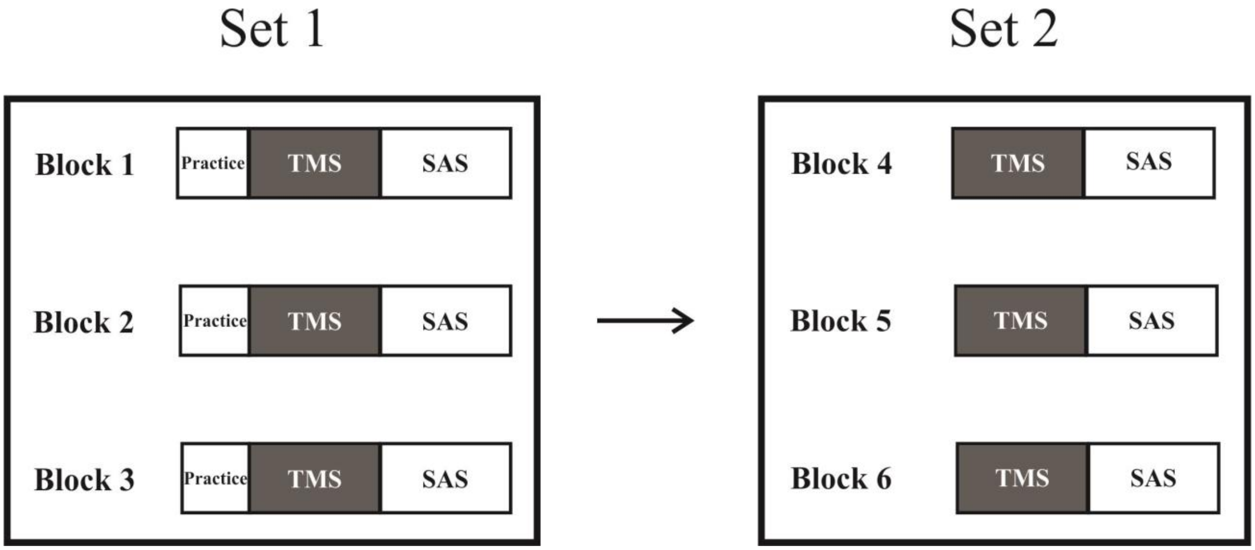

Figure 5. Organizational diagram of the experimental session. Participants completed one of six possible block (difficulty condition) orders, with Sub-block 1 (TMS) always preceding Sub-block 2 (SAS).

All trials began with the presentation of an acoustic warning signal (80 dB, 200 Hz, 100 ms), indicating to participants that they should prepare to perform the wrist extension movement to the target (Figure 6). Following a variable foreperiod (2000 – 2500 ms), an auditory IS (82 dB, 1000 Hz, 40 ms) was presented, at which point participants were required to execute the task as quickly as possible while attempting to maintain movement accuracy within the designated boundaries in each condition. Data was collected for a total of 3000 ms, after which feedback was presented. A variable foreperiod was used in an attempt to prevent participants from anticipating the presentation of the IS. During the TMS sub-blocks, TMS was delivered at 120% of RMT coincident with the IS, and MEP amplitude was recorded. During the SAS sub-blocks, if a SAS (120 dB, 25 ms, white noise waveform) was presented it occurred coincident with the IS. A full trial lasted anywhere from 9.5-10 sec depending on the foreperiod for that trial.

Analog signals were generated using digital to analog hardware (PCIe-6321, National Instruments Inc., Austin, TX) and were amplified and presented via a loudspeaker (M58-H, MG Electronics, Inc., Hauppauge, NY) placed 30 cm directly behind the participant’s head. Stimulus intensity was confirmed using a precision sound level meter (Cirrus Research Optimus, CR:162C) placed at the location of the participant’s left ear prior to testing.

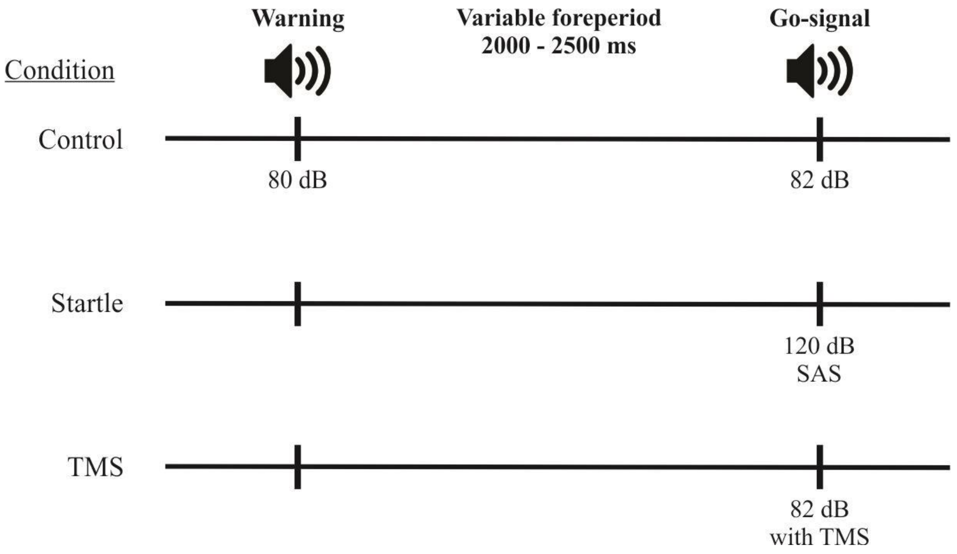

Figure 6. Timeline of the experimental trials. Participants completed trials of three different types. Control trials consisted of an 80 dB warning tone followed at variable latency by an 82 dB IS. During startle trials, the control IS was replaced by a 120 dB SAS. TMS trials were identical to control trials, with the addition of delivery of a single TMS pulse concurrent with the 82 dB IS.

2.4. Feedback. Participants were given online wrist position feedback throughout the trial, as well as feedback regarding final position (accuracy) following the completion of each trial. Position feedback was provided by displaying a virtual marker showing the position of the wrist with respect to the home and target positions. In addition, the target itself turned either red or green based on whether or not the initial movement ended within the target zone. RT feedback was also provided following the completion of the trial, with visual feedback provided on the computer screen for 3500 ms and disappearing upon presentation of the auditory warning tone signalling the beginning of the subsequent trial. If RT was shorter than 50 ms, the trial was deemed anticipatory and a message appeared following trial completion instructing the participant to wait for the IS before responding. The visual display was generated using a customized program written using LabVIEW (National Instruments Inc.).

2.5. Electromyography. Surface EMG data was collected from the muscle belly of the right extensor carpi radialis longus (ECRL; agonist) and flexor carpi radialis (FCR; antagonist) muscles as well as the right SCM (startle indicator) muscles using bipolar preamplified surface electrodes (Delsys Bagnoli DE-3.1, Delsys Inc., Natick, MA). The electrodes were affixed in a parallel orientation with respect to the muscle fibres, and were connected to an external amplifier (Delsys Bagnoli-8) via shielded cabling. The attachment site was cleaned using abrasive gel and an alcohol swab in order to minimize impedance, and the electrodes were attached using two-sided adhesive tape. A grounding electrode (Dermatrode HE-R) was also placed on the left lateral epicondyle. EMG signals were digitally sampled at 4 kHz (PCIe-6321, National Instruments Inc.) for 3 s starting 1 s prior to the go-signal.

2.6. Transcranial Magnetic Stimulation. Excitability of the motor cortex was determined using single-pulse transcranial magnetic stimulation (TMS) applied with a figure-8 coil (70mm, Magstim 200^2, Magstim Company Ltd, UK) to the location on the scalp corresponding to the contralateral M1 representation of the right ECRL. In order to find this location, the midpoints between the nasion and the inion as well as between the left and right preauricular notches were measured to determine the centre of the head (Cz, according to the International 10/20 electrode placement system; Homan, Herman, & Purdy, 1987). From there, a mark was made with a grease pencil on the scalp at a distance of 4.6 cm laterally (to the left) and 1 cm anterior to Cz. Using this mark as an approximate starting point, the optimal coil placement (hotspot) for targeting the right ECRL was determined by locating the site on the scalp at which a consistently large MEP was evoked. Resting motor threshold (RMT) was then be determined to the nearest 1% of stimulator output (Rossini et al., 1994). RMT was defined as the minimum % stimulator output (%MSO) that results in a MEP with amplitude of 100 µV peak-to-peak on 5 out of 10 stimulations. The coil was placed tangentially on the scalp and held at an approximate angle of 45° to induce a current in the posterior-anterior direction. In order to maintain consistent coil placement throughout the experiment, a neuronavigation system (ANT Neuro Visor 2, Madison, WI) was used to record the position of the coil during hot-spotting so that it could be replicated during the experimental protocol.

2.7. Data Reduction and Analysis. EMG parameters were determined from the raw data using a custom LabVIEW analysis program. EMG data for each trial were full-wave rectified and dual-pass filtered using a 25 Hz low-pass second-order elliptical filter. Premotor RT of the ECRL as well as activation of the SCM were determined based on the onset time of EMG activity in each muscle. EMG onsets for all three muscles were initially determined as the first point where the rectified and filtered EMG reached a value greater than 2 standard deviations above the baseline noise (determined from a window of 100 ms of activity preceding the go-signal) and remained elevated for at least 20 ms. These points were then visually inspected and manually adjusted whenever necessary to compensate for the strictness of the algorithm. Trials that had an onset in the ECRL earlier than 50 ms before the go-signal were classified as anticipatory and were excluded from the data set. Trials were also discarded if a movement error occurred (e.g., no movement). If the RT/MEP amplitude of a given trial was greater than that subject’s mean RT/MEP amplitude plus 2 standard deviations, the value was excluded from the analysis. Trials with a SAS were classified as startle/non-startle based on the presence/absence of an EMG burst in the SCM within 120 ms of the presentation of the SAS (Carlsen, Maslovat, Lam, Chua, & Franks, 2011). The proportion of SAS trials exhibiting a startle response was then determined for each SAS sub-block.

Mean MEP amplitude was compared between conditions to detect differences in excitability for different accuracy requirements. MEP amplitude was defined as the largest peak-to-peak amplitude of the baseline corrected raw EMG activity during a 30 ms window beginning 15 ms after the TMS impulse; this 15 ms delay is to allow for neural conduction time. In order to exclude all trials with prior activity in the ECRL, all MEP trials where the root mean square (RMS) of the EMG during the 100 ms preceding TMS delivery exceeded twice the resting value (determined from a mean of the EMG signal determined from a mean of 300 ms of EMG starting 900 ms prior to the go-signal), and whose pre-TMS RMS EMG was also greater than 5 µV were excluded from the analysis. TMS trials were also removed from the analysis if no MEP was detected, which may suggest that the TMS coil placement had shifted from the previously located hotspot and did not elicit a reliable MEP for that trial.

Other measures of interest included RT variability, calculated as the mean SD of RT, and MEP amplitude variability, calculated as the mean SD of MEP amplitude. RT was also examined for trials in the TMS sub-block; however, EMG onset in these trials had to be manually determined, as the algorithm described above did not distinguish between EMG activity resulting from the MEP and activity resulting from voluntary movement initiation. The percentage of correct responses in each condition was also determined by calculating the proportion of trials where the final position of the movement fell within the established boundaries of the target (16° and 48° for the easy target; 32° and 36° for the moderate target; 31.5° and 32.5° for the difficult target). Kinematic measures of interest included movement time (MT), peak displacement, and variability of peak displacement. MT was calculated as the difference between the time of final position and the time of movement onset; the time of movement onset was defined as the first time where displacement changed more than 0.2° following the IS, and the time of final position was defined as the first time where movement velocity remained below 8°/sec for at least 150 ms. Peak displacement was defined as the maximum displacement value recorded during the movement, and peak displacement variability was calculated as the mean SD of peak variability. Finally, movement accuracy was defined as mean absolute error of the movement, which was calculated by taking the absolute value of the difference between the final position on each trial (displacement in degrees at the time of final position, outlined above) and the location of the centre of the target with respect to the home position (32°).

2.8. Statistical Analysis. Mean premotor RT was compared using a 2 (set: Set 1, Set 2) x 3 (difficulty: easy, moderate, difficult) x 2 (stimulus: control, SAS) repeated measures analysis of variance (ANOVA) in order to detect differences resulting from task accuracy requirements during the separate sets of trials and between acoustic stimuli. Mean MEP amplitude was compared using a separate 2 (set: Set 1, Set 2) x 3 (difficulty: easy, moderate, difficult) repeated measures analysis of variance (ANOVA). This same analysis was also completed for the proportion of SAS trials exhibiting a startle reflex, mean RT variability, mean TMS RT, mean MEP amplitude variability, mean MT, mean peak displacement, mean variability of peak displacement, and mean absolute error. In the event that data violated the assumption of normality (as assessed by Shapiro-Wilk's test), non-parametric Friedman's tests were conducted in order to compare data between difficulty conditions within each set, and Wilcoxon signed-rank tests were conducted to compare data between sets within each difficulty condition. The significance value for all statistical tests was set at $p < .05$ unless otherwise indicated. All statistical analyses were completed using SPSS.

3. Results

3.1. Data Reduction. No participants were excluded from the statistical analysis following data reduction and analysis. 21 out of 1260 total control trials were classified as anticipatory and were excluded from data analysis. Another 11/1260 total control trials were classified as movement errors (e.g. no movement, flexion instead of extension, etc.) and were excluded from data analysis. Similarly, 13/540 SAS trials were removed from the analysis as a result of anticipation errors, and 1 trial was classified as "too slow" (RT > 500 ms), which suggested that the subject was not paying adequate attention to the task, and was removed from the analysis. Out of 1800 total TMS trials, 21 trials were excluded as a result of anticipation, and 11 trials were removed as a result of movement error following the TMS pulse. Another 14/1800 TMS trials were excluded if a MEP was not apparent, or due to excessive EMG activity prior to TMS pulse delivery (see Methods section 1.7 for an in-depth explanation). No more than 6.7% of control trials, 8.3% of startle trials, or 8.3% of TMS trials were removed as a result of one of these four types of error for any participant.

Trials were excluded from RT analysis if the premotor RT for the trial was not within 2 standard deviations of the subject's mean RT. This resulted in the removal of 105/1260 control trials and 26/540 SAS trials. This same procedure was completed if the MEP amplitude was not within 2 standard deviations of the subject's mean MEP amplitude. This resulted in the removal of 73/1800 TMS trials. No participant exceeded 8.8% of trials removed as a result of this trimming.

Overall, these data reduction procedures resulted in total trial retention of 89.1% of control trials, 92.6% of SAS trials, and 93.4% of TMS trials.

3.2. Startle response activity. The proportion of SAS trials where SCM activity was detected (indicating a startle response) is shown in Figure 7. Mean startle proportion data were not normally distributed (as assessed by Shapiro-Wilk's test), and this violation of the assumption of normality was not resolved by applying an arcsine-square root transform (Sokal & Rohlf, 1981). Although the repeated measures ANOVA is relatively robust to violations of the assumption of normality, non-parametric analyses were performed on these data. Separate Friedman's tests were performed on data from Sets 1 and 2 in order to identify differences in mean proportion of startle responses observed as a result of task difficulty, and separate Wilcoxon signed-rank tests were performed between difficulty-matched blocks in order to detect any potential effect of set.

