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Corticospinal excitability is reduced in a simple reaction time task requiring complex timing

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Abstract

Increasing the complexity of a movement has been shown to result in longer simple reaction time (RT), which has been attributed to sequencing or timing requirements following the go-signal. However, RT differences may also be due to differences in corticospinal excitability (CE) as previous studies have found an enhanced excitatory state of corticospinal neurons in complex tasks. Transcranial magnetic stimulation (TMS) was used in the present study to probe the excitability of the motor pathway during the simple RT interval for single (simple) versus multiple (complex) key press responses. Premotor RT data indicated that participants responded significantly ($p < .001$) faster in the simple task compared to the complex task, confirming response complexity was manipulated appropriately. Analysis of the CE data indicated that motor evoked potential (MEP) amplitudes increased with time following the go-signal in both conditions and that MEP amplitudes in the simple task were significantly larger than those in the complex task when evoked within 75 ms of movement onset ($p = .009$). These findings suggest that the rate of increase for initiation-related neural activation is reduced for complex as compared to simple movements, which may partially explain differences in RT.

Keywords: Neural activation, Response complexity, Transcranial magnetic stimulation

1. Introduction

The effects of response complexity on the time taken to react to a stimulus has a long history of research, with the common finding that an increase in number of response elements leads to a longer reaction time (RT). Many explanations have been offered for this response complexity effect such as an increased amount of time required to program and retrieve the response from memory (Henry and Rogers, 1960), or an increase in the number of processes occurring after the presentation of the imperative go-signal, such as sequencing or timing (Klapp, 1995; Maslovat et al., 2014). More recent studies have suggested that complexity dependent RT differences may instead relate to levels of neural activation. Models of neural activation have suggested that motor preparation can be envisioned as increasing the activation state of neural networks to a level that is held below the threshold for initiation (Hanes and Schall, 1996; Wickens et al., 1994). Reaction time is thus indicative of the time required to increase neural activation from this preparatory state to a level beyond threshold. Thus differences in RT can be attributed to different rates of activation accumulation (Carpenter and Williams, 1995; Hanes and Schall, 1996), differences in threshold levels (Nazir and Jacobs, 1991), or

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a hybrid of the two (Pacut, 1977). In terms of RT differences due to movement complexity, response initiation may be delayed due to either reduced rate of increase in activation, or a greater amount of required activation. Furthermore, an increased activation requirement could be due to either a higher initiation threshold (Maslovat et al., 2011), or activation beginning from a lower state (or level) of preparatory activation at the time of the imperative signal (Carlsen et al., 2012; Maslovat et al., 2014).

Although response complexity effects have been considered within a neural activation context, few studies have directly assessed cortical activation associated with various movement complexities, and results have been mixed. For example, Kitamura and colleagues (1993) found no activation differences during simple and complex sequential finger movements using electroencephalography, while Shibasaki and colleagues (1993) showed differences in motor cortical cerebral blood blow between simple and complex sequential finger movements through the use of positron emission tomography. The effect of response complexity on corticospinal excitability (CE) has also been examined using transcranial magnetic stimulation (TMS) during static as well as continuous motor tasks, although not in the context of a RT paradigm. TMS applied over primary motor cortex can evoke a short latency excitatory response in a targeted muscle (motor evoked potential, MEP), and activates corticospinal neurons both directly and indirectly through cortico-cortical synapses (Taylor, 2006). Flament and colleagues (1993) compared CE levels between a simple static finger abduction and a variety of more complex static gripping tasks and showed that MEPs were larger in the complex tasks compared to those in the simple finger abduction task. Similarly, Abbruzzese and colleagues (1996) found increased MEP amplitudes for more complex movements during both the production of, or mental simulation of continuous repetitive and sequential finger movements (see also Roosink and Zijdewind, 2010).

The effects of response complexity on CE in a RT paradigm were recently examined by Greenhouse et al. (2015), who used TMS to assess transient motor inhibition during the response preparation phase of a movement in both a simple and choice RT paradigm. The authors varied the complexity of these movements by increasing either the number of muscles or number of elements involved in performing a movement. When the number of muscles involved increased, there was no corresponding increase in RT; however, CE was suppressed for the more complex response. Conversely when the number of movement elements was increased, both RT and CE increased for the more complex movement. While this provides evidence that motor system excitability during movement preparation is sensitive to response complexity, it is unclear why an increase in CE would be associated with longer RT for the sequenced movements. This result appears to be in contrast to the predictions of neural activation models, which predict longer RTs for complex movements related to lower levels of activation with respect to an initiation threshold.

