The Time Course of Motoneuronal Excitability during the Preparation of Complex Movements

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Abstract

■ For a simple RT task, movement complexity increases RT and also corticospinal excitability, as measured by the motor evoked potential (MEP) elicited by TMS of the motor cortex. However, it is unknown if complexity-related increases in corticospinal excitability during the preparation of movement are mediated at the cortical or spinal level. The purposes of this study were to establish a time course of motoneuronal excitability before prime mover activation and to assess task-dependent effects of complex movements on motoneuronal and cortical excitability in a simple RT paradigm. It was hypothesized that motoneuronal and cortical excitability would increase before prime mover activation and in response to movement complexity. In a seated position, participants completed ballistic elbow extension/flexion movements with their dominant arm to one, two, or three targets. TMS and transmastoid stimulation (TS) were delivered at 0%, 70%, 80% or 90% of mean premotor RT for each complexity level. Stimulus intensities were set to elicit MEPs and cervicomedullary MEPs (CMEPs) of ~10% of the maximal M-wave in the triceps brachii. Compared with 0% RT, motoneuronal excitability (CMEP amplitude) was already 10% greater at 70% RT. CMEP amplitude also increased with movement complexity as both the two- and three-movement conditions had greater motoneuronal excitability than the one-movement condition ($p < .038$). Importantly, when normalized to the CMEP, there was no increase in MEP amplitude. This suggests that complexity-related increases in corticospinal excitability are likely to be mediated more by increased excitability at a motoneuronal than cortical level. ■

INTRODUCTION

How the brain controls volitional movement has long been debated and is still not fully resolved. Output from the primary motor cortex is the impetus for goal-directed movements; however, it is important to consider that primary motor cortex output is influenced by a variety of cortical and subcortical inputs and can be modulated at multiple subcortical sites as it travels along the motor pathway toward the target muscle. In humans, the excitability of the motor pathway to a target muscle is often probed using TMS, which activates the motor cortex and produces a short latency excitatory response in the EMG. This motor evoked potential (MEP) reflects the responsiveness of neurons at cortical, spinal, and peripheral levels (Taylor, Butler, & Gandevia, 1999). To account for the possibility of a change in peripheral excitability with an intervention, the MEP is often normalized to the maximal compound muscle action potential (Mmax). Although this process yields an index of corticospinal excitability, the MEP must be normalized to a measure of motoneuronal excitability to isolate the cortical component of the MEP. The H-reflex is the most common such measure; however, its utility as an index of motoneuronal excitability is limited by presynaptic inhibition and other factors that influence the size of the potential (e.g., Zehr, 2002). In humans, the most direct means to assess motoneuronal excitability of an upper limb muscle involves stimulation of the corticospinal tract at the level of the mastoids (Martin, Butler, Gandevia, & Taylor, 2008) to elicit the cervicomedullary MEP (CMEP), a largely monosynaptic response (Petersen, Taylor, & Gandevia, 2002) that is not subject to presynaptic inhibition (Nielsen & Petersen, 1994).

A classic method to assess goal-directed movements is to ask the participant to respond to a stimulus as rapidly as possible. The delay between stimulus presentation and movement (i.e., RT) is indicative of the time necessary for sensory and motor processes to prepare the movement (Wong, Haith, & Krakauer, 2015; Salinas, Scerra, Hauser, Costello, & Stanford, 2014), and is an excellent opportunity to probe neural excitability and the influence of factors such as movement complexity. In the premotor period of a simple RT paradigm, a change in excitability of the peripheral portion of the motor pathway (Mmax) is unlikely, which makes it justifiable to attribute a change in the MEP to altered corticospinal excitability. As such, numerous studies have shown that corticospinal excitability (MEP size) gradually increases ~100 msec before EMG onset (Kennefick, Burma, van Donkelaar, & McNeil, 2019; Kennefick, Maslovat, & Carlsen, 2014; Chen, Yaseen, Cohen, & Hallett, 1998; Hoshiyama