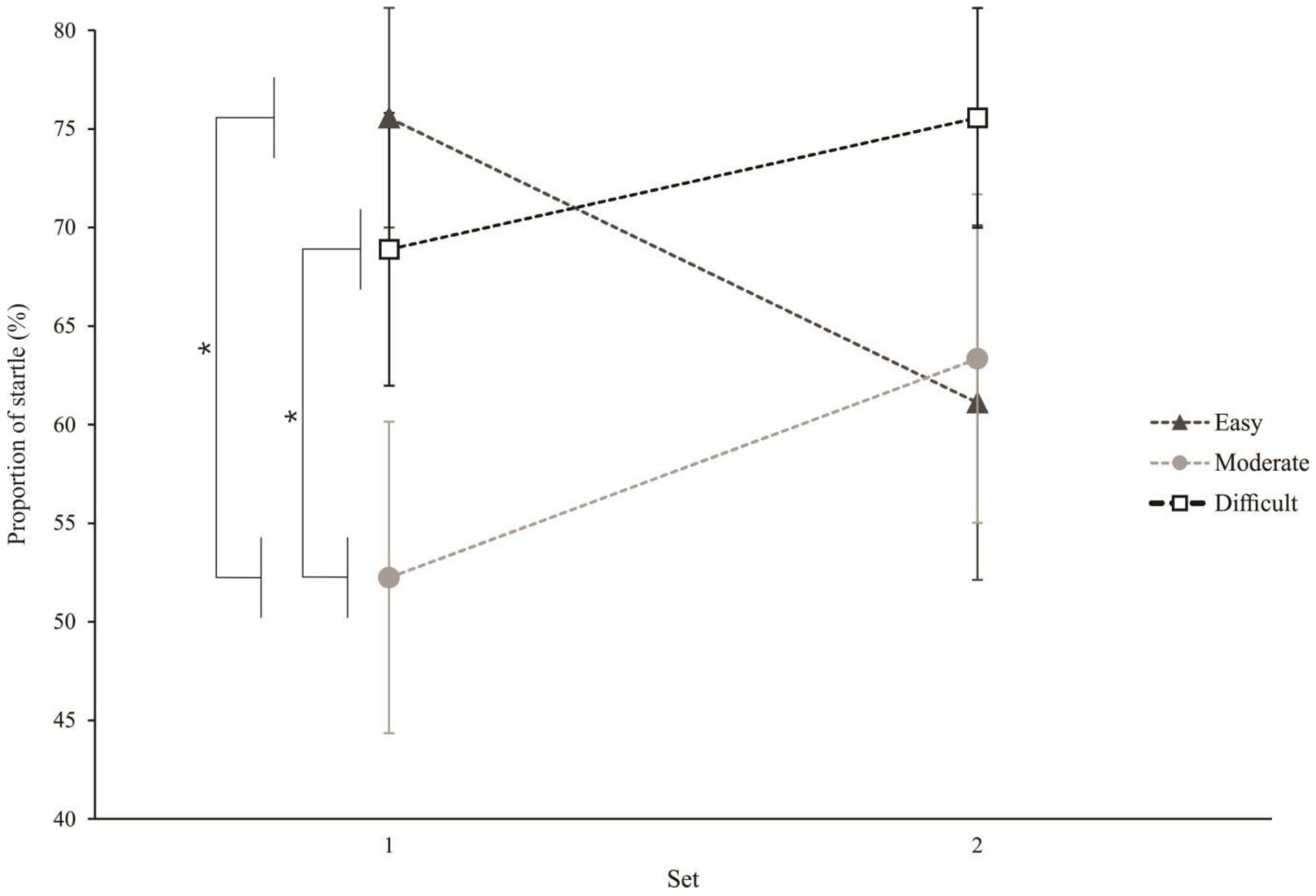

Figure 7. Proportion (%) of SAS trials where a startle response was identified (i.e. SCM activity detected) for the easy (**dark grey triangles**), moderate (**light grey circles**), and difficult (**unfilled squares**) conditions in both sets. Error bars represent standard error of the mean, and * indicates the presence of a significant difference (p < .05).

In Set 1, there was a significant difference in the proportion of startle responses observed between the different difficulty conditions, $\chi^2(2) = 10.773$, p = .005. Post-hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a significance level set at p < .017; mean startle proportion was significantly lower in the moderate task compared to the easy task, Z = -2.606, p = .009, as well as the difficult one, Z = -2.689, p = .007. No significant difference was detected between the easy and difficult task conditions, Z = -1.149, p = .250. In Set 2, there was no statistically significant difference in the proportion of startle between the different difficulty conditions, $\chi^2(2) = 3.435$, p = .180. For the easy task, a Wilcoxon signed-rank test comparing the proportion of SAS trials exhibiting a startle response between sets showed that mean startle proportion was not significantly different between Sets 1 and 2, Z = -1.602, p = .109. Similarly, mean startle proportion in the moderate task was not significantly different in Set 1 compared to Set 2, Z = -1.018, p = .309. For the difficult task, no significant difference in mean control RT variability was seen, Z = -1.149, p = .250.

3.3. Premotor RT. Mean premotor RT for all conditions is shown in Figure 8. In all 6 SAS sub-blocks, there were cases where the incidence of startle in certain participants was less than 50%; in these cases, the mean RT value for the corresponding block was removed from the analysis. This occurred in 8/15 subjects, for a total of 22 removed data points out of a possible 90 (15 subjects multiplied by 6 RT sub-blocks). Subject 15 had a startle incidence <50% for SAS trials in all 6 RT sub-blocks and was therefore completely removed from this analysis only. A linear regression-based multiple imputation was completed in SPSS to fill the removed RT data points in SAS sub-blocks, with each missing experimental value replaced by the mean from 5 imputed values.

These data were log-transformed to account for violations in the assumption of normality; mean log-transformed RT was normally distributed for all difficulties across both time points and for both stimulus types, as assessed by Shapiro-Wilk's test ($p > .05$ for all values). Once this was completed, a 2 (set; Set 1, Set 2) x 3 (difficulty; easy, moderate, difficult) x 2 (stimulus; control, SAS) repeated measures ANOVA was conducted on the transformed data. There was no significant main effect of set, $F(1, 13) = 1.654$, $p = .221$, $\eta_p^2 = .113$, and no main effect of difficulty, $F(2, 26) = .073$, $p = .930$, $\eta_p^2 = .006$. A significant main effect of stimulus was detected, $F(1, 13) = 85.414$, $p < .001$, $\eta_p^2 =.901$, with mean SAS RT being significantly faster than mean control RT. There was also a significant interaction between set and stimulus, $F(1, 13) = 10.389$, $p = .007$, with control but not SAS RTs decreasing from Set 1 to Set 2. No other interactions between the factors were significant ($p > .05$ for all other interactions).

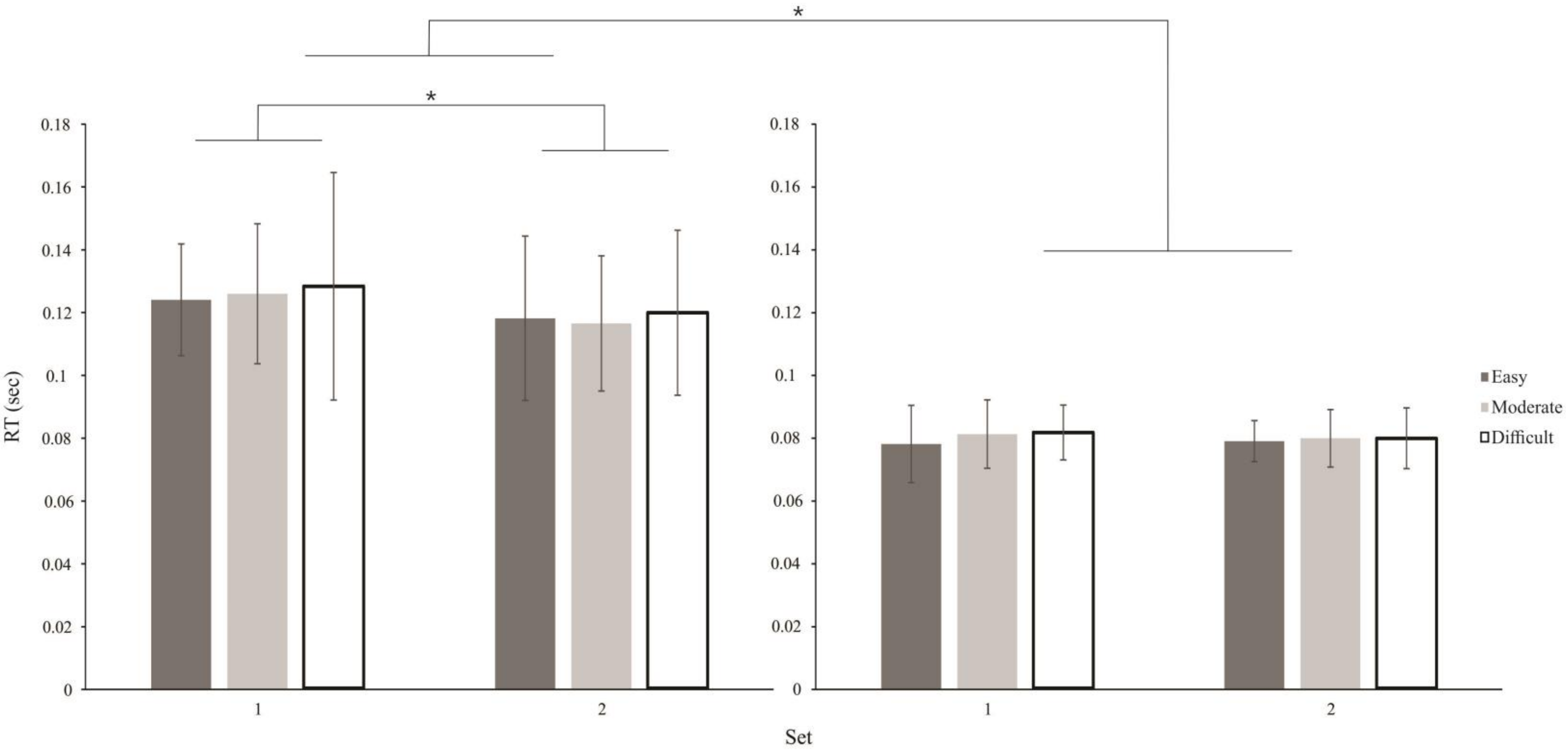

Figure 8. Mean control premotor RT (N = 15; left) and mean SAS premotor RT (N = 14; right) in seconds for all three difficulty conditions in both sets. Error bars represent standard deviation, and * indicates the presence of a significant difference (p < .05).

A second analysis of mean premotor RT was performed separately for control trials only, since the complete analysis above involved the imputation of 22 values for mean startle premotor RT as well as the removal of one subject from the data set. Mean control log-transformed premotor RT was analyzed for all 15 subjects using a 2 (set; Set 1, Set 2) x 3 (difficulty; easy, moderate, difficult) repeated measures ANOVA. Results showed a significant main effect of set, F(1, 14) = 8.582, p = .011, η_p^2 = .380, with mean RT decreasing from Set 1 to Set 2 (see Figure 8). No significant main effect of difficulty was detected, F(2, 28) = .074, p = .929, η_p^2 = .005, and there was no significant interaction between the two factors, F(2, 28) = .053, p = .948, η_p^2 = .004.

3.3.1. Variability of RT. As was the case for the startle proportion data, mean control RT variability was not normally distributed (as assessed by Shapiro-Wilk’s test). Non-parametric analyses were therefore performed. Separate Friedman’s tests were performed on data from Sets 1 and 2 in order to identify differences in mean control RT variability as a result of task difficulty, and separate Wilcoxon signed-rank tests were performed between difficulty-matched blocks in order to detect any potential effect of set.

In Set 1, there was no significant difference between difficulty levels in the mean rank of control RT variability, $\chi^2(2)$ = 2.533, p = .282, nor was there a significant difference in mean rank in Set 2, $\chi^2(2)$ = .533, p = .766. For the easy task, a Wilcoxon signed-rank test comparing control RT variability between Sets 1 and 2 showed that mean variability was significantly higher in Set 1 compared to Set 2, Z = -2.442, p = .015. Similarly, mean variability in the moderate task was significantly higher in Set 1 compared to Set 2, Z = -3.237, p = .001. For the difficult task however, no significant difference in mean control RT variability was seen, Z = -1.704, p = .088.

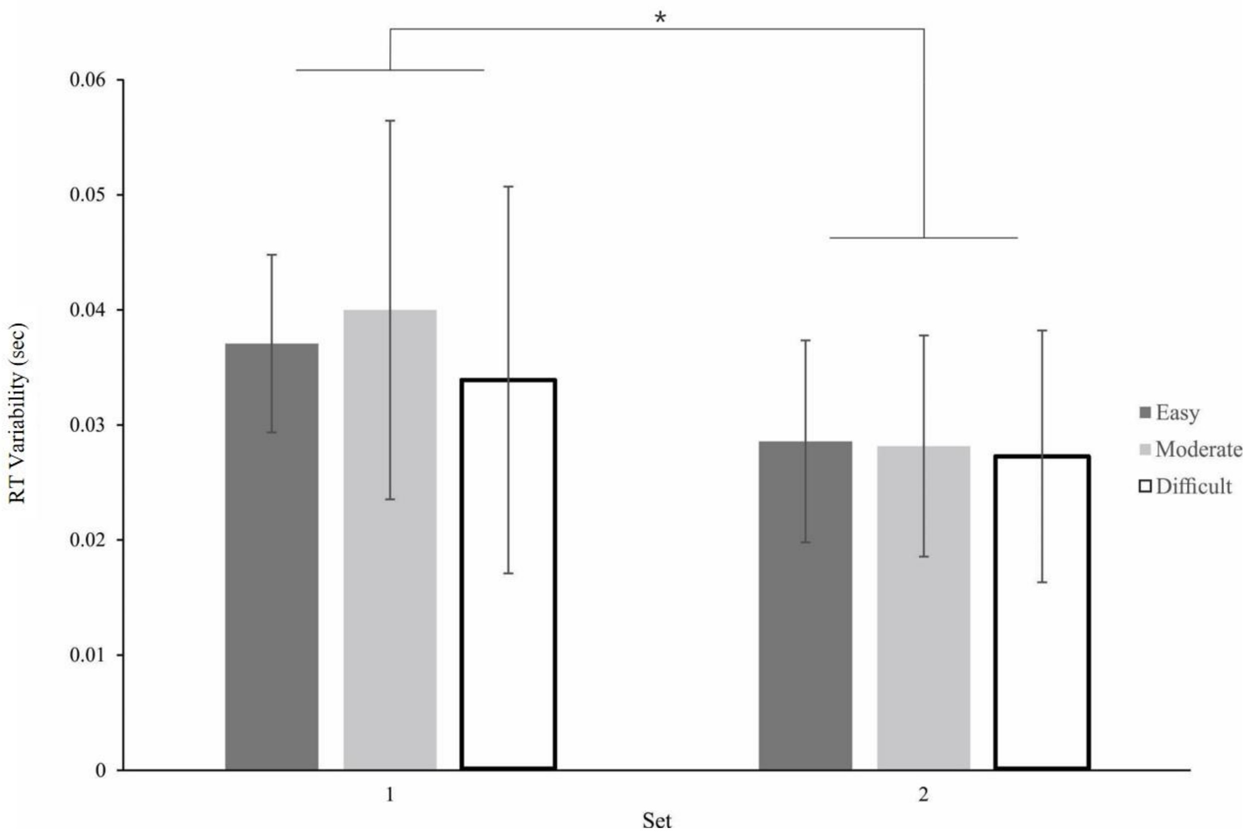

Figure 9. Mean control premotor RT variability (N = 15) in seconds for all three difficulty conditions in both sets. RT variability was defined as the mean standard deviation of premotor RT for all participants. Error bars represent standard deviation, and * indicates the presence of a significant difference (p < .05).