The evidence above suggests that response complexity can affect neural activation levels; yet the relationship between CE and response latency as a function of complexity of the required movement is still unclear. Therefore, the purpose of the current study was to investigate how response complexity affects CE in a simple RT paradigm. Participants performed simple RT tasks requiring either a single key press or a three key press sequence with a non-isochronous timing structure, as this has shown to be a robust method to manipulate response complexity and thus increase simple RT (Maslovat et al., 2014). TMS was used to probe CE in 25 ms intervals between 0 and 125 ms following the go-signal (i.e., during the RT interval) in order to quantify changes in the time course of excitability during the response initiation phase, rather than assessing excitability during the preparation phase (e.g., Greenhouse et al., 2015). It was expected that the more complex movement would result in longer simple RTs, and that CE would increase prior to the onset of both simple and complex movements. However, of greater interest was a comparison between activation curves for the two movements between the go-signal and response onset. Based on neural activation models (Carlsen et al.,

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2012; Maslovat et al., 2014), it was hypothesized that if the MEP amplitude was lower at presentation of the imperative stimulus (IS) the longer RTs observed for more complex movements could be attributed to a lower overall preparatory level. In contrast, if preparatory MEPs were not different between tasks, longer RTs observed in a more complex task may be attributed to either differences in activation onset latencies or accumulation rates - evidenced by either a later increase from baseline MEP or activation increase occurring at slower rate following the IS.

2. Results

2.1 Voluntary response measures

In order to determine if the complexity manipulation led to differences in RT and/or EMG characteristics, response output measures for the simple and complex movements were compared at the baseline time point (i.e., TMS stimulation at the IS - see 4.7 for details). Analysis of mean premotor RT (Figure 1A) confirmed that RT in the complex task was significantly longer ($T(15) = 4.24$, $p < .001$, $r = .74$) than in the simple task, similar to what has been shown previously (Maslovat et al., 2014). Analysis of peak EMG between simple and complex conditions (Figure 1B) revealed that peak EMG was significantly greater ($T(15) = 3.25$, $p = .005$, $r = .64$) in the simple compared to the complex movement. Similarly, the simple movement had a significantly larger ($T(15) = 3.06$, $p = .008$, $r = .62$) integrated EMG over the entire burst (iEMG) as compared to the more complex movement (Figure 1C). Finally, analysis of integrated muscle activation in the rising phase (first 30 ms) of the EMG burst (Q30) also revealed that the early rate of increase in the simple movement was significantly greater ($T(15) = 5.57$, $p < .001$, $r = .82$) than that of the complex movement (Figure 1D).

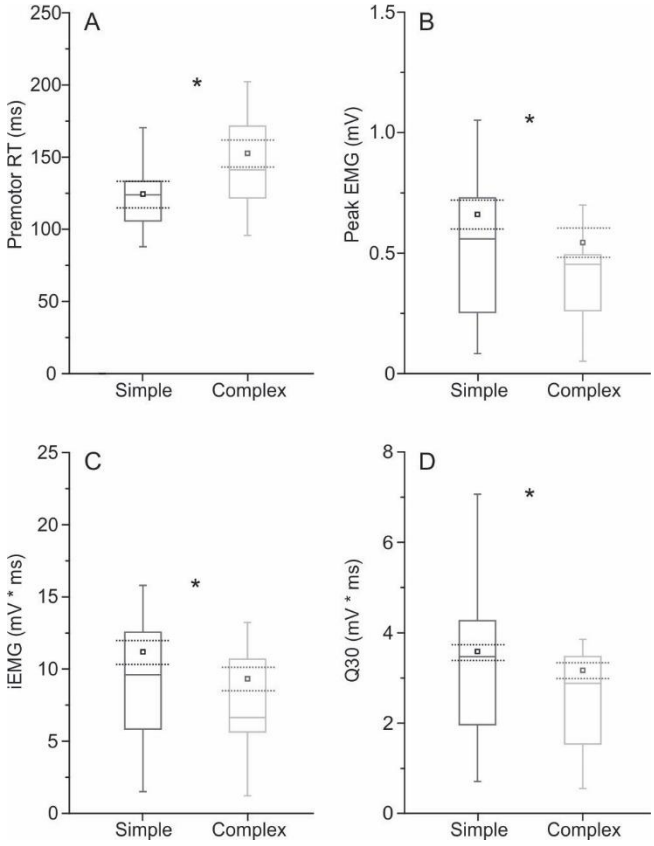

Figure 1. *Boxplots of voluntary response measures for the simple and the complex tasks. Box boundaries represent between-participant first and third quartiles, solid horizontal lines represent medians, the small square inside the box plots represents mean, and error bars represent the farthest outliers within 1.5 times the inter-quartile range from the box boundaries. Horizontal dashed lines represent within-participant 95% confidence intervals from the mean. A) Premotor reaction time (RT); B) Peak value of rectified and filtered EMG from first voluntary agonist burst; C) Integrated EMG (iEMG) from full duration of raw rectified first voluntary agonist EMG burst; D) Integrated EMG from the first 30 ms of raw rectified voluntary activity in first agonist burst. Asterisks (*) denote a significant difference between the simple and complex movements.*