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et al., 1996; Tarkka, McKay, Sherwood, & Dimitrijevic, 1995; Pascual-Leone, Brasil-Neto, Valls-Solé, Cohen, & Hallett, 1992; Pascual-Leone, Valls-Solé, et al., 1992; Tomberg & Caramia, 1991). None of these studies included a measure of motoneuronal excitability so it was not possible to separate corticospinal excitability into cortical and spinal components. The one study to consider spinal contributions to the increase in MEP size before movement (rapid wrist flexion) found that the H-reflex only increased with the onset of EMG, which led the authors to attribute the facilitation of the MEP during the premotor RT (i.e., the time from stimulus to EMG onset) to cortical mechanisms (MacKinnon & Rothwell, 2000). Conversely, studies using static contractions demonstrate a progressive facilitation of the H-reflex, beginning ~50 msec before movement onset (Day, Rothwell, & Marsden, 1983; Pierrot-Deseilligny, Lacert, & Cathala, 1971; Gottlieb, Agarwal, & Stark, 1970).

Movement complexity influences preparatory processes, which allows researchers to gain additional insight with respect to corticospinal excitability during the premotor period. Complex motor behaviors have been shown to increase (Kennefick et al., 2014, 2019; Greenhouse, Saks, Hoang, & Ivry, 2015; Kennefick, Maslovat, Chua, & Carlsen, 2016; Roosink & Zijdewind, 2010; Abbruzzese, Trompetto, & Schieppati, 1996; Flament, Goldsmith, Buckley, & Lemon, 1993) or decrease (Kennefick et al., 2016) corticospinal excitability. Although these findings are largely in agreement, it should be noted that complexity can be manipulated in a variety of ways (e.g., the number of movement components, the number of controllable degrees of freedom, more complex movement dynamics, the number of possible task solutions) and the implications for the mechanisms of neural control may vary with the paradigm. The paradigm whereby complexity is increased by the addition of movement components (Henry & Rogers, 1960) is appealing because it permits the same initial movement to be performed in each condition, and the movement pattern is known before the imperative stimulus. As a result, a participant can prepare fully for the upcoming response, which means that responses to stimulation offer relatively direct insight about the effects of movement complexity on corticospinal excitability in the premotor period.

Although the aforementioned studies indicate that corticospinal excitability is sensitive to movement complexity, no studies have investigated separately the influence of complexity on cortical and motoneuronal excitability during the preparation phase of a movement. Therefore, the purpose of the current study was twofold. The first purpose was to establish a time course of motoneuronal excitability during the premotor period of a simple RT task. The second purpose was to assess separately the influence of additional movement components on motoneuronal and cortical excitability. It was hypothesized that both cortical and motoneuronal excitability would increase before movement onset, as well as in response to movement complexity.

METHODS

Participants

Seventeen healthy (11 women, age range = 18–42 years), self-declared right-handed participants with normal or corrected-to-normal vision and no history of neurological, sensory, or motor disorders participated in this study. Testing of each participant took place during a single session and required approximately 1.5 hr to complete. The study was approved by The University of British Columbia's clinical research ethics board (CREB approval: H17-00796) and conformed to the guidelines of the Declaration of Helsinki, except for registration in a database. Written informed consent was obtained from each participant.

Experimental Setup

Participants sat in front of the KINARM End-Point Lab (BKIN Technologies Ltd.) and grasped the handle of the right manipulandum, linked to robotic motors, with their right hand. The participant could not see their hand or the manipulandum and instead saw a virtual representation of their hand, that is, a circle that mirrored the movement of the handle. Of note, the manipulandum does not raise or lower, which means the participants could only move in two-dimensional space (i.e., forward–backward and left–right). Surface EMG data were recorded from the lateral head of the triceps brachii via adhesive Ag–AgCl electrodes (10 mm diameter, Cleartrace; ConMed), with the active lead positioned over the muscle belly and the reference over the distal tendon. Data were sampled at 2000 Hz using a 16-bit A/D converter (CED Power1401-3; Cambridge Electronic Design Ltd.) and Spike2 software (Version 7.10; Cambridge Electronic Design). Signals were amplified (×100) and bandpass filtered (16–1000 Hz) using CED 1902 amplifiers (Cambridge Electronic Design).