***3.3.2. First block analysis*.** In order to determine whether RT differences between difficulty conditions were being obscured by a potential order effect, a separate analysis was conducted on RT in the first block of trials only (i.e. before substantial practice of the task had occurred; Figure 10). Participants completed the experiment in one of 6 possible orders; however, for the purpose of this analysis, participants were grouped together based on the difficulty of the first block completed, irrespective of the order of the second and third blocks. This led to three possible classifications (Easy-first [N=6], moderate-first [N=5], and difficult-first [N=4]; see Appendix C), each of which contained participants having completed one of two possible experimental orders. Mean control premotor RT in the first block was normally distributed for all three classifications, as assessed by Shapiro-Wilk's test ($p > .05$ for all values). A one-way ANOVA with three levels of difficulty was performed on the mean premotor RT of these three groups of participants. Levene's test revealed no significant violation of the assumption of homogeneity of variances ($F = .115$, $p = .893$). The analysis revealed no significant difference in mean premotor RT in the first experimental block, $F(2, 14) = .289$, $p = .754$. In other words, no mean premotor RT differences were detected between difficulties, even as early as the first block of RT trials.

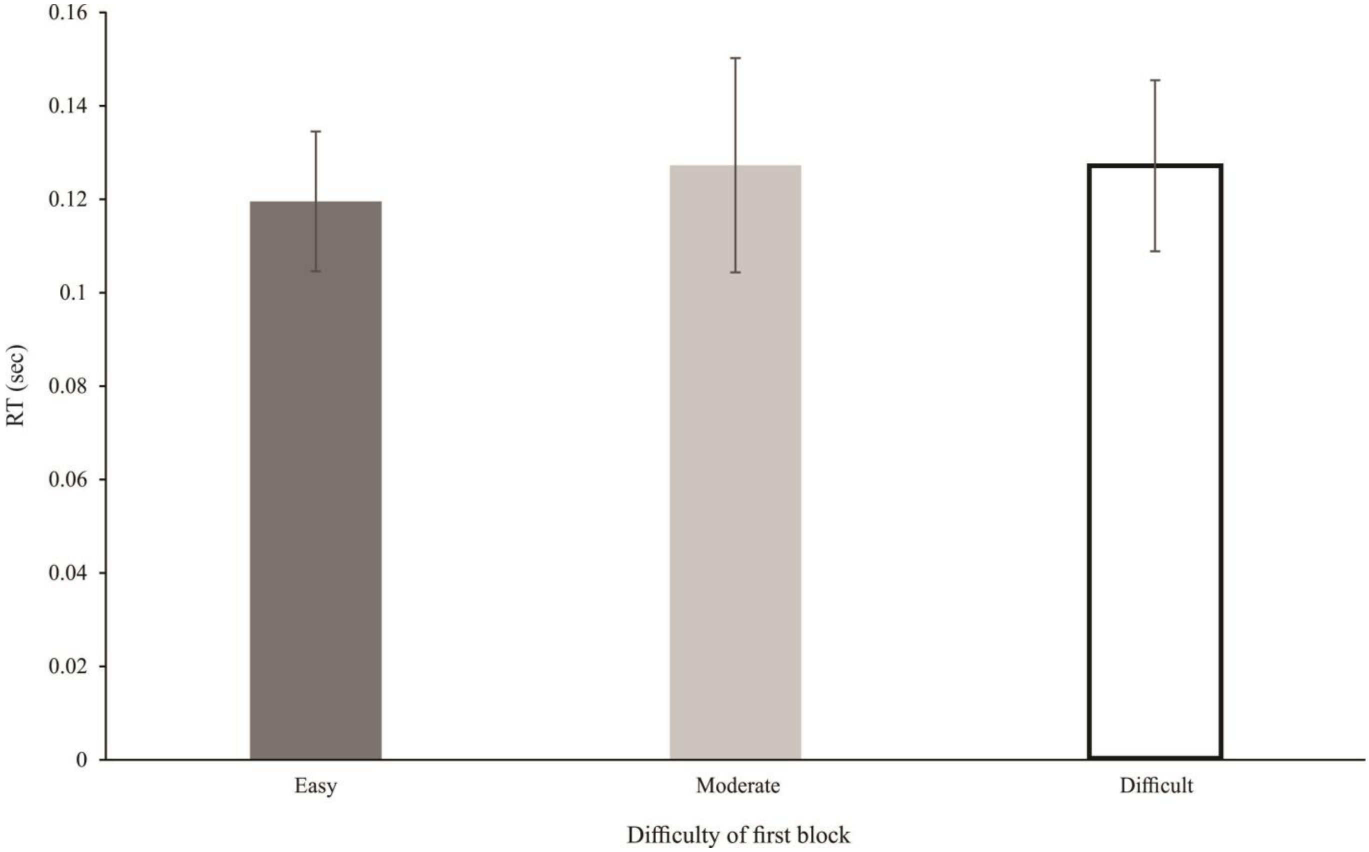

Figure 10. Mean control premotor RT in seconds in the first block of trials (SAS sub-block, see Figure 5) for the **easy** (N = 6), **moderate** (N = 5), and **difficult** (N = 4) conditions. Error bars represent standard deviation.

3.3.3. Premotor RT in TMS trials. Mean premotor RT was assessed separately in TMS trials to account for any possible TMS effects on RT. Mean TMS RT data were log-transformed to account for violations of the assumption of normality; mean log-transformed TMS RT was normally distributed for all difficulties across both time points, as assessed by Shapiro-Wilk's test ($p > .05$ for all values). Data were then analyzed using a 2 (set; Set 1, Set 2) x 3 (difficulty; easy, moderate, difficult) repeated measures ANOVA. No significant main effect of set, $F(1, 14) = .576$, $p = .460$, $\eta_p^2 = .040$, or difficulty, $F(2, 28) = .220$, $p = .804$, $\eta_p^2 = .015$, were detected, and there was no significant interaction between the factors, $F(2, 28) = .599$, $p = .556$, $\eta_p^2 = .041$.

3.4. MEP Amplitude. Across participants, mean RMT was 37.6 ± 7.6% MSO, which resulted in a mean TMS intensity of 45.1 ± 8.7% MSO being used throughout the experiment (120% of RMT). Mean control MEP amplitude was normally distributed for all difficulties across both time points, as assessed by Shapiro-Wilk's test ($p > .05$ for all values). The mean peak-to-peak MEP amplitude for all 3 difficulties within Sets 1 and 2 can be seen in Figure 11. A 2 (set; Set 1, Set 2) x 3 (difficulty; easy, moderate, difficult) repeated measures ANOVA revealed no significant main effect of set, $F(1, 14) = .503$, $p = .409$, $\eta_p^2 = .051$, and no significant main effect of difficulty, $F(2, 28) = 2.439$, $p = .106$, $\eta_p^2 = .130$, on MEP amplitude. Additionally, there was no significant interaction effect between the two factors, $F(2, 28) = .472$, $p = .629$ $\eta_p^2 = .024$.

3.4.1. Variability of MEP amplitude. The standard deviation of mean MEP amplitude was also analyzed in order to assess MEP variability (Figure 12). Mean MEP amplitude SD data were log-transformed to account for violations of the assumption of normality, after which mean transformed SD of MEP amplitude was normally distributed for all difficulties across both time points, as assessed by Shapiro-Wilk's test ($p > .05$ for all values). A 2 (set; Set 1, Set 2) x 3 (difficulty; easy, moderate, difficult) repeated measures ANOVA revealed no significant main effect of set, F(1, 14) = .1.285, p = .276, η_p^2 = .084, and no significant main effect of difficulty on the SD of MEP amplitude, F(2, 28) = 2.702, p = .085, η_p^2 = .162. No significant interaction between the two factors was seen, F(2, 28) = .428, p = .656, η_p^2 = .030.

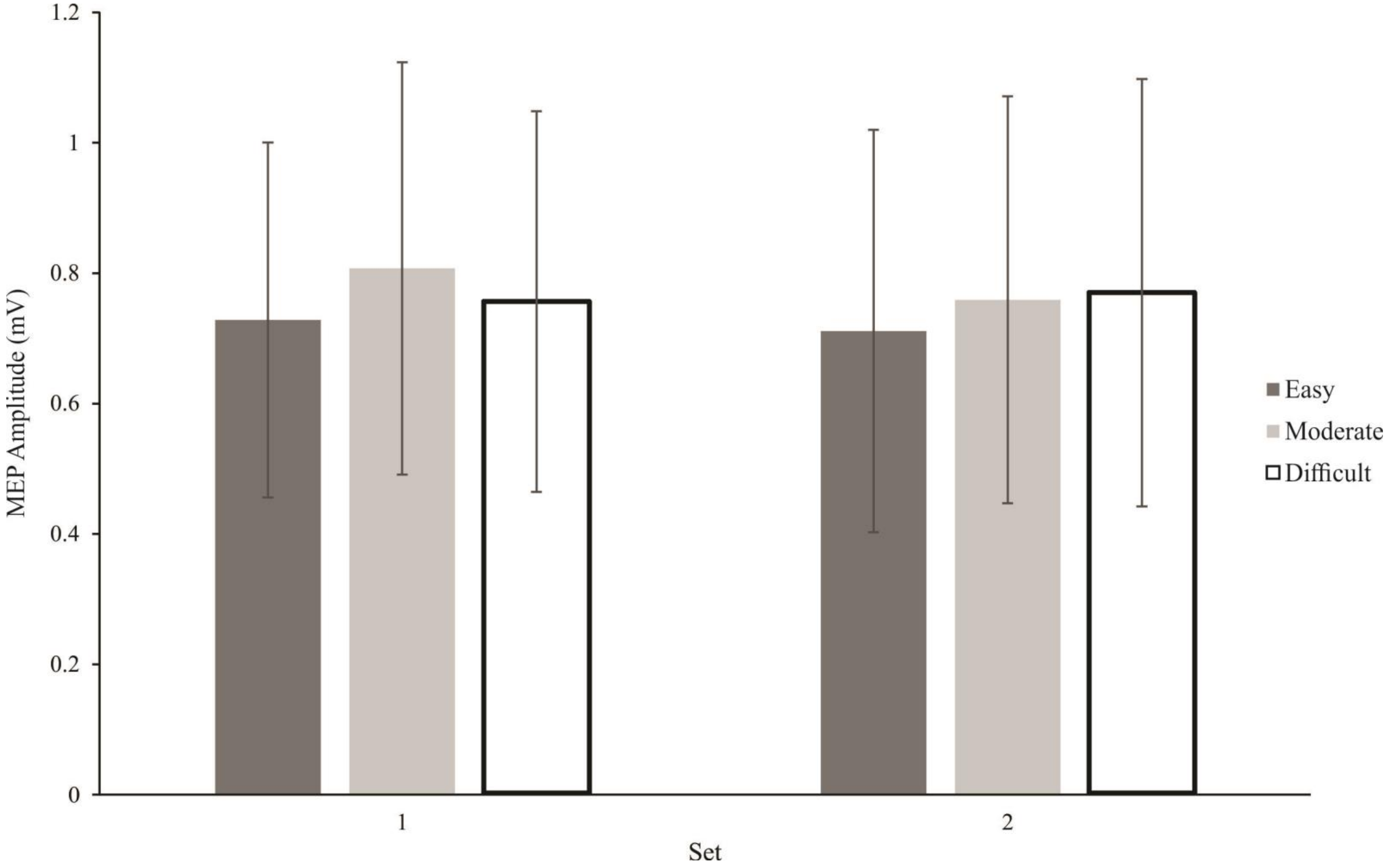

Figure 11. Mean MEP Amplitude (N = 15) in millivolts for all three difficulty conditions in both sets. Error bars represent standard deviation.

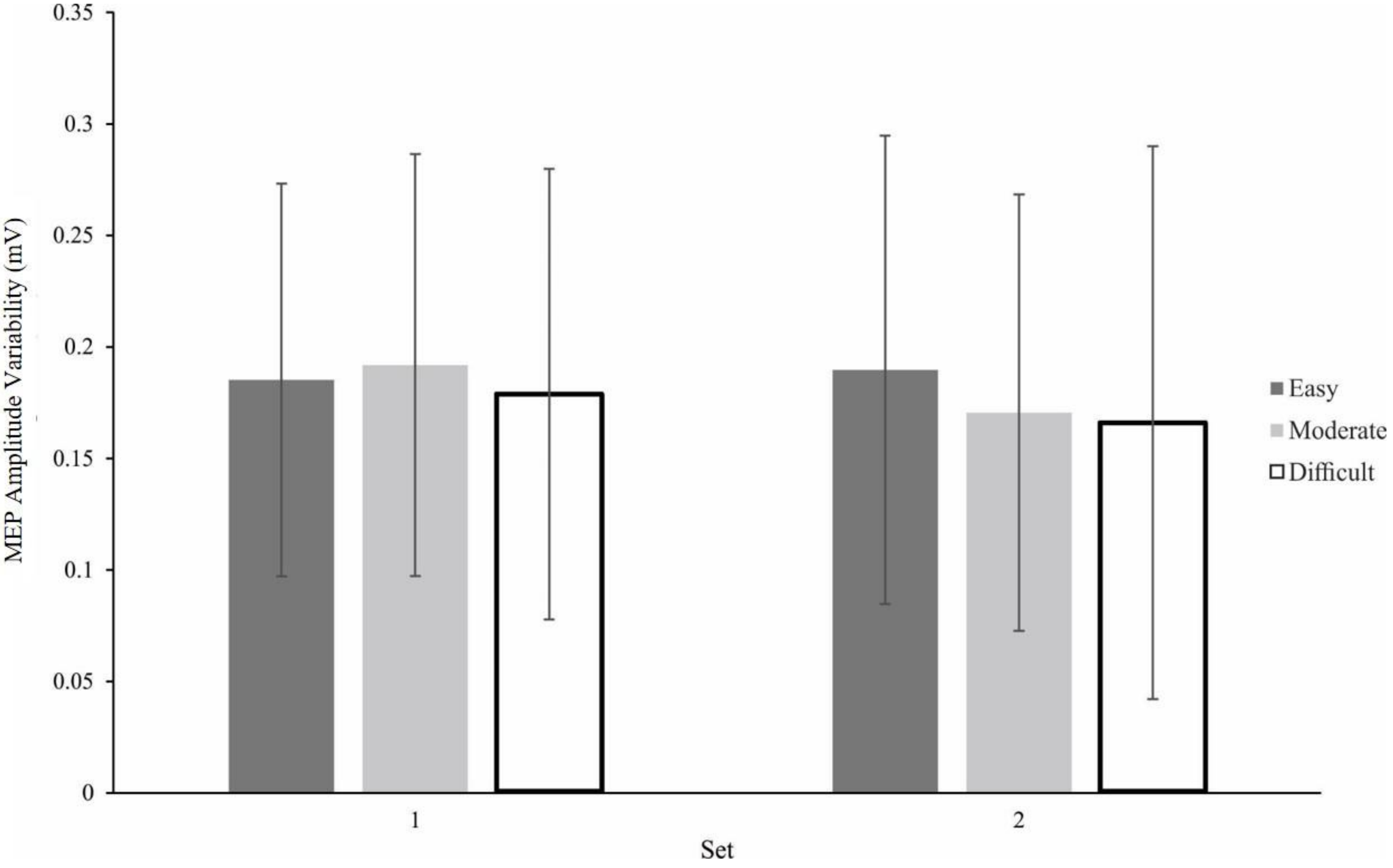

Figure 12. Mean variability of MEP Amplitude (N = 15) in millivolts for all three difficulty conditions in both sets. Variability of MEP amplitude was defined as the mean standard deviation of MEP amplitude for all participants. Error bars represent standard deviation.