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2.2 MEP measures

Representative individual EMG traces from a single participant at each analyzed stimulation time point (0 – 75 ms, see 4.7), for both simple and complex movement tasks are shown in Figure 2. Mean MEP amplitudes for the stimulation time points 0 to 75 ms following the IS are shown in Figure 3. Although there was no significant main effect for task ($F(1,15) = 0.890$, $p = .361$, $\eta_p^2 = .056$), there was a significant main effect for time ($F(3,45) = 20.346$, $p < .001$, $\eta_p^2 = .576$) indicating that MEP amplitudes increased along with time following the go-signal. Post-hoc tests analyzing differences in MEP amplitude between baseline (0 ms) and subsequent time points (collapsed across movement type) indicated that there were no significant differences between the 0 ms and 25 ms time point ($p = .319$), but MEP amplitude increased significantly compared to all previous time points for the 50 ms and 75 ms TMS stimulation times (all t ratios > 3.36, all corrected p values < .026).

There was no significant Task x Time interaction for MEP amplitude with respect to the IS, although the result approached conventional levels of significance ($F(3,45) = 2.519$, $p = .070$, $\eta_p^2 = .144$). Thus in order to directly test the hypothesis that CE would be lower for the complex task at the time of the go-signal, peak-to-peak MEP amplitudes measured at baseline (IS onset, 0 ms) were analyzed separately. No difference was observed ($T(15) = 0.09$, $p = .927$, $r = .02$) in MEP amplitude between the simple (M = 0.389 mV, SD = 0.27) and the complex (M = 0.388 mV, SD = 0.27) movements (see Figure 3, time 0).

Normalized MEP amplitudes in time bins prior to EMG onset are shown in Figure 4. Analysis at each time bin confirmed a significant difference between the simple and the complex condition only at 75 ms prior to EMG onset ($U = 8553$, $z = -2.62$, $p = .009$, $r = -.154$) with all other p values > .35.

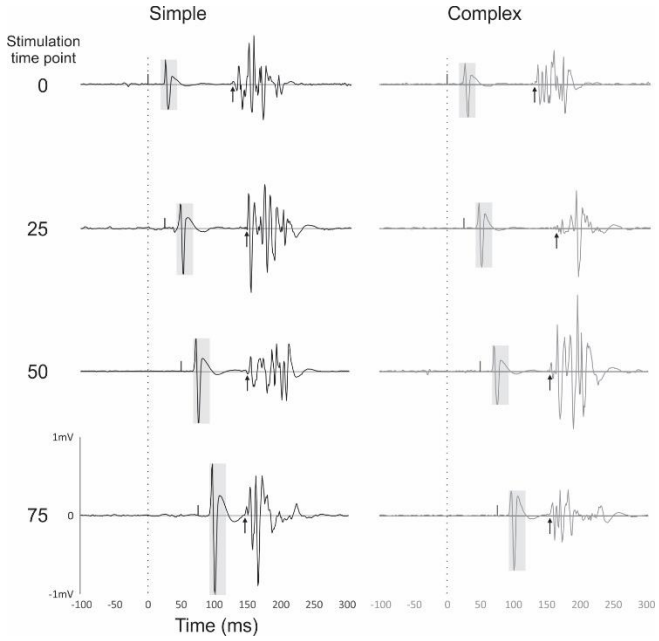

Figure 2. *Representative EMG traces for the time period of -100 ms to +300 ms with respect to the go-signal (0 ms). The first 300 ms of individual trials are shown from a single subject in both the simple (black) and complex (grey) tasks, for TMS stimulation time points 0, 25, 50 and 75ms following the imperative go-signal. Go-signal is shown as dashed line, and TMS pulse as short vertical bar in each trace. Motor evoked potential (MEP) measurement windows are highlighted with a grey area for each trial, and time of voluntary EMG onset (premotor RT) is indicated in each trial with a black arrow.*

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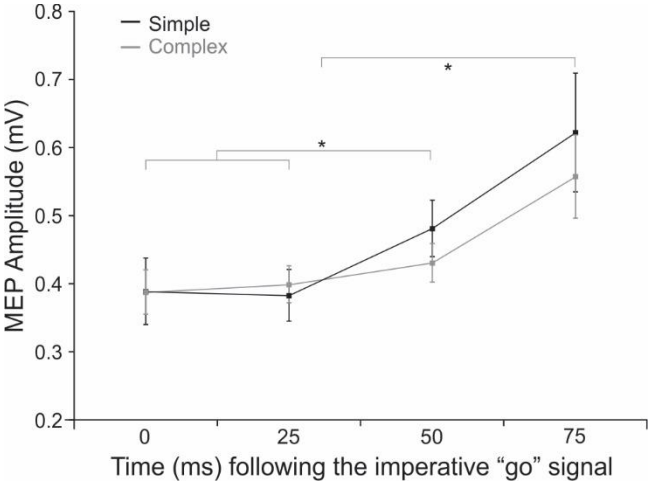