Task Details

Before testing, participants were informed the upcoming experimental task required them to react as fast as possible to an imperative stimulus (details below). Based on the number of targets (one, two, or three) presented on the KINARM display at the beginning of each testing trial, the participant needed to perform one of three movement conditions (see Kennefick, Wright, Smirl, & van Donkelaar, 2018, Figure 1). These movement conditions consisted of either a single movement component, two movement components, or three movement components. All three movement conditions were performed with participants grasping the right robotic handle with

a neutral grip. For each trial, once the target(s) appeared, the participant moved the circle representing their hand within a larger red circle that indicated the home position. After a random delay between 1000 and 3000 msec, the red circle changed to green (imperative stimulus), and the participant moved their hand forward to the first target. For the one-movement condition, the participant tried to end their movement at the target. For the two-movement condition, the participant was to hit the first target then change course to terminate their movement at the second target. For the three-movement condition, the participant needed to make another change in direction after the second target and finish their movement at the third target. As this study is concerned only with movement preparation processes and not with the online control of movement, it was stated explicitly that it was most important to react as quickly as possible to the imperative stimulus and complete the movement pattern without any correction for missed targets. Accuracy was not recorded, and no trials were removed due to missed targets.

Brachial Plexus Stimulation

To determine the EMG response to simultaneous activation of the entire triceps brachii motoneuron pool, electrical stimulation was applied to the brachial plexus to evoke the Mmax. Single stimuli were delivered by a constant current electrical stimulator (DS7AH; Digitimer Ltd.) at a pulse duration of 200 μsec and continuously variable voltage between 100 and 400 V. The cathode and anode (adhesive Ag–AgCl electrodes; Cleartrace) were placed over the supraclavicular fossa and acromion, respectively. Stimuli were delivered as the participant held the manipulandum at the home position and prepared as if to move. Current was increased gradually with successive stimuli until the M-wave reached a plateau (Mmax). Once a plateau was established, an additional two stimuli were delivered at that current to establish a mean Mmax value.

Transmastoid Stimulation

To elicit a CMEP from the triceps brachii, the corticospinal tract was stimulated with a high-voltage electrical current (DS7AH; 200-μsec pulse duration, 100–400 V) passed between adhesive Ag–AgCl electrodes (Cleartrace) fixed to the skin ~1 cm superior and medial to the mastoid processes (Gandevia, Petersen, Butler, & Taylor, 1999; Ugawa, Rothwell, Day, Thompson, & Marsden, 1991). Stimulus intensity was set to elicit a CMEP amplitude equivalent to ~10% of the Mmax obtained under the same conditions (i.e., at the home position, prepared to move).

TMS

To elicit an MEP from triceps brachii, TMS was applied to the motor cortex using a circular coil (Forman, Monks, & Power, 2019; Spence, Alcock, Lockyer, Button, & Power, 2016; Pearcey, Power, & Button, 2014; Lévénez, Garland, Klass, & Duchateau, 2008; Martin, Weerakkody, Gandevia, & Taylor, 2008) with a 13.5-cm outer diameter attached to a Magstim 200^2 stimulator (Magstim). The coil was held over the vertex, with the handle pointing backward. Stimulus intensity was set to elicit a MEP amplitude equivalent to ~10% of the Mmax obtained under the same conditions (i.e., at the home position, prepared to move).