3.5. Correlational Analysis. The relationship between the difference in mean premotor RT and the difference in mean MEP amplitude was analyzed for all three possible combinations of difficulty conditions (Difficult-Easy; Difficult-Moderate; Moderate-Easy) using Pearson's correlation analyses. The change in RT (ΔRT) was calculated as the difference in mean premotor RT between two conditions, such that a positive value indicated an increase in RT with an increase in difficulty, and a negative value indicated a decrease in RT with an increase in difficulty. The change in MEP amplitude (ΔMEP) was calculated as the difference in mean MEP amplitude between two conditions, such that a positive value indicated an increase in MEP amplitude with an increase in difficulty, and a negative value indicated a decrease in MEP amplitude with an increase in difficulty.

No significant correlation was seen between $\Delta RT_{\text{Difficult-Easy}}$ and $\Delta MEP_{\text{Difficult-Easy}}$, r = -.114, N = 15, p = .685 (Figure 13a). There were also no significant correlations detected between $\Delta RT_{\text{Moderate-Easy}}$ and $\Delta MEP_{\text{Moderate-Easy}}$, r = -.292, N = 15, p = .291 (Figure 13b), or between $\Delta RT_{\text{Difficult-Moderate}}$ and $\Delta MEP_{\text{Difficult-Moderate}}$, r = -.096, N = 15, p = .733 (Figure 13c).

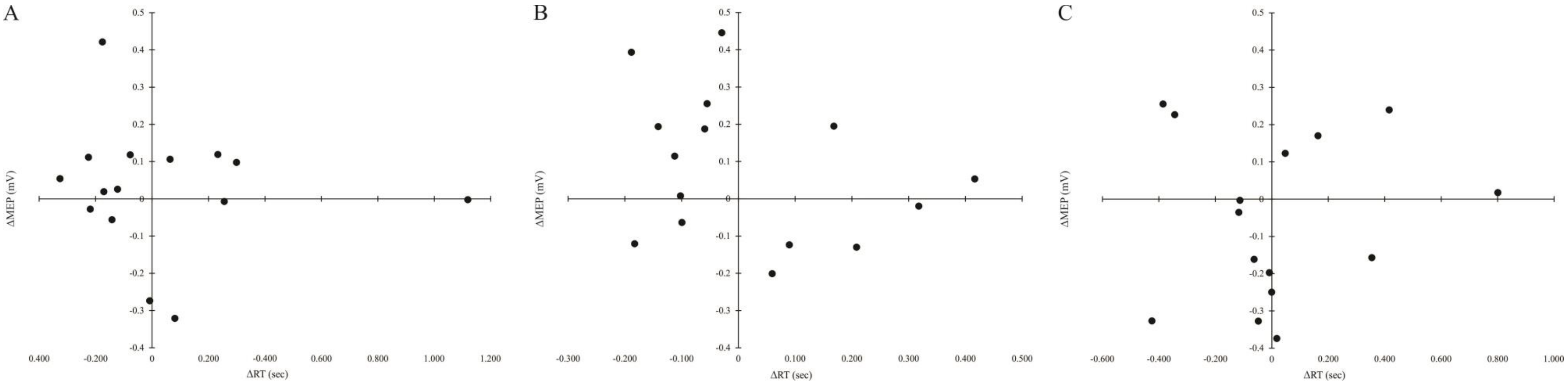

Figure 13. Relationship between the difference in premotor RT (ΔRT; sec) and the difference in MEP amplitude (ΔMEP; mV) for all three possible comparisons between difficulty conditions. a) Calculated differences in MEP and RT between the difficult and easy conditions; b) calculated differences in MEP and RT between the moderate and easy conditions; c) calculated differences in MEP and RT between the difficult and moderate conditions.

3.6. Subset analysis. While no significant effect of difficulty was seen when comparing RT between difficulties and variability of RT between difficulties for all 15 subjects, a subset of subjects (N = 7) showed a positive difference in RT between the difficult and easy conditions at Set 1 (indicating that mean control RT was longer in the difficult condition; mean $\Delta RT_{Difficult\text{-}Easy}$ = 0.0292 sec, SE = 0.0148 sec [Figure 14a]). Mean control RT was normally distributed for all difficulties across both time points for this subset, as assessed by Shapiro-Wilk's test ($p > .05$ for all values). RT data from these 7 subjects were compared using a one-tailed t-test because the direction of the difference in RT was predetermined, as these subjects were all selected because they showed an increase in RT as a result of increasing difficulty. There was a significant difference in mean control premotor RT between the easy and difficult conditions in Set 1, t(6) = 2.531, p = .045, d = .866. In Set 2, no significant difference in mean control premotor RT between easy and difficult conditions was detected, t(6) = 1.440, p = .200, d = .119. Similarly, mean control RT variability in the easy condition was significantly higher than in the difficult condition in this subset of subjects in Set 1, t(6) = 2.821, p = .030, d = .947, but not between these conditions in Set 2, t(6) = 0.474, p = .652, d = .184 (Figure 14b).

Even though these 7 subjects showed a positive $\Delta RT_{Difficult\text{-}Easy}$ (N = 7), when mean control MEP amplitude in Set 1 was analyzed in this subset no significant difference was detected, t(6) = 1.157, p = .291, d = .124 (Figure 15a). Similarly, in Set 2 no significant difference in mean control MEP amplitude between easy and difficult conditions was seen, t(6) = 1.665, p = .147, d = .269. This same pattern was seen when analyzing the mean variability of MEP amplitude in control trials for the same 7 subjects in Set 1, t(6) = 1.545, p = .173, d = .123, and Set 2, t(6) = 1.366, p = .221, d = .261 (Figure 15b).

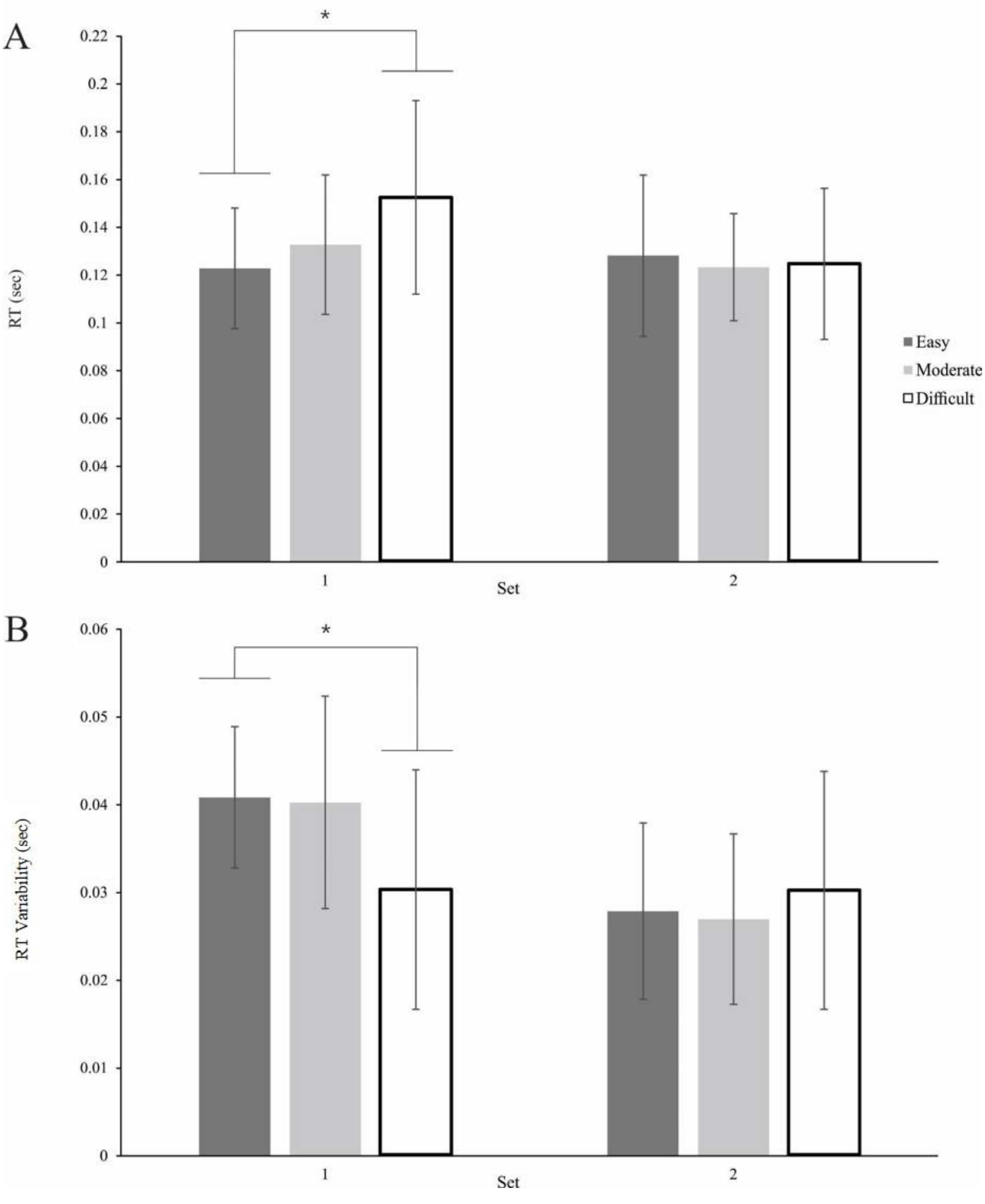

Figure 14. Premotor RT data from the three target accuracy conditions for Set 1 and Set 2. a) Mean control premotor RT (sec) of the subset of subjects (N=7) that exhibited an increase in RT from the easy to the difficult condition for both sets; b) Mean variability of control premotor RT (sec) of the same subset of subjects (N=7) for both sets. Error bars represent standard deviation, and * indicates the presence of a significant difference (p < .05).

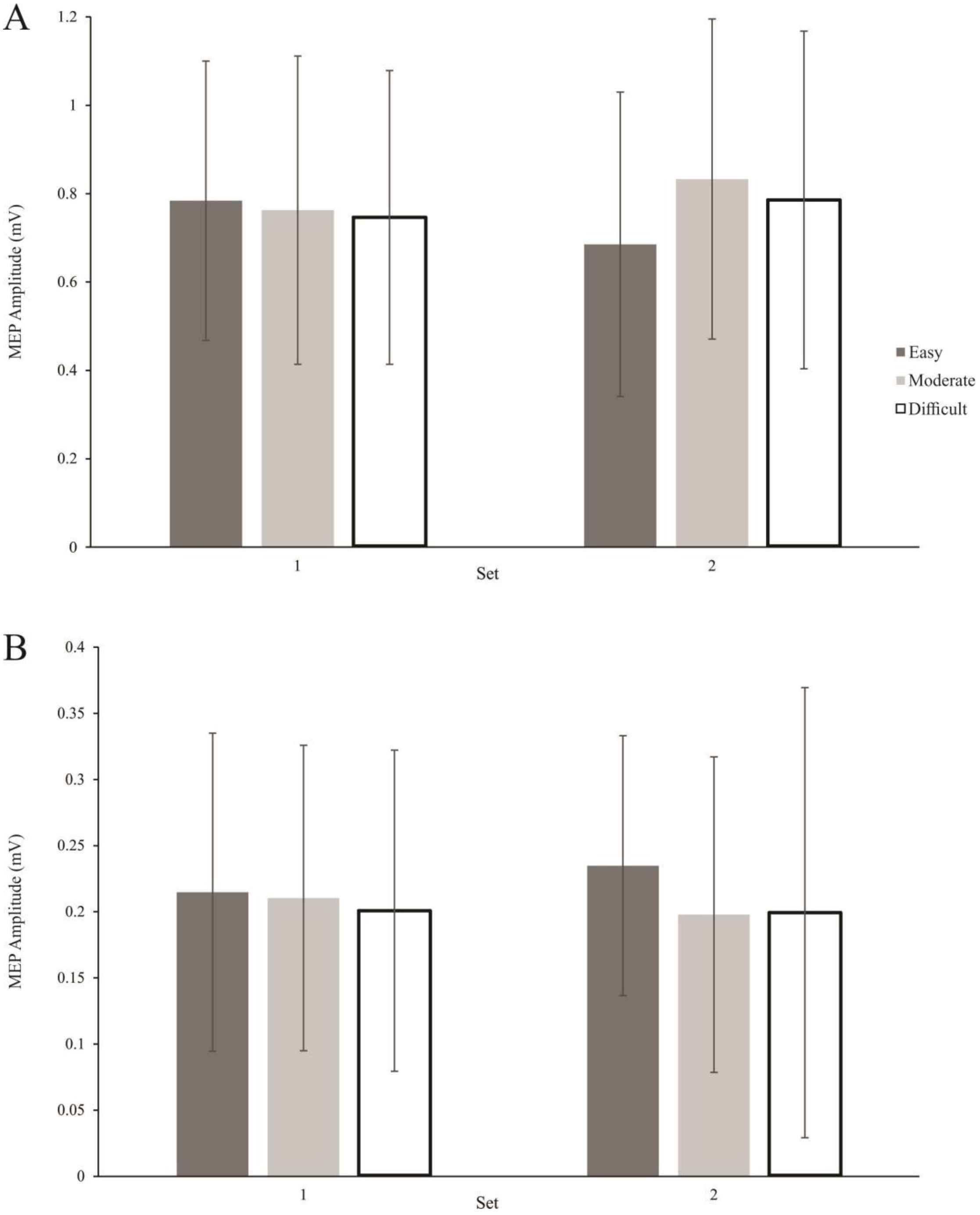

Figure 15. MEP data from the three target accuracy conditions for Set 1 and Set 2. a) Mean control MEP amplitude (mV) of the subset of subjects (N=7) that exhibited an increase in RT from the easy to the difficult condition for both sets; b) Mean variability (standard deviation) of MEP amplitude (mV) of the same subset of subjects (N=7) for both sets. Error bars represent standard deviation, and * indicates the presence of a significant difference ($p < .05$).