Figure 3. *Mean (± within participant 95% CI) MEP amplitudes at TMS delivery times following the imperative go-signal for both the simple (black) and the complex (grey) tasks. Note that at time zero the means are very similar, and the 95% confidence intervals fully overlap. Asterisks (*) denote a significant difference between presentation times (collapsed across task).*

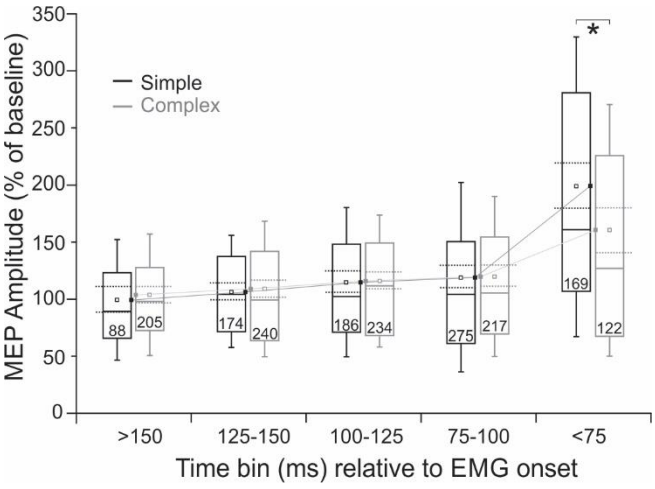

Figure 4. *Boxplot showing MEP amplitudes for both the simple (black) and the complex (grey) tasks as a percentage of baseline and grouped into time bins relative to EMG onset (numbers in each box denote how many observations contribute to each distribution). Box boundaries represent inter-quartile range, horizontal lines show medians, and small squares show means. Error bars represent one standard deviation from the mean, and horizontal dashed lines represent 95% confidence intervals from the mean. An asterisk (*) denotes a significant difference in MEP amplitude between the tasks at 75 ms prior to EMG onset. Thin lines are shown connecting mean values for each condition (Note: connected means, shown as filled squares, are offset from center of boxes for visual alignment).*

3. **Discussion**

The purpose of this study was to examine the relationship between simple RT and the excitability of the motor pathways prior to initiation of movements, as a function of differing levels of complexity. Previous work has shown that RT for a complex task is typically longer than that for a simple task, a result that has been attributed to additional programming (Henry and Rogers, 1960), sequencing requirements (Klapp, 1995), or timing preparation (Maslovat et al., 2014). The results of the current study show that task complexity was successfully manipulated within a simple RT paradigm (confirmed by a significantly longer RT following the more complex task, Figure 1A), allowing a novel comparison of CE between the two movement tasks during the RT interval. Even though there were no differences in the initial preparatory activation state between the two movement tasks (i.e., at the IS), neural activation levels increased in both tasks following the go-signal (i.e., during the RT interval). Although the change in CE with time following the IS was not significantly different between the tasks (Figure 3), a lower level of excitability was apparent for the complex task when the data were examined with respect to EMG onset (Figure 4).

Although a lower level of neural activation for a more complex movement may initially seem counter-intuitive, these results are consistent with neural activation models that suggest that RT differences may be in part related to cortical activation level prior to response output - although these models had yet to be tested for movements involving different complexities. Within the context of a neural activation framework, several possible explanations may account for the observed delayed response initiation that accompanies more complex movements. For example, a longer RT could be the result of a lower level of preparatory activation (Carlsen et al., 2012; Maslovat et al., 2014), a higher initiation threshold (Maslovat et al., 2011; Nazir and Jacobs, 1991), a decreased rate of activation accumulation (Carpenter and Williams, 1995; Hanes and Schall, 1996), or some combination of these.

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In the current study, mean raw MEP amplitude data indicated that CE measured at the IS was not different between the two movements ($p = .927$), signifying that initial cortico-spinal preparatory activation level was not affected by differing response complexity. This contrasts with previous studies that have shown that CE is increased for movements of greater complexity (Abbruzzese et al., 1996; Flament et al., 1993), although these were not conducted within the context of a RT framework. Those studies showing an increase in CE with response complexity have used tasks which required a greater degree of motor unit recruitment such as simple finger abduction versus gripping (Flament et al., 1993). As such, during more complex ongoing static tasks, increased CE may simply be related to the number of muscles and/or motor units involved in the action. The current study manipulated complexity in a qualitatively different way by requiring additional movement elements from the same effector. Because the preparatory CE was not different between levels of complexity, this suggests that the preparatory CE may be primarily determined by the task requirements of the first element in a movement sequence.