Experimental Procedures

Before testing, participants completed a practice block consisting of 10 trials for each condition. Practice blocks were done in ascending order (one, then two, then three movements) and used to establish the mean premotor RTs for each participant. Mean premotor RT was calculated for each condition after excluding the fastest and slowest trials and was defined as the time between the imperative stimulus and the onset of EMG activity. The 144 testing trials were identical to practice trials, with the exception that TMS or transmastoid stimulation (TS) was presented at four time points following the imperative stimulus. Most previous studies (Chen et al., 1998; Kennefick et al., 2016; Leocani, Cohen, Wassermann, Ikoma, & Hallett, 2000) have presented TMS at absolute time points following the imperative stimulus; however, as RTs differ between participants, these set time points would not measure the same preparatory processes across all participants. Therefore, similar to the work of Summers and colleagues (Fujiyama et al., 2012; Hinder, Fujiyama, & Summers, 2012), the four time points in the current study were based on a percentage of the average premotor RT calculated during the practice session for each participant. In this case, we delivered stimuli at 0%, 70%, 80%, and 90% of premotor RT. Testing consisted of three blocks of 48 trials. Each block included 24 pseudorandomized trials for each stimulation type (TMS and TS) across each level of complexity (i.e., two trials at each of the four time points for the one-, two-, and three-movement conditions with both TMS and TS).

Data Analysis

All data from the testing trials were analyzed offline using Signal software (Version 6.03, Cambridge Electronic Design). The root mean square (RMS) of the background voluntary EMG was measured in the 100 msec before the TMS or TS pulses in the right triceps brachii muscle (i.e., the prime mover). A limitation of the current study is that the evoked potentials of interest are only recorded from the triceps brachii. Although we acknowledge that evoked potentials from other contributing muscles (e.g., biceps brachii or deltoid) could be informative, it would be imprudent with these stimulation intensities because

the data would not be properly normalized to the Mmax of these muscles. The amplitude of each evoked potential (Mmax, CMEP, and MEP) was measured between the initial deflection from the baseline to the second crossing of the horizontal axis (Martin, Gandevia, & Taylor, 2006). To determine if motoneuronal or corticospinal excitability was altered by complexity or the percentage of premotor RT, for each participant, CMEP and MEP amplitudes were expressed relative to their respective baselines (0% premotor RT) for each movement complexity. For comparison to previous work, absolute MEP amplitude was also used to evaluate corticospinal excitability in each condition. To assess the cortical contribution to any changes in corticospinal excitability, mean MEP amplitude was normalized to the mean CMEP amplitude obtained under the same conditions (i.e., MEP at baseline/CMEP at baseline). For both CMEPs and MEPs, potentials with an amplitude greater than 2 *SD*s from the overall mean of each participant were removed from the analysis. Furthermore, if the RMS EMG was greater than 2 *SD*s from each individual's overall mean, the entire trial was removed from the analysis. Overall, data from 100 trials (3.9% of all trials) were removed from the analysis.

Statistical Analysis

Data were analyzed using repeated-measures analyses of variance (RM-ANOVAs). All analyses were conducted using SPSS Version 23 (SPSS, Inc.). RMS EMG was analyzed using a 2 (Stimulation type) × 3 (Movement complexity) × 4 (Stimulation time) three-way RM-ANOVA. Evoked potentials were assessed for violations of normality using the Shapiro–Wilk's test on the studentized residuals. Both the MEP and CMEP amplitudes were significantly nonnormal ($p < .05$), so were subjected to a square-root transform (Stuart-Hamilton, 2007). The transformed MEP and CMEP data were analyzed using 3 (Movement complexity) × 4 (Stimulation time) two-way RM-ANOVAs. For all RM-ANOVAs, Greenhouse–Geisser epsilon was used to adjust degrees of freedom for violations of sphericity, when necessary. Post hoc tests were performed using Bonferroni-corrected paired samples Student's t tests, where appropriate. Differences with a $p < .05$ were considered significant. Data are presented as mean ± *SD*.

RESULTS

Control Measures

Across participants, the mean peak-to-peak amplitude of Mmax was 25.2 ± 5.25 mV. Measured before testing while the participant held the manipulandum at the home position and prepared to move, MEP and CMEP amplitudes were 2.40 ± 0.64 mV (9.5% of Mmax) and 2.51 ± 0.58 mV (10.0% of Mmax), respectively.