3.7. Movement Time. Mean control trial MT data (Figure 16a) were log-transformed to account for violations of the assumption of normality; mean log-transformed MT was normally distributed for all difficulties across both time points, as assessed by Shapiro-Wilk's test ($p > .05$ for all values). Data were then analyzed using a 2 (set; Set 1, Set 2) x 3 (difficulty; easy, moderate, difficult) repeated measures ANOVA. Mauchly's test indicated that the assumption of sphericity had been violated in the case of the set by difficulty interaction ($\chi^2(2) = 7.128$, $p = .028$), and degrees of freedom were therefore corrected for this factor using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .578$). No significant main effect of set, $F(1, 14) = 3.347$, $p = .089$, $\eta_p^2 = .193$, or difficulty, $F(2, 28) = .308$, $p = .737$, $\eta_p^2 = .022$, were detected, and there was no significant interaction between the factors, $F(1.406, 19.690) = 2.500$, $p = .121$, $\eta_p^2 = .152$.

3.8. Peak Displacement. Mean control trial peak displacement (Figure 16b) was not normally distributed as assessed by Shapiro-Wilk's test; non-parametric analyses were therefore performed. Separate Friedman's tests were performed on data from Sets 1 and 2 in order to identify differences in mean control RT variability as a result of task difficulty, and separate Wilcoxon signed-rank tests were performed between difficulty-matched blocks in order to detect any potential effect of set.

In Set 1, there was no significant difference in the mean rank of control peak displacement between difficulties, $\chi^2(2) = 1.200$, $p = .549$, nor was there a significant difference in mean rank in Set 2, $\chi^2(2) = .533$, $p = .766$. For the easy task, a Wilcoxon signed-rank test comparing control peak displacement between Sets 1 and 2 showed no significant difference in Set 1 compared to Set 2, $Z = -.170$, $p = .865$. Similarly, mean peak displacement in the moderate task was not significantly different between Sets 1 and 2, $Z = -.852$, $p = .394$. Finally, for the difficult task no significant difference in mean peak displacement was seen, $Z = .000$, $p = 1.000$.

3.8.1. Peak Displacement Variability. Mean control trial peak displacement variability (Figure 16c) was not normally distributed as assessed by Shapiro-Wilk's test; non-parametric analyses were therefore performed. Separate Friedman's tests were performed on data from Sets 1 and 2 in order to identify differences in mean control RT variability as a result of task difficulty, and separate Wilcoxon signed-rank tests were performed between difficulty-matched blocks in order to detect any potential effect of set.

In Set 1, no significant difference in the mean rank of control peak displacement variability was seen, $\chi^2(2) = 2.800$, $p = .247$, nor was there a significant difference in mean rank in Set 2, $\chi^2(2) = 1.733$, $p = .420$. The comparison of Set 1 and Set 2 by Wilcoxon signed-rank tests revealed no significant differences in mean peak displacement in the easy task, $Z = -1.306$, $p = .191$, or in the moderate task, $Z = -.114$, $p = .910$. However, for the difficult task a significant difference in mean peak displacement variability was seen, $Z = -2.045$, $p = .041$.

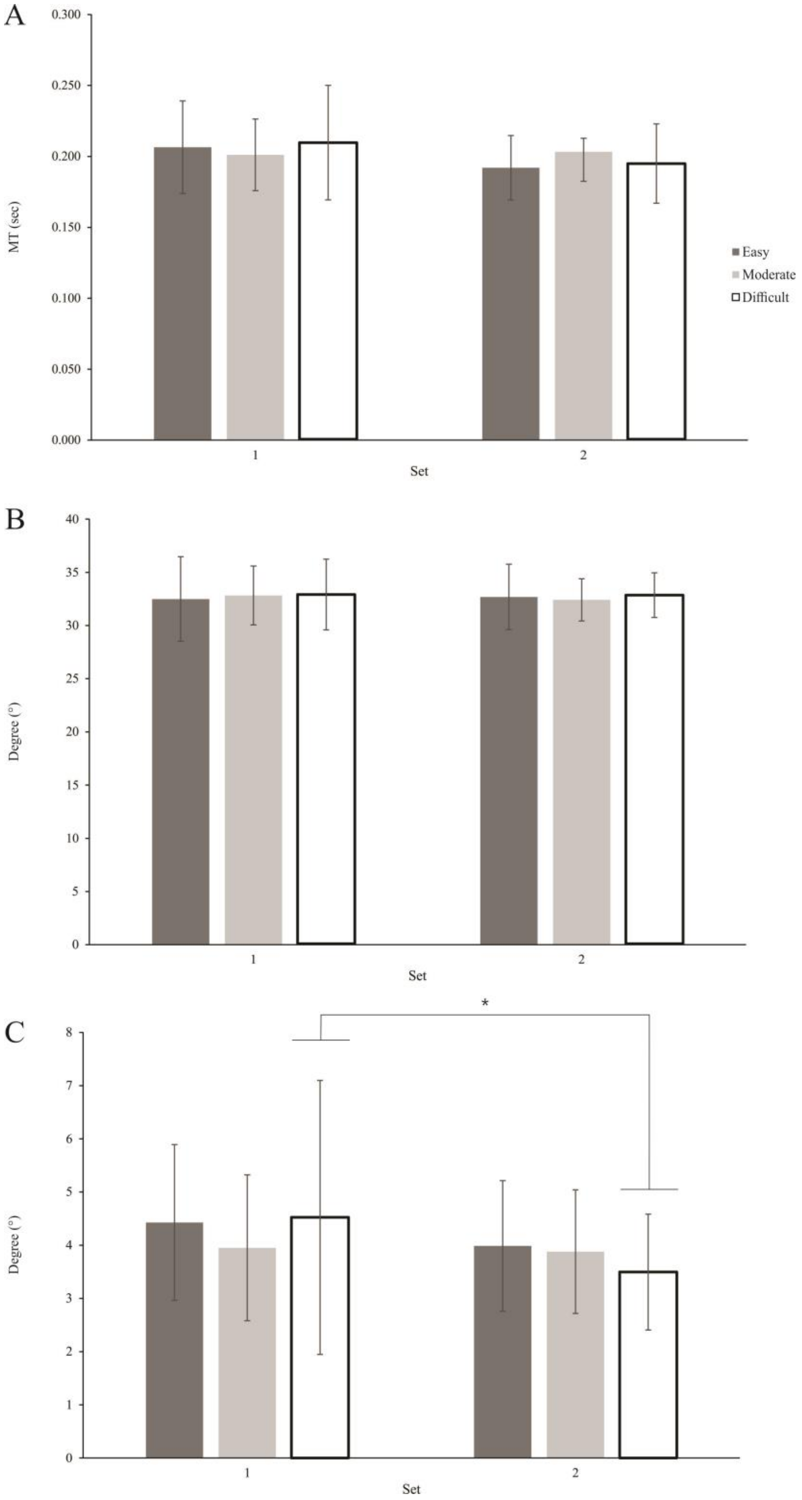

Figure 16. Kinematic variables observed in the three target accuracy conditions for Set 1 and Set 2. a) Mean control motor RT (sec), b) mean control peak displacement (°), and c) mean control variability of peak displacement (°) for all three difficulty conditions in both sets (N = 15). Error bars represent standard deviation, and * indicates the presence of a significant difference ($p < .05$).

3.9. Accuracy. The percentage of responses that resulted in the target turning green (i.e. correct response) was determined based on the mean control final position of the movement. For the easy task condition, the rate of correct responses was 94.2 ± 4.5% in Set 1 and 96.7 ± 3.5% in Set 2. For the moderate task condition, the rate of correct responses fell to 58.5 ± 17.5% in Set 1 and 60.7 ± 10.4% in Set 2. Finally, the rate of correct responses in the difficult condition was 8.3 ± 4.3% and 10.8 ± 4.8% in Sets 1 and 2, respectively.

The mean absolute error was also analyzed in order to assess movement accuracy, with lower mean absolute error indicating higher accuracy (i.e. smaller absolute value of the difference between the performed movement and the centre of the target; Figure 17).

Mean absolute error was not normally distributed as assessed by Shapiro-Wilk’s test. As a result, separate non-parametric Friedman’s tests were performed on data from Sets 1 and 2 in order to identify differences in mean absolute error as a result of task difficulty, and separate Wilcoxon signed-rank tests were performed between difficulty-matched blocks in order to detect any potential effect of set.

In Set 1, analyses revealed a significant difference between the different difficulty conditions, $\chi^2(2) = 12.133$, $p = .002$. Post-hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a significance level set at $p < .017$; mean absolute distance to the centre of the target was significantly smaller in the moderate task compared to the easy task, Z = -2.726, p = .006. No significant differences were seen when comparing between the easy and difficult tasks, Z = -2.215, p = .0.27, or between the moderate and difficult ones, Z = -1.136, p = .256. In Set 2, there was no statistically significant difference in the absolute distance to the target centre between the different difficulty conditions, $\chi^2(2) = 2.133$, $p = .344$. When comparing between sets, a Wilcoxon signed-rank test comparing the mean absolute distance to the target centre between Sets 1 and 2 for the easy task showed a significant difference between sets, Z = -2.442, p = .015. No significant difference in mean absolute distance in the moderate task between Sets 1 and 2 was seen, Z = .000, p = 1.000, or in the difficult task, Z = -1.874, p = .061.

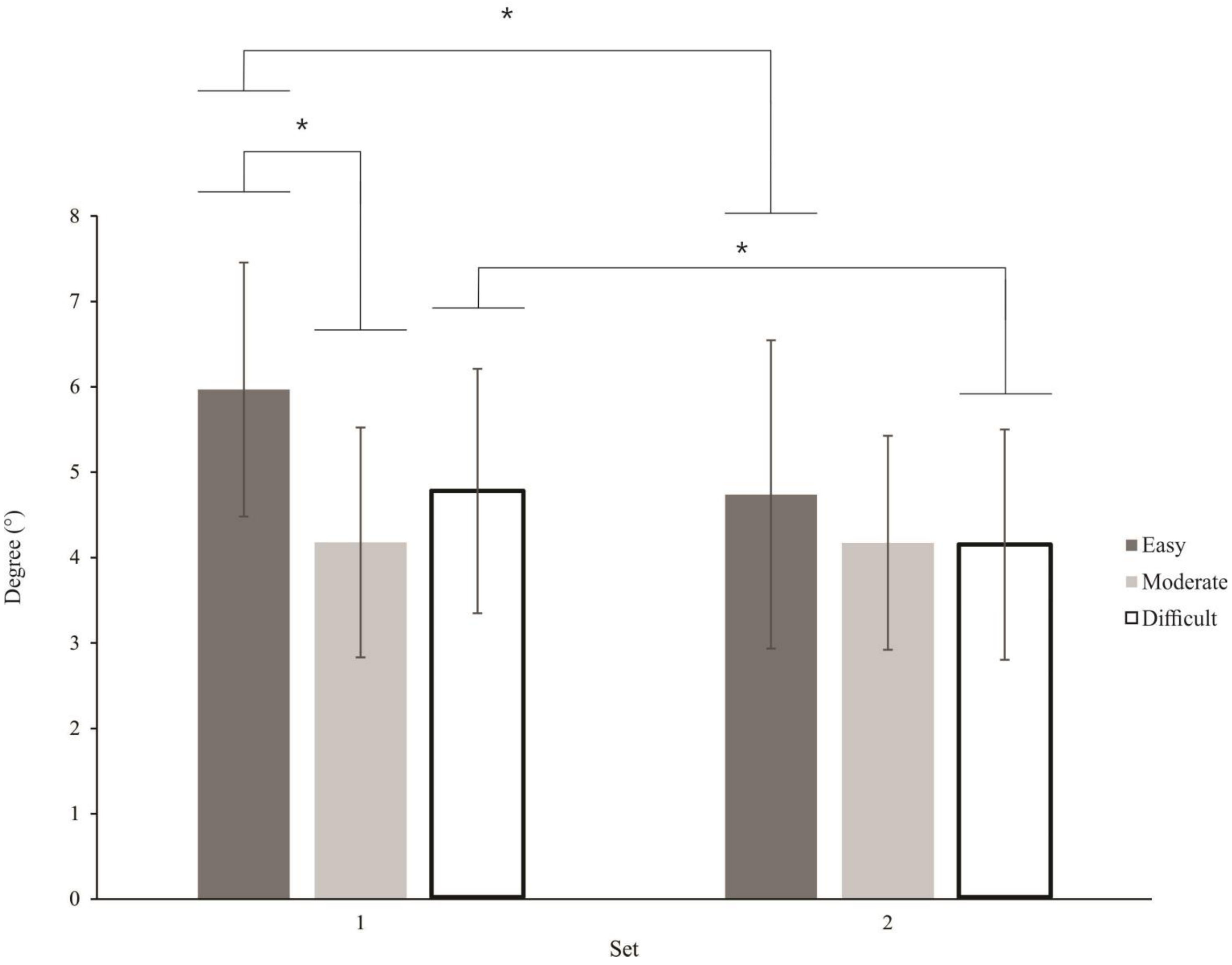

Figure 17. Mean absolute error of peak displacement (°) in control trials with respect to the 32° target centre for all three difficulty conditions in both sets (N = 15). Error bars represent standard deviation, and * indicates the presence of a significant difference (p < .05).

4. Discussion

The purpose of the present experiment was to study the effects of varying accuracy requirements on the level and variability of neural activation during movement preparation using both TMS and RT measures to infer neural activation during different conditions. Based on the predictions made by Carlsen and colleagues (2012) regarding the characteristics of their neural accumulator model, it was first hypothesized that MEP amplitude would decrease as accuracy demands increased, indicating a decrease in neuromotor preparatory levels, and that the variability of MEP amplitude would increase concurrently with accuracy requirements. Secondly, it was hypothesized that the proportion of SAS trials where there was involuntary movement triggering (i.e., presence of the StartReact effect) would decrease as accuracy demands increased, potentially due to lower levels of preparation. Finally, it was hypothesized that both RT and RT variability would increase with increasing accuracy demands. These hypotheses were predicated on the assumption that the task used in this experiment would result in RT differences between accuracy conditions, as has been shown previously in similar tasks (Castellote & Valls-Solé, 2015; Lajoie & Franks, 1997). However, premotor RT in the different difficulty conditions was not significantly affected by the varying accuracy requirements used in this particular task, nor was there a significant effect of accuracy requirements on the variability of RT. When analyzing a subset of subjects that *did* show an increase in RT with increasing accuracy requirements, no significant effect of difficulty on MEP amplitude was detected, nor was there a significant effect on variability of MEP amplitude. The methodological factors that may account for the absence of RT differences between accuracy conditions, and the limited conclusions that can be drawn from the subset analysis, will be discussed in the following sections.

4.1. Reaction Time. Contrary to previous studies, the results of this experiment showed no significant differences in RT between the different accuracy conditions when all 15 participants were considered (Figure 8). There are several possible features of the methodology used in this experiment that may explain why no significant RT slowing was seen as a result of increases in accuracy demands.