Similarly, examining the normalized MEP data time-locked to EMG onset (Figure 4) indicated that threshold levels were not higher for the complex movement, as CE was lower in the complex task as compared to the simple task just prior to response output. This is in contrast to a recent study that provided evidence that increasing the number of movement elements led to a marginally reliable increase in MEP amplitudes ($p = .06$) (Greenhouse et al., 2015). However, important differences in methods between the two studies may have contributed to this discrepancy. First, the study by Greenhouse et al. (2015) examined CE during the RT foreperiod (i.e. preparatory phase), whereas in the current study the time course of CE was examined during the RT interval (i.e. initiation phase). Secondly, the sequenced movement used by Greenhouse et al. (2015) required the use of multiple effectors with no specific timing requirement. Conversely, in the current study increased complexity was achieved by requiring multiple button presses of a single key with an imposed timing structure, using the same effector.

While the present data indicate that the change in CE following the go-signal was not different between the tasks (Figure 3), it is prudent to note that a marginal interaction effect ($p = .070$) was found between the tasks and TMS delivery points. This interaction hints at a slower rate of increase in CE for the complex compared to the simple movement following the IS (Figure 3). Although speculative, the data in Figure 3 suggest that CE may have begun to increase above baseline at a later time point for the complex movement, although the relatively large 25 ms time bins make it difficult to identify the precise point of increase from baseline and loss of short RTs for the later TMS stimulation points may contribute to this finding. On the other hand, because RTs were approximately 20 ms longer in the complex condition, the rise in CE observed in Figure 4 would have been delayed on average by ~20 ms. While a delayed increase in neural activation for the complex movement may partially contribute to the longer RT value, when data were aligned to EMG onset, it is clear that the amount of excitability increase is significantly lower ($p = .009$) for the complex movement in the final time bin (< 75 ms) preceding movement initiation (Figure 4), suggesting a possible lower rate of increase in excitability for more complex movements.

Although the current data provide novel evidence that the lengthened RT for a complex movement is accompanied by a reduction in CE, these results cannot in isolation identify the site(s) responsible for a change in MEP amplitude, which has previously been shown to depend on the excitability of both cortical and spinal neurons (Taylor, 2006). It is also worth noting that the reduction in CE observed leading up to voluntary response onset in the complex task was also reflected in the voluntary response outcome measures. Specifically, while a lowered level of activation for the sequenced movement during the RT interval was associated with longer RT (Figure 1A), the reduced CE also led to a less forceful response for the complex task, as evidenced by reduced peak EMG amplitude (Figure 1B), as well as a reduced overall integrated EMG area for the muscle burst (Figure 1C), and a reduced rate of rise of the initial EMG activation (Figure 1D). As such, it is worth considering why a more complex

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movement resulted in a differentially lower CE compared to a more simple task, as the current data cannot distinguish between whether decreased CE led to increased RT and a diminished voluntary response output, or whether a less forceful planned response is responsible for the diminished CE and hence, increased RT. That is, while there does appear to be a correlation between activation levels and response output measures, it is not possible to determine the direction of causality. One possibility for the diminished CE is that additional inhibitory mechanisms are involved in the production of a sequenced movement. Because the complex movement involved a specific and complex timing structure, cerebellar input may be responsible for this inhibition, resulting in longer RT and diminished response output measures. The cerebellum has long been considered to play a major role in the planning, initiation and organization of timed movement (Allen and Tsukahara, 1974) with the olivocerebellar system identified as a unique timing control system that has strong inhibitory connections to the motor cortex (see Llinas, 2014 for a recent review); however, testing this possibility would require further investigation. While increased inhibition is one explanation for the lowered MEP amplitudes for the complex task, it is also possible that a shift rather than reduction in activation is responsible for our observed results. That is, it may be the case that the reduced forcefulness of response (as evidenced by the decreased EMG activity for the more complex movement) resulted in less activation required leading up to response execution.

In conclusion, the findings of the current study indicate that increasing movement complexity by adding additional elements with a specific timing requirement results in reduced CE during the RT interval, but only in the final 75 ms leading up to response production. The observed slower RT and reduced EMG output in the complex task therefore appear to be related to a reduction in CE, providing a novel correlate to previously reported response complexity effects.

4. Experimental Procedure

4.1 Ethical approval

Fully informed, written consent was obtained from all participants prior to the study. The study was conducted in accordance with ethical guidelines approved by the University of Ottawa's Research Ethics Board (REB approval: H03-12-03) and conformed to the guidelines of the Declaration of Helsinki.