RT Measures

To determine the effect movement complexity had on premotor RT, a one-way RM-ANOVA was performed on data from the practice trials. The analysis (Figure 1A) revealed a significant main effect of Complexity, $F(1.14, 20.5) = 12.4$, $p < .001$, $\eta_p^2 = .408$. The post hoc analysis indicated that premotor RT was faster for the one-movement condition compared with both the two-movement ($M = 37.4$ msec, 95% CI [9.51, 65.3], $p = .002$) and three-movement conditions ($M = 41.7$ msec, 95% CI [11.6, 71.9], $p = .002$). However, premotor RT did not increase from the two- to three-movement conditions ($M = 4.32$ msec, 95% CI [−4.84, 13.5], $p = .668$). To ensure the complexity effect on premotor RTs remained during the testing protocol, premotor RT data from the TS trials were subjected to the same analysis. TMS trials were not included in this analysis because suprathreshold TMS has been shown to prolong RT (Pascual-Leone, Valls-Solé, et al., 1992; Day et al., 1989). This analysis revealed (Figure 1B) a similar main effect of Complexity, $F(2, 32) = 16.9$, $p < .001$, $\eta_p^2 = .513$. The post hoc analysis indicated that premotor RT was faster for the one-movement condition compared with both the two-movement ($M = 17.1$ msec, 95% CI [6.46,

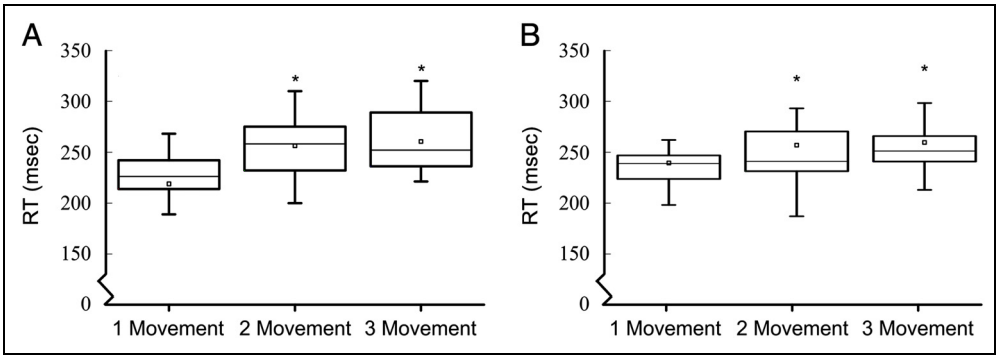

Figure 1. Boxplot of the mean premotor RT across the three complexity levels in both the practice (A) and TS testing (B) sessions. Box boundaries represent the 25th and 75th percentiles, solid horizontal lines represent medians, the small squares within the box represent means, and error bars represent the farthest outliers within 1.5 times the interquartile range from the box boundaries. The asterisk (*) denotes a significantly slower RT compared with the one-movement condition.

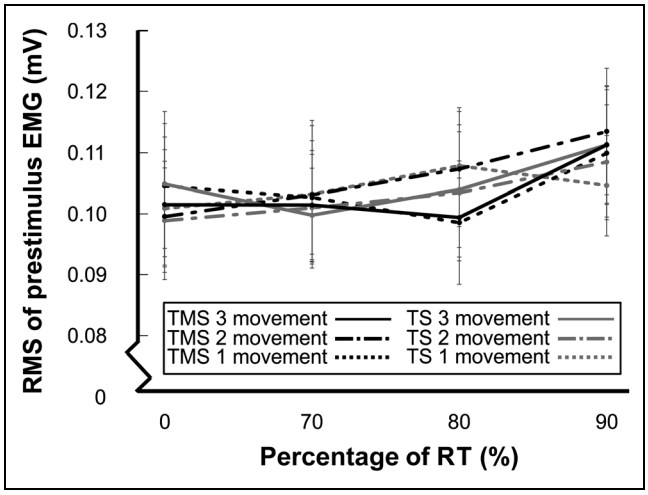

Figure 2. The RMS of the voluntary EMG 100 msec before TMS (black lines) or TS (gray lines) across the three-movement complexities. Error bars represent *SEM*.