4.1.1. Order effect. The order in which the various blocks were presented within each set was first considered as a possible explanation for the observed lack of RT differences between difficulty conditions. The order in which participants completed the three difficulty conditions was counterbalanced to mitigate any possible order effect. Overall, 6 participants completed the easy block first, 5 completed the moderate block first, and 4 completed the difficult block first. However, it is reasonable that by exposing some participants to the easiest task first, they were afforded more practice with the movement in a less demanding environment and thereby were able to perform the more challenging task with reduced RTs compared to those whose first exposure to the task involved the smallest target (i.e. most challenging task). Similarly, participants who practiced with the hard task first may have continued to prepare a similar movement when moving on to the easier tasks later. Nonetheless, results showed no significant difference in mean premotor RT when comparing mean RT in the first block between subjects that completed the easy, moderate, or difficult condition first (see Figure 10). Therefore, although the order in which participants completed the three accuracy conditions may have been a contributing factor, it is unlikely that an order effect was the principle cause of the lack of RT slowing observed with increasing accuracy.

It should also be noted that when the difference in RT between the difficult and easy conditions ($\Delta RT_{\text{Difficult-Easy}}$) was examined on a subject-by-subject basis, there was no apparent relationship between the condition that was completed first and the value of $\Delta RT_{\text{Difficult-Easy}}$ (see Appendix C). In fact, only three of the six subjects that completed the easy block first had longer RTs in the difficult condition (positive $\Delta RT_{\text{Difficult-Easy}}$), while the other three had longer RTs in the easy condition (negative $\Delta RT_{\text{Difficult-Easy}}$). Similarly, half of those that completed the difficult condition first exhibited a positive $\Delta RT_{\text{Difficult-Easy}}$ while the other half exhibited a negative difference. Although the number of subjects in each post hoc grouping is too small to draw any definitive conclusions from this pattern, it would nevertheless appear that the effect of difficulty condition order is inconsistent and unlikely to account for the consequent lack of significant effect of difficulty on RT seen in this experiment.

4.1.2. Easy target size. Another possible explanation for the lack of significant difference in RT between difficulty conditions is that the size of the target used in the easy condition was too large, potentially altering subjects' movement strategies as a result. During the experiment, the centre of the target was located 32° away from the home position. Participants were instructed to aim for the centre of each target in an attempt to ensure similar movements were prepared in each condition; however, if the movement ended within the target, participants were given positive feedback (i.e. target turning green). In the easy condition, the target measured 32° wide, meaning that subjects had a large margin of error where they would still receive positive feedback. During the initial design of the experiment, it was reasoned that this might allow subjects to prioritize RT to a greater degree, thereby resulting in faster RT and greater movement variability for this condition. However, it is also possible that the extreme ease with which subjects could complete the task successfully instead altered the strategy that was used to successfully complete the movement during the experiment. The easy task could be completed successfully by performing only a 16° wrist extension movement. In theory, participants may have begun to decrease the amplitude of their movements over time (either consciously or unconsciously) as they became more familiar with the parameters of this task condition. Aiming to the inner edge of the target and producing a smaller movement would likely be less costly than aiming to the centre of the target, while still enabling successful trial completion. This change in movement strategy may have affected the overall level of preparatory activity being achieved during the easy task, and this decreased movement preparation may have in turn resulted in slower RTs in the easy condition, and thereby resulted in a lack of significant RT differences between difficulty conditions.

The use of an alternate movement strategy and subsequent potential decrease in movement preparatory level is partially supported by the startle proportion data, with the proportion of SAS trials with SCM activity trending towards a significant decrease from Set 1 to Set 2 for the easy condition only ($p = .106$); no significant differences were detected between Sets 1 and 2 for the other two accuracy conditions. Although not conclusive, this result hints at a decrease in preparation during the easy task that is not seen in the other conditions, which may be the result of a change in the movement strategy as subjects gained more experience with the task. It should be noted here that this trend is not mirrored in the MEP data, with no significant difference in MEP amplitudes between difficulty conditions being observed; however, MEPs are notably variable within subjects and therefore may not be the most sensitive measure of motor preparation as a result (see section *3.2.2 Variability of MEP amplitude* below for further discussion). Furthermore, mean absolute error was significantly higher in the easy condition than in the moderate condition in Set 1, and the difference between absolute error in the easy and difficult conditions was trending towards significance ($p = .027$ with a corrected level of significance set at $p < .017$). These results indicate a less accurate movement in the easy condition and suggest that participants may not have been aiming to the centre of the target to the same degree as in the harder conditions. However, it should be noted that there was a significant decrease in mean absolute error in the easy condition between Sets 1 and 2, which would indicate that subjects were in fact landing closer to the target as they were able to practice with the task, instead of adapting their movement to land closer to the outer bound of the target in the easy condition. Additionally, mean peak displacement in the easy condition for both sets was in fact extremely close to the 32° movement that subjects were instructed to produce ($M_{Set1} = 32.5 \pm 4.0°$; $M_{Set2} = 32.7 \pm 3.1°$). There was also no significant difference in mean peak displacement between difficulties during either set, further suggesting that participants were not in fact altering their movement strategies. Overall, participants did seem to produce similar movements on average between all three difficulty conditions once they became more familiar with the task condition, rather than neglecting accuracy demands in the easy condition as a result of the large acceptable margin of error for successful task completion.

4.1.3. Difficult target size. On the other hand, it is also possible that the target in the difficult task was too small, which may have had an adverse effect on participants' motivation during the experiment. The difficult target measured only 0.5° wide and had an ID of 7, and as a result participants were extremely unlikely to hit the target. Since there was no negative penalty for missing the target, it was originally believed that this would result in longer RTs as participants attempted to be more accurate. However, it is possible that the frequency with which the negative feedback (target turning red) was displayed during the difficult condition may have resulted in reprioritization of speed over accuracy, since the percentage of trials where the target was successfully was very low regardless of how much of an effort was made to hit the target (8.3 ± 4.3% in Set 1, 10.8 ± 4.8% in Set 2). As mentioned above, the mean absolute error in the difficult task was not significantly different from the moderate or from the easy conditions (although the difference between the easy and difficult targets was trending toward significance), indicating that participants were similarly accurate during all of the condition despite the increased accuracy needed to hit the target in the difficult one. Although this explanation seems plausible, it is not fully supported by the results. In the case of the difficult task, the proportion of SAS trials where a startle was elicited did increase from Set 1 to Set 2 (Figure 7), indicating that movement preparation may have increased as subjects became more familiar with the task conditions and potentially put more emphasis on RT and less emphasis on accuracy requirements; however, this difference did not reach significance (p = .250). Therefore, it cannot definitively be concluded that participants were neglecting the accuracy constraints of the task in order to react quickly to the IS.

4.1.4. Attentional explanation. Another explanation for the lack of significant difference in RT between accuracy conditions may be that, rather than a different movement strategy being used, participants' level of focus during the easy condition may have decreased. Given the large target size and low level of difficulty (ID = 1), the ease with which participants were able to complete the easy task may have resulted in them losing the focus required to fully prepare the movement. However, there were also no significant differences seen in mean control premotor RT between the moderate and difficult conditions; if a lack of focus was the cause of decreased preparation in the easy task, one would expect that this would be remedied by using an intermediate target size. Although levels of movement preparation in the moderate condition are relatively stable between sets (as suggested by similar rates SCM activation in SAS trials for this difficulty), no RT differences between the moderate and difficult condition were evident despite presumably maintaining focus throughout the condition. This in turn suggests that a lack of focus in the easy condition may not explain why difficulty had no significant effect on RT, since no significant differences exist in RT between the moderate and difficult conditions. That being said, the target used in the moderate task measured only 4° wide, which may in turn be too small and therefore too similar to the difficult target (0.5°) to detect any significant effect of difficulty on RT. While a decrease in the level of focus may fit the pattern of results described above, it is impossible to conclude definitively that this was the case, as focus was not explicitly measured/monitored during this experiment.

4.1.5. Position Feedback. Finally, the lack of significant RT differences between accuracy conditions may be attributable to the provision of position feedback throughout the entire trial. Participants' hand position with relation to the target was displayed in real time on-screen during each trial. Although they were instructed to complete a ballistic wrist extension movement following the IS to ensure preprogramming of the entire movement, having access to this online position feedback during the movement may have lessened the degree to which participants prepared the movement in its entirety, instead using the provided online feedback to successfully home in on and hit the target.

Traditionally, movements that are completed with close to maximal speed are considered to be preprogrammed, while those that are performed at slower rates are thought to involve at least some degree of online control (Rosenbaum, Hindorff, & Munro, 1986; Klapp, 1975). Although MT at maximal movement speed was not measured (as a way of ensuring that movement preprogramming was fully occurring), MTs reported for this experiment (Figure 16a) were longer than those found in other experiments that used the same movement task but that did not provide online feedback (Smith et al., under review). No significant differences were seen in MT between the easy and difficult conditions for either set; the lack of differences between the two task conditions may hint at similar degrees of online movement control, irrespective of the target size. Furthermore, increasing the complexity of a task has been shown to have a commensurate effect on RT only in instances where movements do not involve online control (van Donkelaar & Franks, 1991b; Klapp, 1975; Fitts & Petersen, 1963). Therefore, the use of some degree of online movement control using the provided position feedback may partially explain the lack of RT differences by preventing the complete preprogramming of the movement.

Future studies could examine this possibility by removing the provision of continuous position feedback in favour of information regarding the success of the trial only (i.e. target turning either green or red). By removing online position feedback, participants may be encouraged to prepare the full movement in advance, which in turn may potentially lead to differences in RT becoming evident.

4.2 TMS. This experiment used single-pulse TMS delivered at the go-signal in order to compare motor preparation levels between difficulty levels. To do this, MEPs were recorded and the amplitude of these MEPs was compared between conditions as well as between sets. The MEP amplitude analysis was completed in order to test the hypothesis that changes in RT resulting from changing accuracy might be explained by differing levels of preparation in the motor cortex at the time of IS presentation. However, given that there were no differences in mean premotor RT between difficulty conditions when the full complement of subjects was considered, no relationship between MEP amplitude and RT differences can be drawn.

No significant differences were detected in mean MEP amplitude between difficulty conditions. The first possible explanation for this result is that movements requiring greater accuracy can still be prepared to the same degree as those with lower accuracy demands;

however, since not all participants exhibited increasing RT with increasing accuracy demands, it is not possible to state this conclusively. Instead, it is more likely that the methodological factors identified above (e.g., target size, position feedback provided; see section 3.1 Reaction Time), which likely resulted in the lack of RT differences between conditions, could have in turn influenced the level of preparation in the different difficulty conditions such that no differences in MEP amplitude were apparent in this experiment. As such, a new experiment involving modified methods will be required in order to determine whether differences in neural excitability levels truly exist when varying degrees of movement accuracy are required.

4.2.1. Subset analysis. Given that approximately 50% of the subjects did show an increase in mean premotor RT with increasing task difficulty, a separate analysis was completed on these 7 subjects. By selecting only the subjects that showed the desired effect, the results of this analysis are no longer generalizable to the population as a whole; however, completing this analysis allows the neural characteristics of the effect in question to still be studied, albeit with a markedly reduced sample size.

Within the subset of 7 subjects, RT in the difficult condition was on average 29.2 ms slower than in the easy condition. It should be noted that this difference in RT was very variable, with a range of 0 – 111 ms difference between the two conditions (Appendix B). It appears that even within the group of subjects that did have slower RT in the difficult condition compared to the easy one, the effect of varying accuracy demands is still not uniform. Even with this large degree of variability in mind, premotor RT in the easy condition was significantly faster than that in the difficult condition for these 7 subjects. MEP amplitude for the same subjects was compared between the easy and difficult targets, and no significant difference between conditions was found. Although far from conclusive due to the small sample size, this would suggest that the movements in the easy and difficult conditions were prepared to similar degrees despite significant RT slowing as a result of increased accuracy demands. As well, this same comparison of MEP amplitude between difficulty conditions was made in subjects that showed a difference in RT between moderate and difficult as well as moderate and easy task conditions, and similar results were found; despite significant differences in RT, no differences in MEP amplitude were apparent (Appendix D).

In this experiment, it was hypothesized that the level of neural activation, which in this case was used as a measure of motor preparation, would not rise as high during the preparatory foreperiod in the difficult condition due to the possible constraining effect of increased neural noise in this condition (see Figure 1 and Literature review section 4.3). The lack of difference in MEP amplitude despite RT differences between tasks with varying accuracy demands may suggest that some *other* feature of the neural accumulator model not yet considered may be responsible for the differences in RT that are observed in other experiments. It is possible that neural activation levels may reach similar levels during the preparatory foreperiod, and that RT differences are instead the product of a smaller rate of increase in initiation-related activation following the go-signal in the condition with higher accuracy demands (see Figure 18a). The shallower slope of activation in the more difficult condition would result in activation levels reaching threshold at a later time point compared to the easier condition, which would theoretically yield slower RTs despite similar levels of preparation at the time of IS presentation. This explanation is in agreement with the original neural accumulation model of saccadic movements put forth by Hanes and Schall (1996). Alternatively, it is possible that preparatory levels are similar for both high and low accuracy tasks, but that in the task with higher accuracy requirements the relative initiation threshold for the movement was also higher (Figure 18b).

This would result in overall later initiation of the movement despite similar levels of preparation at the time of IS presentation. Although it is unclear from the results of this experiment which alternate model may explain the RT differences between accuracy conditions seen in other studies, the results of the subset analysis described above do suggest that preparatory levels at the time of IS presentation did not vary significantly; therefore, another explanation may be necessary.