4.2 Participants

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

4.3 Experimental set-up and task

Participants sat comfortably facing a 23-inch LCD computer monitor with their right arm pronated and resting on a flat surface, and were informed that the upcoming task was a simple RT task consisting of a button press movement of a single telegraph key (Ameco AM-K4B) using only the right index finger. For the simple movement, the participant was required to press the telegraph key once for a duration of 150 ms, while the complex movement required a three key press sequence using only the right index finger in which the first two presses were separated by 150 ms and the second and third presses were separated by 450 ms (individual key press durations of 150 ms). These movements were chosen as they have previously been shown to exhibit large simple RT differences (Maslovat et al., 2014). All trials began with the word "Ready!" appearing on the screen, followed by a visual cue in the center of the monitor (simple = "dit"; complex = "dit dit ___ dit") and an auditory template of the movement in which tones (80 dB, 150 ms, 500 Hz) represented the movement pattern including the amount

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of time the telegraph key should be pressed and time between presses. The visual precue stayed on the screen for the duration of the foreperiod, which was randomly selected between 2500-3500 ms, and was followed by an auditory IS (82 dB, 40 ms, 1000 Hz), at which time the participant initiated their movement. All auditory tones were generated using digital to analog hardware (National Instruments PCI-6024E), with the signal amplified and presented via a loudspeaker (MG Electronics Model M58-H) located in front of the participant.

Participants were instructed to initiate the required movement as quickly as possible in response to the IS while executing the timing pattern as accurately as possible. Feedback was provided on the computer monitor after each trial, consisting of RT and timing accuracy. Accuracy represented the participant's ability to replicate the auditory template played prior to the IS, and consisted of a visual display of the closing and opening of the telegraph key displayed beneath a template of the movement. If the participant incorrectly replicated the auditory template (defined as greater than 100 ms error on any key press duration or interval), the on-screen feedback would be red and the trial was omitted from analysis (total 101 trials, 49 in the simple task, 52 in the complex task; total 3.5% of trials across participants); otherwise it would appear green indicating a correct response. A reward structure was provided to the participant whereby five points were awarded to the participant when the response feedback turned green and five points were awarded when displacement RT was below a predetermined criterion (which was initially set at 250 ms, but adjusted based on the participant's practice performance). These points had no monetary significance and were simply used as a means to increase participant motivation to perform accurate movements initiated at short latency. Each participant began by completing a practice session consisting of a block of 10 simple movements followed by a block of 10 complex movements. Following practice, participants performed testing trials which were identical to the practice trials, with the exception that TMS was applied at six time points following the IS (0 ms, 25 ms, 50 ms, 75 ms, 100 ms, 125 ms). Previous research involving TMS delivery during the RT interval typically either used a protocol in which TMS is time-locked to a proportion of individual RT (Tandonnet et al., 2012) or fixed intervals following the IS (Duque et al., 2010). The current study time-locked the TMS to the IS as the primary research question involved the examination of the relative difference in activation levels between the movements both at the IS and moving forward in time until response output (i.e., during the RT interval). Testing consisted of 180 trials separated into 5 blocks of 36 trials, including 18 simple and 18 complex movements (i.e., 3 trials of each of the 6 TMS stimulation points for each level of complexity). The order of the trials within a block was randomized and controlled by a computer. The task is visually represented in Figure 5.

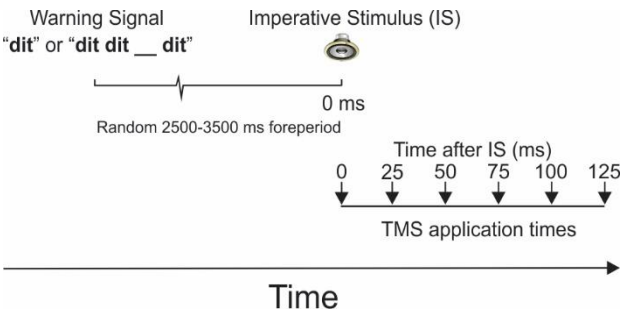

Figure 5. *Visual representation of the timeline of a testing trial, including TMS stimulation points which are represented by downwards pointing arrows.*

4.4 Transcranial magnetic stimulation

TMS pulses were applied using a figure-8 magnetic coil (70mm; Magstim 2002, Magstim Company Ltd, UK). Prior to testing, the coil was placed over the optimal location for eliciting MEPs from the right flexor digitorum superficialis (FDS), with the handle of the coil pointing backwards at a 45˚ angle. The starting location was found by first finding the midpoint between the nasion and inion, and the left and right preauricular notches. From this midpoint, a location 5 cm lateral and 1 cm posterior was marked on the participant's scalp using a red grease crayon. The optimal location was then found by delivering single test pulses at

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various scalp locations around this mark and determining the location that resulted in consistently large MEPs. Resting motor threshold (RMT) was determined at rest to the nearest 1% of stimulator output using the Rossini-Rothwell (Rossini et al., 1999) method (defined as the minimum intensity required to evoke MEPs above 50 μV in at least 5 out of 10 trials). The magnetic coil was held stationary over the optimal location by the experimenter and the position was maintained by holding the coil in the reference position on the head with the assistance of neuronavigation hardware and software (ANT Neuro Visor 2, Madison, WI). During experimental trials, stimulus intensity was adjusted to 110% of RMT as similar intensities have been used previously to probe changes in CE (Chen et al., 1998; MacKinnon and Rothwell, 2000). Across participants RMT was 47% (SD = 10) of maximal stimulator output, and the mean test stimulus was 52% (SD = 11) of maximal stimulator output.