27.8], p = .002) and three-movement conditions (M = 19.7 msec, 95% CI [10.4, 30.0], p < .001). The post hoc analysis once again demonstrated no increase in premotor RT between the two- and three-movement conditions (M = 2.56 msec, 95% CI [−6.97, 12.1], p = 1.000).

Voluntary EMG

Before stimulus onset, there was minimal RMS EMG under any condition (Figure 2), indicating that the triceps brachii muscle was largely relaxed before the TMS or TS pulse. The analysis revealed no main effects of Stimulus intensity, $F(1, 17) = 0.034$, p =.856, η_p^2 = .002; Complexity, $F(2, 34) = 0.021$, p =.979, η_p^2 = .001; or Time, $F(1.23, 20.9) = 2.47$, p = .126, η_p^2 = .127.

Evoked Potentials

Mean values for all raw data in absolute units are summarized in Table 1. As this study used a simple RT paradigm, participants knew which movement they were to perform when the imperative stimulus was presented. To test if complexity could affect motoneuronal or corticospinal excitability as early as the imperative stimulus, a 2 (Stimulus type) × 3 (Movement complexity) two-way RM-ANOVA was performed for absolute MEP and CMEP amplitudes at 0% RT. This analysis revealed no effect of Movement complexity, $F(1.39, 23.6) = 0.238$, p = .708, η_p^2 = .014, which indicated that complexity of the upcoming movement did not influence motoneuronal or corticospinal excitability at the timing of imperative stimulus. Representative EMG traces for each movement condition at 0% RT are shown for a single participant in Figure 3. Despite the excellent matching of the MEP and CMEP during setup (9.5% vs. 10.0% of Mmax, respectively), there was a main effect for Stimulation type, $F(1, 17) = 8.00$, p = .012, η_p^2 = .320, such that the MEP amplitude was larger than that of the CMEP at 0% RT (2.9 vs. 2.0 mV, respectively). Although this finding was unexpected, the potentials still represent a similar proportion of the motoneuron pool.

The analysis of the CMEP normalized to 0% premotor RT (Figure 4) revealed a main effect of Complexity, $F(2, 34) = 4.87$, p = .014, η_p^2 = .223, and Time, $F(1.86, 31.7) = 20.1$, p < .001, η_p^2 = .542, but no interaction, $F(2.80, 48.1) = 2.29$, p = .094, η_p^2 = .119. The post hoc analysis indicated greater motoneuronal excitability in the two- compared with one-movement condition (M = 5.12%, 95% CI [0.291, 10.9], p = .037) and in the three-movement compared with one-movement condition (M = 5.23%, 95% CI [0.273, 11.2], p = .038). Furthermore, the post hoc analysis of the time effect (data pooled across conditions)