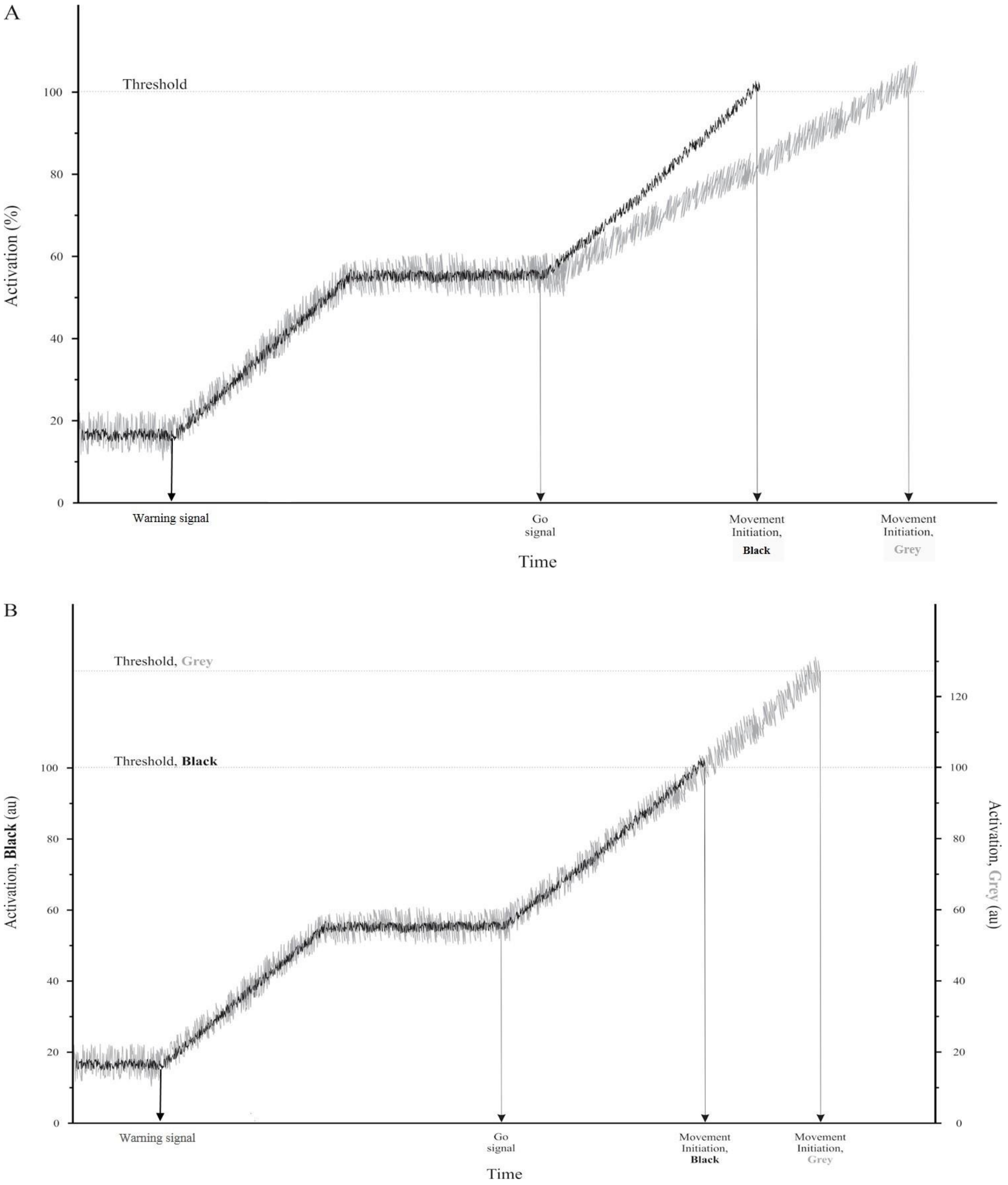

Figure 18. Representation of a neural accumulation model illustrating alternate explanations for the differences in reaction time resulting from varying movement complexity. A) Preparatory phase activation levels reach a similar plateau level, but the rate of increase of activation is slower for the more difficult task (**grey**) compared to the easier one (**black**). B) Activation is similar for both movements, but initiation threshold is higher in the more difficult condition (**grey**) compared to the easier one (**black**). Note that in Panel B, activation is represented in arbitrary units (au) and not %, as this explanation posits that magnitude of the movement initiation threshold for the more difficult movement is higher than in the easy condition.

4.2.2. MEP variability. In this experiment, there was no significant effect of difficulty condition or of set on recorded MEP amplitude. No significant differences were seen when data from all 15 participants were included, or in the subset of subjects that exhibited an RT increase from the easy to the difficult conditions (N = 7). Although the lack of significant effect of difficulty condition on MEP amplitude may indicate that neural activation levels in M1 were not different when accuracy demands were low compared to when they were high, it is also possible that the lack of differences in MEP amplitude between difficulties was simply the result of high intra-individual variability in MEP amplitude. While inter-individual variability is accounted for by the nature of the repeated measures analysis used, the variability of MEP amplitude between trials within the same subject may still be preventing the detection of any true differences in neural activation in the different difficulty conditions.

The high intra-individual variability of MEPs as elicited by TMS delivered to M1 is well documented in previous studies. When excitability in M1 is higher, the delivery of a single TMS pulse causes a greater number of neurons to reach firing threshold, and consequently results in the recruitment of more motor units (van der Kamp et al., 1996; Rothwell et al., 1991). When a larger number of motor units are activated, MEP amplitude increases as a result. However, rapid, spontaneous fluctuation of excitability levels in the corticospinal tract as well as within the motor neurons themselves results in MEP amplitude variability (Kiers, Cros, Chiappa, & Fang, 1993). Increased intensity of the TMS pulse has been shown to reduce this variability (van der Kamp et al., 1996); in this experiment, the TMS intensity used was less than half that of the maximum stimulator output (M = 45.1 ± 8.7% MSO). Although future studies may consider the use of a higher intensity in order to reduce intra-individual variability in order to better detect any effect of task accuracy requirements on MEP amplitude, the use of increased TMS intensity also has a commensurate effect on RT (e.g. Ziemann et al., 1997), possibly leading to confounds in the results.

Another possible factor affecting the variability of MEP amplitude may be the number of trials that was used in each condition. Although 40 MEPs were collected for each difficulty condition, these were collected in two different sets, with the difficulty condition changing every 20 trials. It is possible that increasing the number of MEPs collected in each sub-block may result in decreased intra-individual variability. However, it should be noted that previous research has shown little benefit to increasing the number of MEPs recorded beyond ~30 trials/condition; after this point, no additional improvement in within-session reliability was seen (Goldsworthy, Hordacre & Ridding, 2016). Therefore, there may be limited benefit of increasing the number of TMS trials in each sub-block beyond the current 20 trials.

4.3. Practice effect. Although the manipulation of difficulty in this experiment did not have a significant effect on many of the variables of interest, multiple outcome measures exhibited significant changes from Set 1 to Set 2. Within Set 1, participants completed 45 trials of each condition, with the order of conditions assigned to each participant at random. Within each block, 20 trials involved delivery of single-pulse TMS and another 6 involved the presentation of a SAS. Another 5 were familiarization trials, and the remaining 14 were simply control trials. Despite the seemingly small amount of exposure that participants had to each target (85 trials/target over the course of the experiment; see Appendix B), on average they were still able improve their performance across a variety of outcome measures.

Mean premotor RT decreased in control trials from Set 1 to Set 2, indicating that participants were responding to the IS more quickly following practice irrespective of the size of the target. This was not the case for premotor RT in SAS trials, which is in line with previous work showing that the RT of movements involuntarily triggered through startle cannot be significantly reduced any further, possibly due to a neurophysiological floor effect (Smith & Carlsen, 2017). The variability of control premotor RT also decreased from Set 1 to Set 2. Based on the predictions made by the neural accumulator model discussed earlier, it is possible that the overall amount of stochastic noise in the neural network associated with the movement decreased with repeated practice, since decreased noise in the signal was hypothesized to lead to less variable RTs (see Figure 2). Although this is not supported by the analyses of MEP amplitude and MEP amplitude variability between sets (there was no main effect of Set for either RT or RT variability), the possibility that elevated levels of within-subject variability may have masked certain significant differences has already been discussed above. Therefore, the absence of an increase in MEP amplitude (which would have suggested higher levels of preparation) or a decrease in MEP amplitude variability (which would have suggested more consistent levels of preparation) does not necessarily preclude the possibility that activation levels were indeed becoming less variable (i.e. less noisy) and that movement preparation was potentially increasing as a result.

Additionally, there is a significant decrease in the variability of peak displacement from Set 1 to Set 2 in the difficult condition; however, this decrease is not seen in the easy or moderate conditions. This decrease in variability indicates that participants became more consistent with their movements as they practiced the task, but only when the accuracy demands of the task were high. Although this does not provide an indication of whether or not participants were becoming more accurate with practice overall, this may serve as further evidence that they were in fact attempting to constrain the amplitude of their movements in order to move to the target more accurately despite the extreme difficulty of the task (ID = 7). Since this significant decrease in the variability of peak displacement was not seen in the easy or moderate tasks, it would appear that participants may not have been as concerned with accuracy in these conditions. This pattern of results suggests that participants were prioritizing accuracy to a different degree when demands were higher, as was originally intended during the design of the experiment. Despite this however, there did not appear to be a significant trade-off between accuracy and RT, as evidenced by the lack of significant effect of difficulty condition on RT.

Moreover, there was a significant decrease in the mean absolute error of peak displacement with respect to the 32° target centre from Set 1 to Set 2 for both the easy and difficult conditions. This increasingly accurate performance in the difficult condition seems to mirror the results discussed above with respect to the mean variability of peak displacement; participants were not only becoming more consistent with their movements, but were also becoming more successful at aiming to the target (despite only landing in the target 8-10% of the time). This further supports the idea that subjects are prioritizing accuracy in the difficult condition. However, the mean absolute error of peak displacement also significantly decreased from between Sets 1 and 2 in the easy condition, despite a lack of commensurate decrease in variability in this condition. This may result from the exceedingly large target size used in the easy condition; in Set 1, participants may have had a harder time aiming for the centre of the largest target compared to in the smaller target, as it may have been harder to estimate its location. Following exposure to the other targets, participants may have gained a better sense of the location of the centre of the target, resulting in more accurate performance in the easy condition as a result. This is supported by the significant difference in accuracy that was detected within Set 1 between the easy and moderate tasks, where movements in the easy task were on average less accurate compared to the moderate one. In Set 2, this difference disappeared as the mean absolute error in the easy condition decreased significantly. As such, it is possible that by practicing the movement under conditions where the centre of the target was more readily estimated (i.e. the moderate and difficult conditions), movement performance in the easy condition became more accurate in Set 2 without becoming less variable.

5. General Discussion & Conclusion

In the present experiment, no differences in premotor RT were apparent when data from all subjects were considered. When only data from the subset of subjects that showed positive RT differences between the difficult and easy conditions was included, the effect of difficulty on RT was significant. Although the analysis of only the subjects that demonstrated the desired effect may on the surface appear to simply be confirmation bias (i.e. “cherry-picking” the data), it is important to consider that the purpose of this experiment was to investigate the neural underpinnings of movement preparation specifically when varying accuracy demands led to RT differences. If the phenomenon in question does not occur in a certain sample, then the conclusions that are drawn from that sample do not necessarily reflect the neural mechanisms that underlie said phenomenon, and the subjects in this sample should therefore be excluded for this specific purpose, as was done here. This subset analysis is somewhat analogous to what is a commonly accepted practice when studying the StartReact effect; when no consistent startle reflex is detected for a particular subject (as determined by the proportion of SAS trials that exhibit SCM activity), data from that subject is excluded as it cannot reliably be confirmed that the StartReact effect did in fact occur when the SAS was presented (Carlsen, Maslovat, & Franks, 2012). With this type of analysis, it is important to acknowledge that the generalizability of the findings to the population as a whole is severely compromised. Only 7/15 subjects in this experiment showed a positive $\Delta RT_{Difficult\text{-}Easy}$, likely do to methodological issues; the low number of subjects in this experiment that demonstrated the phenomenon of interest is a limitation of this study that future studies may address by resolving some of the methodological issues that have previously been outlined.

The results of this study differ from those seen in the literature, where increases in RT as accuracy demands increase have been demonstrated (Castellote & Valls-Solé, 2015; Lajoie & Franks, 1997). A few key differences should be highlighted in the tasks used in these two experiments, as compared to the task used in this experiment, which may partially explain the discrepant results in terms of RT differences. The task used by both Castellote and Valls-Solé (2015) and Lajoie and Franks (1997) involved a reach-and-point to a target that was located directly in front of the participant. It is possible that this task, which is more prevalent in everyday life compared to the targeted wrist extension task used in the present experiment, felt more natural and therefore may be more representative of a real-world phenomenon.

Additionally, the movement amplitude itself may have contributed to the differences seen in the various experiments. In the study by Lajoie and Franks (1997), movement amplitude averaged ~80 mm. In the present study, the average movement amplitude was measured in degrees and is therefore difficult to estimate without subjects' hand measurements. However, it is likely that movement amplitudes greater than 80 mm were produced, especially in the easy task where a larger margin of error was provided to overshoot the target centre. It has previously been shown that the relationship between RT and target size (i.e. accuracy requirement in this experiment) is affected by the length of the movement being produced, with a smaller effect of target size on RT for longer movements (Klapp, 1975). The lack of RT differences between difficulty conditions in this experiment, despite previous demonstrations of this effect, may therefore be the result of discrepant movement amplitudes between experiments. It should be noted however that the recent study by Castellote and Valls-Solé (2015) does not report movement amplitudes, and it is therefore difficult to further substantiate this explanation.

Another possible confounding effect of increased movement amplitude may lie in the types of movement control that are being used. It has previously been suggested that, when movement complexity increases without any commensurate increase in RT, this may indicate that some form of online control is occurring since theoretically no additional preparation is occurring in the more complex task (Rosenbaum, Hindorff, & Munro, 1986). Although this is a very simplistic explanation of the complex neural processes that are occurring during movement preparation and initiation, this interpretation may suggest that in the context of the present experiment, an increase in online control may be responsible for the lack of RT differences with changes in accuracy requirements. If online movement control was being used to home in on the target, it is possible that little to no effect of task difficulty would be seen. This is also supported by the movement time results for this experiment, which are somewhat longer than those that have been previously seen in wrist extension movements with no accuracy requirement; this may hint at increased online control (Smith et al., under review).

However, there are two caveats to considering increased online control as a possible explanation for the discrepancies in the relationship between accuracy requirements and RT that must be considered. Firstly, the relationship between RT and movement complexity has primarily been investigated in tasks where complexity was manipulated by increasing the number of movement elements as opposed to the accuracy of a single movement element (Henry & Rogers, 1960); as a result, this relationship may not be generalizable to the task in the present experiment. Secondly, it should be noted that comparing movement duration alone is a rather rudimentary way of establishing the mode of control used during the movement; it is generally agreed upon that examining the average acceleration traces is a better measure of whether participants are relying on online control to complete their movements (van Donkelaar & Franks, 1991a). It is therefore important to acknowledge these two factors when considering this explanation.

Overall, the conclusions that can be drawn from the results of this experiment are minimal given that no RT differences were seen between the different difficulty conditions when examining the entire sample of participants. While the lack of significant effect of difficulty condition on MEP amplitude within the subset of subjects that did show a positive $\Delta RT_{Difficult-Easy}$ may hint at similar levels of movement preparation despite increased accuracy requirements, it is also possible that this may simply be the result of methodological issues that future studies will need to address. To that end, the current experiment may serve as a pilot study on which to build off of for further investigations into the neural accumulator model and its implications for movement preparation and initiation.

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Appendix A: Pilot Data

	Mean Reaction Time (sec)		
Subject #	**Easy**	**Moderate**	**Difficult**
1	0.114	0.129	0.142
2	0.136	0.142	0.173
3	0.128	0.153	0.165
4	0.146	0.175	0.175

Appendix B: Trial Type Breakdown

Set 1				**Set 2**		
	Practice	TMS Sub-block	SAS Sub-block		TMS Sub-block	SAS Sub-block
Block 1	5	20	20	**Block 4**	20	20
Block 2	5	20	20	**Block 5**	20	20
Block 3	5	20	20	**Block 6**	20	20

Appendix C: Breakdown of the differences in premotor RT for all subjects

Subject #	**Difficulty of first block**	**ΔRT$_{\text{Difficult-Easy}}$ (ms)**	**ΔRT$_{\text{Moderate-Easy}}$ (ms)**	**ΔRT$_{\text{Difficult-Moderate}}$ (ms)**
1	Easy	25.64	20.84	4.80
2	Easy	6.43	-9.94	16.38
3	Moderate	-21.85	-10.20	-11.65
4	Moderate	-17.04	-18.84	1.79
5	Difficult	23.33	-18.28	41.61
6	Difficult	-17.54	16.83	-34.36
7	Easy	-7.69	-2.92	-4.77
8	Easy	-0.081	41.66	-42.46
9	Moderate	8.13	8.98	-0.85
10	Moderate	-32.56	5.95	-38.51
11	Difficult	111.87	31.80	80.08
12	Difficult	-12.18	-5.92	-6.26
13	Easy	-14.15	-14.11	-0.03
14	Easy	-22.48	-11.23	-11.25
15	Moderate	29.94	-5.52	35.46

*Note: ΔRT represents the difference in premotor RT between the indicated conditions in Set 1 only.