4.5 Recording equipment

Surface electromyography (EMG) data were collected from the muscle belly of the primary effector muscle, the right FDS, using a bipolar preamplified (gain = 10) surface electrode (Delsys Bagnoli DE-2.1) connected via shielded cabling to an external amplifier (Delsys Bagnoli-8, bandwidth 20-450 Hz). The electrode was placed parallel to the muscle fibres, and attached to the skin using double-sided adhesive strips. A ground electrode (Dermatrode HE-R) was placed on the participant's right lateral epicondyle. The site of the electrode was cleaned using abrasive skin prepping gel and alcohol wipes. The telegraph key was connected to a DC power source such that +5V was produced when the switch was closed (depressed) and 0V was produced when the button was open. Data collection for each trial was initiated by the computer 500 ms prior to the warning signal and continued for 2000 ms. Unfiltered EMG and telegraph key signals were digitally sampled at 1kHz (National Instruments PCI-6024E) using a custom-made program written in LabVIEW (National Instruments Inc.) and stored for offline analysis.

4.6 Dependent Measures

Practice trials were omitted from analysis. Voluntary EMG onset in the agonist was defined as the first point (non-MEP related) where the rectified and filtered (25 Hz low pass elliptical filter) EMG activity first reached a value of two standard deviations above baseline levels (mean EMG activity in a 100 ms interval following the warning signal) and was maintained for a minimum of 20 ms. EMG onset points were first determined using a custom program written in LabVIEW (National Instruments Inc.) and then were visually confirmed and manually adjusted (if necessary) to compensate for any errors due to the strictness of the algorithm and to dissociate between voluntary EMG and MEPs. EMG offset point was determined in a similar fashion.

Response output measures included premotor RT (time between the go-signal and the EMG onset), peak EMG (maximum value obtained between onset and offset), initial rise of the EMG agonist burst (Q30; integration of rectified raw EMG for first 30 ms), and size of initial EMG burst (iEMG; integration of rectified raw EMG for entire burst duration). Reaction times that were longer than 350 ms (36 trials: 4 simple response, 32 complex response) or shorter than 50 ms (6 trials: 5 simple response, 1 complex response) were excluded from the analysis. These were considered to be bad trials where the participants either reacted too slowly to have been properly preparing for the task or anticipated the IS.

MEP amplitude was defined as the largest peak-to-peak amplitude recorded in a 25 ms window starting 20 ms after the TMS pulse (to allow for neural conduction). Trials in which a MEP was not detectable were rejected (often due to the MEP occurring during the EMG burst), which resulted in the removal of 25.2% of trials across participants (435 simple, 291 complex), or 726 total trials (0 ms = 30; 25 ms = 26; 50 ms = 34; 75 ms = 101, 100 ms = 219; 125 ms = 316). This breakdown indicates that the MEP occurred during the EMG burst much more frequently in trials with TMS applied at 100 ms and 125 ms following the IS. As such this led to missing data points for these time conditions for 5 of the 16 participants and an overall

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rejection rate of over 56% of the trials in these conditions. Additionally, trials in which a MEP was measurable when TMS was presented at 100 ms or 125 ms following the IS were necessarily trials with longer RTs, and thus activation at these time points was less representative of that occurring in a typical trial. This is evidenced by the mean RT values being considerably longer for both simple and complex movements in trials where a measureable MEP was detected when TMS was delivered at 100 or 125 ms following the IS (simple M = 208 ms, complex M = 220 ms) compared to the earlier TMS time points (simple M = 145 ms, complex M = 164 ms). As such, data from these two time points were discarded from the primary analysis. The loss of these data points is a potential drawback to time-locking the TMS delivery to the IS, rather than delivering TMS based on individual mean RT values (as previously outlined). Given the variability associated with typical RTs, TMS delivery time-locked to individualized mean RT values would also likely have resulted in a relatively large number of discarded trials. Nevertheless, to compensate for this limitation in our IS-locked protocol, MEP amplitudes were analyzed both with respect to the IS and EMG onset such that activation timelines could be determined following the IS and prior to movement onset (see section 4.7 below).