Table 1. Absolute Values for RT, Evoked Potential Amplitude, and Background EMG

	1-Movement Complexity	*2-Movement Complexity*	*3-Movement Complexity*
RT (msec)	229 (23)	256 (31)	261 (30)
Peak-to-peak MEP amplitude (mV)			
0 % RT	2.85 (1.65)	2.89 (1.66)	2.86 (1.62)
70% RT	3.21 (2.01)	3.60 (2.40)	3.41 (2.51)
80% RT	3.67 (2.51)	3.90 (2.55)	3.75 (2.68)
90% RT	4.13 (2.96)	4.69 (3.81)	4.07 (2.91)
Peak-to-peak CMEP amplitude (mV)			
0 % RT	2.03 (0.91)	1.99 (0.87)	1.94 (0.93)
70% RT	2.17 (1.05)	2.57 (1.57)	2.61 (1.39)
80% RT	2.85 (1.51)	3.12 (2.13)	2.92 (2.15)
90% RT	3.23 (2.39)	3.75 (2.73)	3.34 (2.29)
RMS of 100 msec before TMS pulse (mV)			
0% RT	0.105 (0.04)	0.100 (0.03)	0.101 (0.04)
70% RT	0.103 (0.04)	0.103 (0.04)	0.101 (0.04)
80% RT	0.099 (0.04)	0.107 (0.04)	0.100 (0.03)
90% RT	0.110 (0.04)	0.113 (0.04)	0.111 (0.04)
RMS of 100 msec before TS pulse (mV)			
0% RT	0.101 (0.04)	0.100 (0.04)	0.105 (0.05)
70% RT	0.103 (0.05)	0.100 (0.04)	0.100 (0.03)
80% RT	0.108 (0.03)	0.103 (0.04)	0.104 (0.04)
90% RT	0.104 (0.03)	0.108 (0.04)	0.111 (0.04)