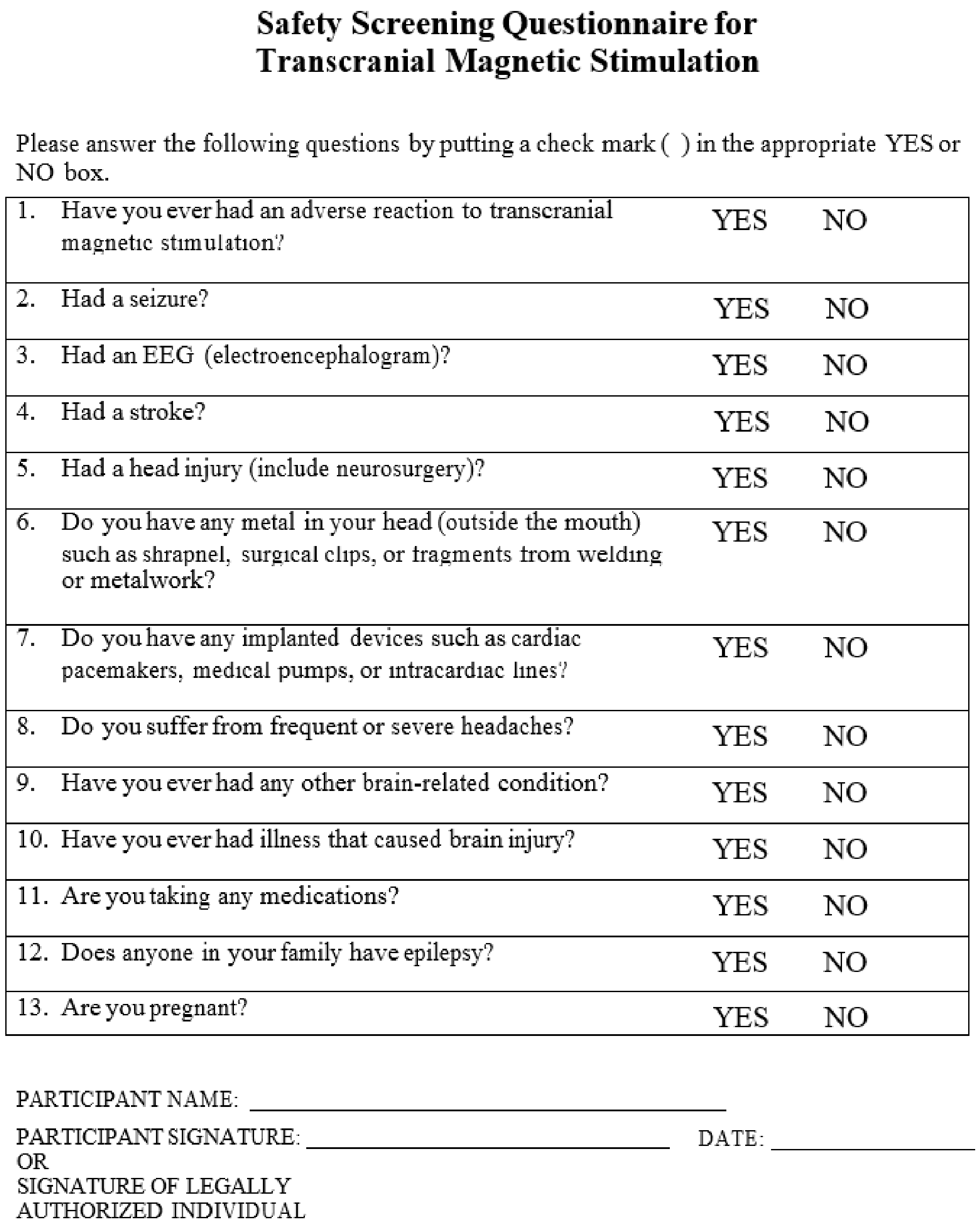
Appendix D:

Breakdown of the differences in MEP amplitude for all subjects

Subject #	**ΔMEP$_{\text{Difficult-Easy}}$ (mV)**	**ΔMEP$_{\text{Moderate-Easy}}$ (mV)**	**ΔMEP$_{\text{Difficult-Moderate}}$ (mV)**
1	-0.007	-0.130	0.123
2	0.106	-0.064	0.170
3	-0.028	0.008	-0.036
4	0.019	0.393	-0.374
5	0.119	-0.121	0.239
6	0.421	0.195	0.227
7	0.118	0.446	-0.327
8	-0.274	0.053	-0.327
9	-0.321	-0.124	-0.198
10	0.054	-0.201	0.255
11	-0.002	-0.020	0.017
12	0.026	0.187	-0.162
13	-0.056	0.194	-0.250
14	0.111	0.115	-0.003
15	0.098	0.255	-0.157

*Note: ΔMEP represents the difference in MEP amplitude between the indicated conditions in Set 1 only.

Appendix E: TMS Safety Questionnaire



Safety Screening Questionnaire for Transcranial Magnetic Stimulation

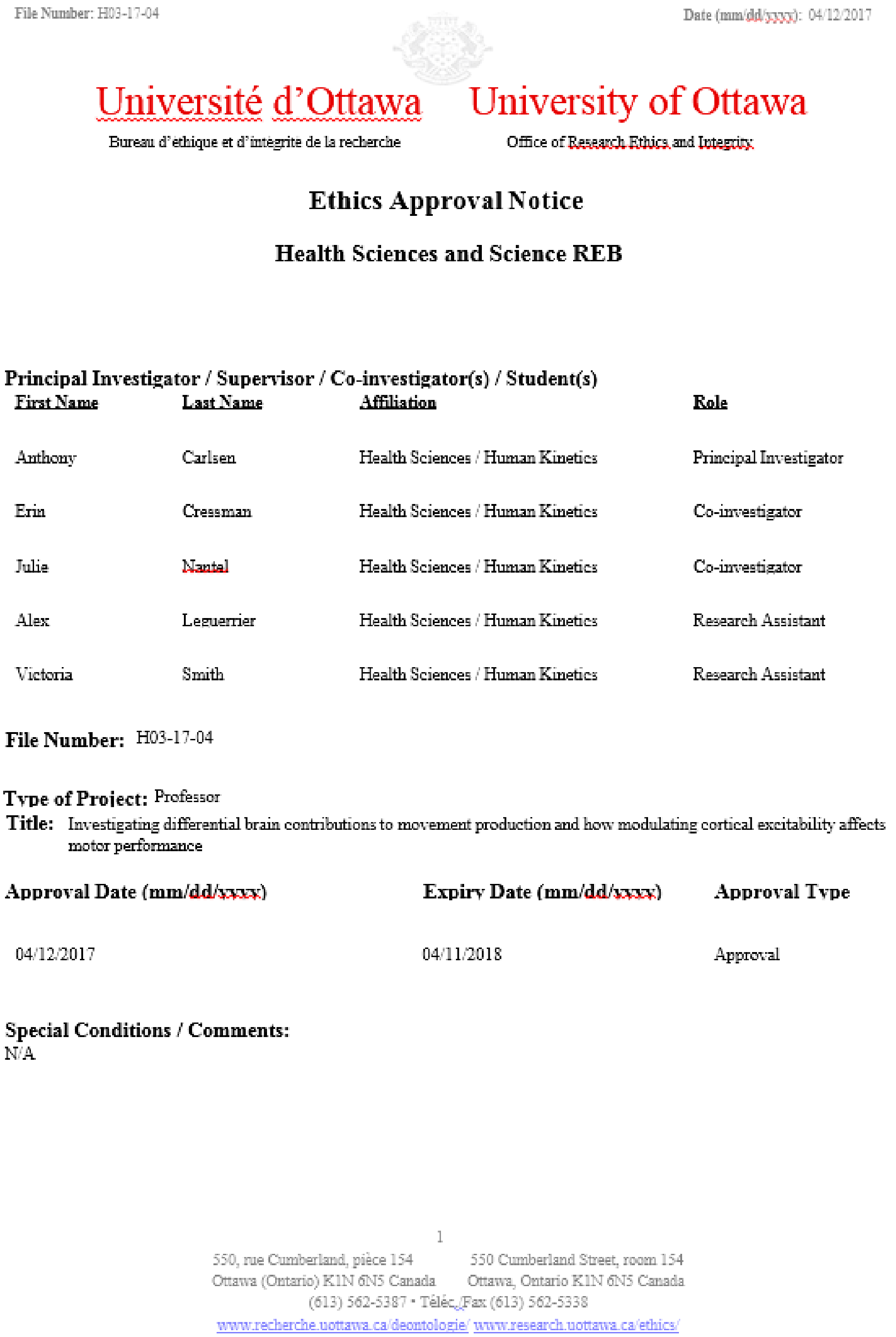
Please answer the following questions by putting a check mark ( ) in the appropriate YES or NO box.

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
11. Are you taking any medications?	YES	NO
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 F: Ethics Certificate



File Number: H03-17-04

Date (mm/dd/yyyy): 04/12/2017

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	Cressman	Health Sciences / Human Kinetics	Co-investigator
Julie	Nantel	Health Sciences / Human Kinetics	Co-investigator
Alex	Leguerrier	Health Sciences / Human Kinetics	Research Assistant
Victoria	Smith	Health Sciences / Human Kinetics	Research Assistant

File Number: H03-17-04

Type of Project: Professor

Title: Investigating differential brain contributions to movement production and how modulating cortical excitability affects motor performance

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
04/12/2017	04/11/2018	Approval

Special Conditions / Comments:
N/A

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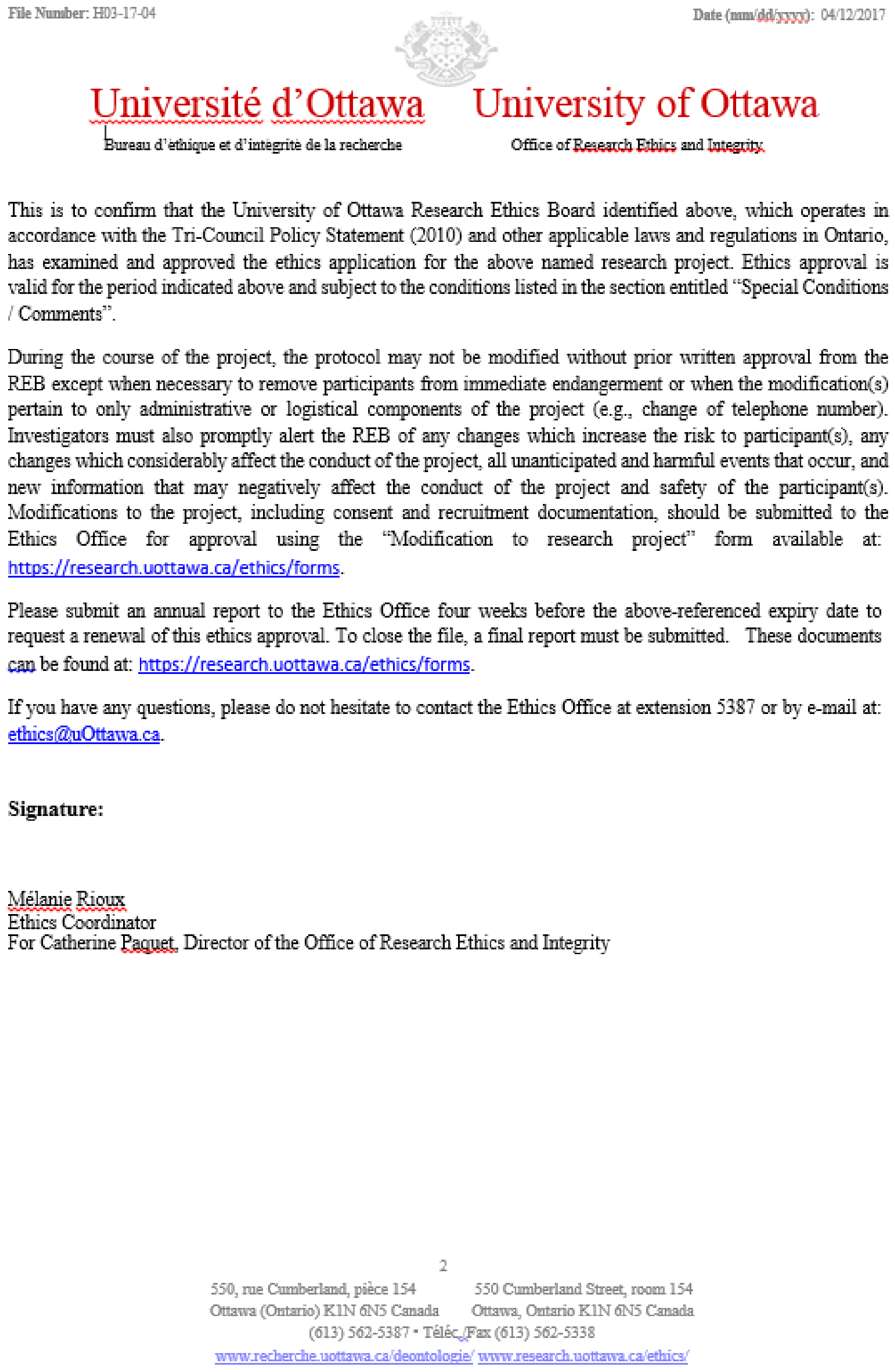

File Number: H03-17-04

Date (mm/dd/yyyy): 04/12/2017

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University of Ottawa
Office of Research Ethics and Integrity

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement (2010) and other applicable laws and regulations in Ontario, has examined and approved the ethics application for the above named research project. Ethics approval is valid for the period indicated above and subject to the conditions listed in the section entitled “Special Conditions / Comments”.

During the course of the project, the protocol may not be modified without prior written approval from the REB except when necessary to remove participants from immediate endangerment or when the modification(s) pertain to only administrative or logistical components of the project (e.g., change of telephone number). Investigators must also promptly alert the REB of any changes which increase the risk to participant(s), any changes which considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project and safety of the participant(s). Modifications to the project, including consent and recruitment documentation, should be submitted to the Ethics Office for approval using the “Modification to research project” form available at: https://research.uottawa.ca/ethics/forms.

Please submit an annual report to the Ethics Office four weeks before the above-referenced expiry date to request a renewal of this ethics approval. To close the file, a final report must be submitted. These documents can be found at: https://research.uottawa.ca/ethics/forms.

If you have any questions, please do not hesitate to contact the Ethics Office at extension 5387 or by e-mail at: ethics@uOttawa.ca.

Signature:

Mélanie Rioux
Ethics Coordinator
For Catherine Paquet, Director of the Office of Research Ethics and Integrity

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