Finally, trials were excluded from analysis if root mean square (RMS) EMG activity in the 100 ms preceding the TMS pulse in any individual trial exceeded twice the resting RMS value for that trial (determined from a mean of 100 ms EMG prior to the warning signal); trials that fit this criterion must have also had a pre-TMS RMS EMG value greater than 10 microvolts. This procedure resulted in the exclusion of 56 additional trials (1.9%). Finally, MEP amplitudes greater than 3 standard deviations from each individual's overall mean were removed from the analysis, which resulted in the removal of an additional 10 trials. Overall the primary analyses included 1562 of the 1920 total trials when the TMS was presented between 0 to 75 ms following the IS (81% inclusion rate).

4.7 Statistical Analyses

In order to examine the effects of the complexity manipulation on production of the movement independent of any effects of late TMS, paired samples Student's t-tests were performed for each of the response output measures (premotor RT, peak EMG, iEMG, and Q30), comparing the simple versus complex movement at the first TMS delivery time point. Shapiro-Wilk tests showed that data for peak EMG, iEMG, and Q30 were all significantly non-normal ($p < .05$) and thus were subjected to a Log10 transform prior to analysis, which corrected for significant violations (all post transformed p values > .1). Only the data when the TMS was presented concurrent with the IS was considered for this analysis as removal of trials where EMG onset preceded MEP onset were more prevalent for the later TMS time points which may have led to non-representative data for these later time points.

Prior to examination of MEP amplitudes, RMS of background EMG in the 100 ms preceding TMS onset was collapsed across TMS delivery times and compared between complexity conditions using a single paired samples Student's t-test. This analysis confirmed that there was no significant difference in background EMG between conditions ($T(15) = 0.133$, $p = .896$, $r = .03$).

MEP amplitudes were subjected to two separate analyses in order to accurately describe the time course of CE for the simple and complex movement with respect to both the IS and EMG onset. Shapiro-Wilk tests showed that raw MEP data were significantly non-normal ($p < .05$) and thus were subjected to a Log10 transform prior to the first analysis, which corrected for significant violations (all post-transformed p values > .14). First, to investigate any changes in activation following the IS, raw MEP amplitudes were analyzed using a 2 Task (simple, complex) x 4 Time (0 ms, 25 ms, 50 ms, 75 ms) repeated measures ANOVA. While the analysis above captures activation changes following the IS, it does not provide comparable information relative to movement onset as RT differences were expected between the simple and complex movement. Thus, a second analysis investigated MEP amplitude data time-locked to the onset of EMG by

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calculating the time difference between the MEP and the onset of EMG. These data were organized into time bins in which MEP onsets occurred either <75 ms prior to EMG onset or in 25 ms time bins at increasing intervals up to >150 ms prior to EMG onset (see Chen et al., 1998 for a similar analysis). Note that for this analysis, trials from all TMS delivery times where a MEP was noted were used (including 100 ms and 125 ms); error trials, trials with RTs outside of 50-350 ms, and trials with excessive RMS activity preceding the TMS were excluded (see above). Thus this analysis included a total of 1910 of 2880 trials (66.3% inclusion rate). Because not all participants had data values for all time bins, each trial was considered as an individual observation resulting in what can be considered between-group analysis. As such, in order to compensate for inter-individual variability in MEP amplitudes, raw peak-to-peak MEP values were normalized for each participant by expressing them as a percentage of their own mean MEP amplitude at time 0 (i.e. baseline, 100%) in both the simple and complex tasks. The resultant distributions were analyzed for normality using Shapiro-Wilk tests revealing that MEP distributions all bins were significantly non-normal (all p values < .002). Transforming the data led to no improvement in normality, thus untransformed binned MEP amplitudes were analyzed separately using Mann-Whitney U tests for rank ordered data between the simple and complex conditions at each time bin (Bonferroni-corrected for multiple comparisons). Note that the MEP normalization procedure employed is different to previous research examining the time course of CE, which have often used time points prior to the IS as a baseline measure (Duque et al., 2010; Tandonnet et al., 2012; Touge et al., 1998). Although these studies have shown a reduction in CE at the IS as compared to the warning period, the current study was primarily interested in a comparison of CE timelines between task complexities from the IS until response output, and thus the IS time point was used as a baseline indicator.

For repeated measures ANOVAs, the Greenhouse-Geisser Epsilon factor was used to adjust the degrees of freedom for violations of sphericity if necessary. Uncorrected degrees of freedom are reported, with the corrected p-values and partial eta squared (η_p^2) and *r* values reported as measures of effect size. Post-Hoc tests were performed using Bonferroni-corrected paired samples Student's t-tests where appropriate. Untransformed means, standard deviations, and within-participant confidence intervals (see Cousineau, 2005; Morey, 2008) are reported and/or presented in figures where null hypothesis significance tests were performed on transformed variables. Differences with a probability of less than .05 were considered significant.

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