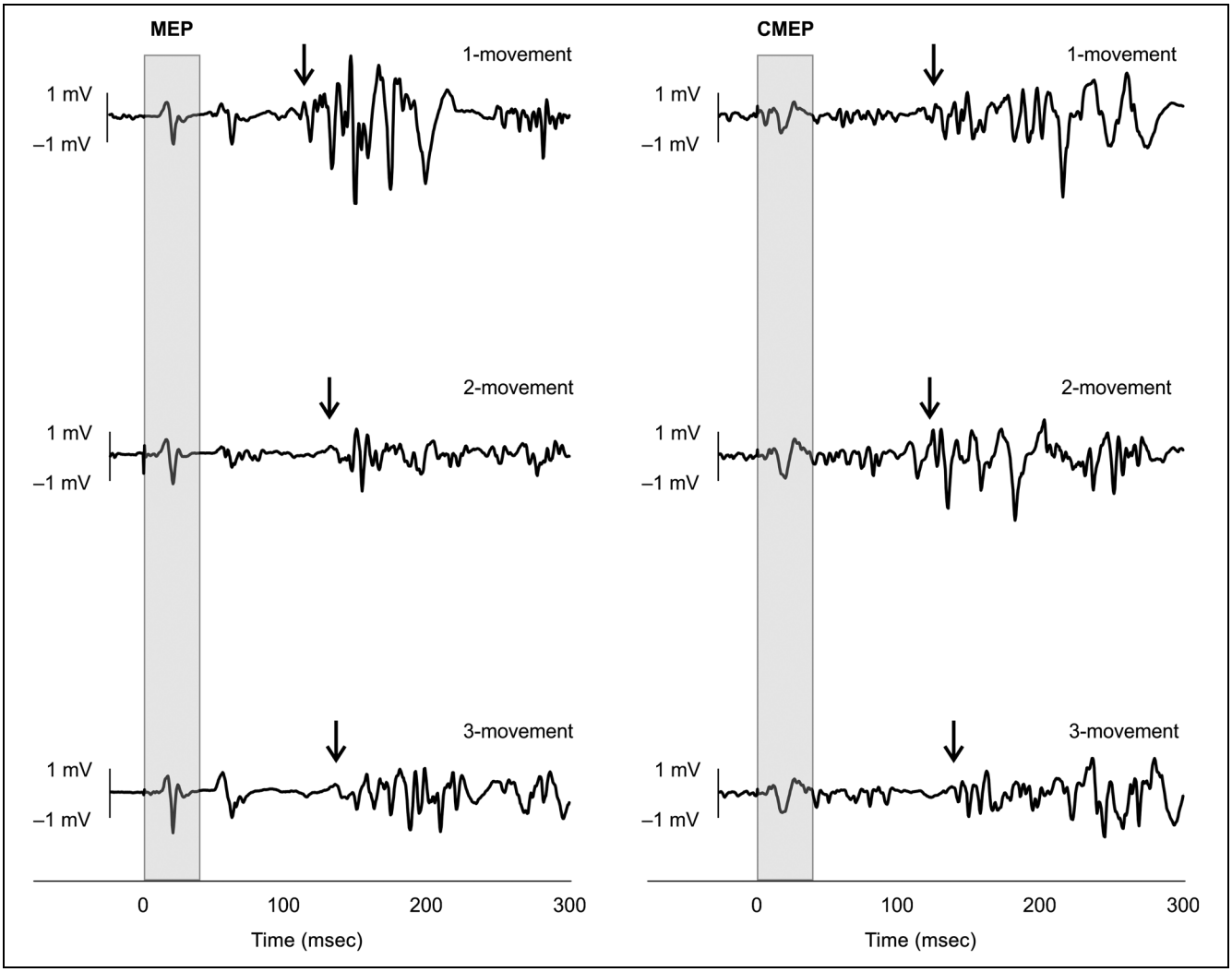

Figure 3. Representative EMG traces from a single participant for the one-, two-, and three-movement conditions for both TMS (left) and TS (right) conditions. The shaded box highlights the MEP or CMEP. In all trials, the stimulus was delivered at 0% RT (i.e., at the time of the imperative stimulus), represented by the left edge of the shaded box. Time to voluntary EMG onset (premotor RT) is indicated in each condition by an arrow.

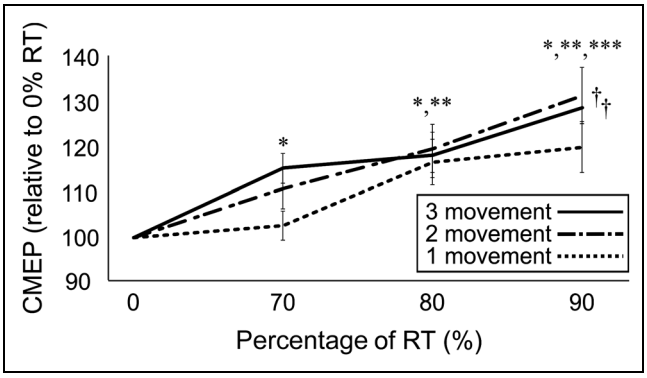

Figure 4. CMEP amplitude expressed as a percentage of the 0% RT condition in each movement condition. For both the two- and three-movement conditions, motoneuronal excitability was greater than the one-movement condition (†). For data pooled across complexities, motoneuronal excitability was higher at 70% RT compared with 0% RT (*), higher at 80% RT compared with 0% and 70% RT (**), and higher at 90% RT than 0%, 70%, and 80% RT (***). Error bars represent *SEM*.

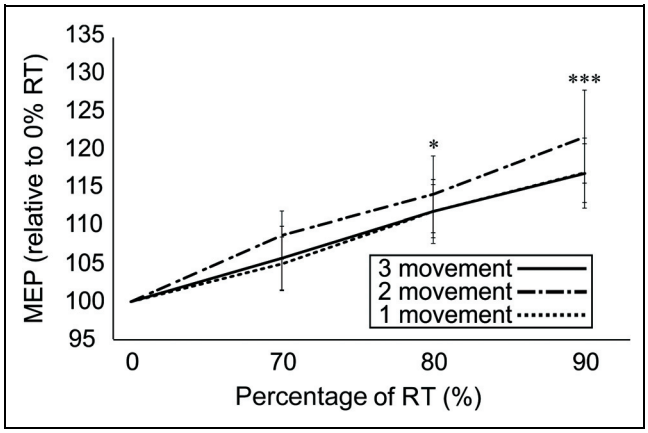

Figure 5. MEP amplitude expressed as a percentage of the 0% RT condition in each movement condition. For data pooled across complexities, corticospinal excitability was higher at 80% RT compared with 0% RT (*) and higher at 90% RT than 0%, 70%, and 80% RT (***). Error bars represent *SEM*.

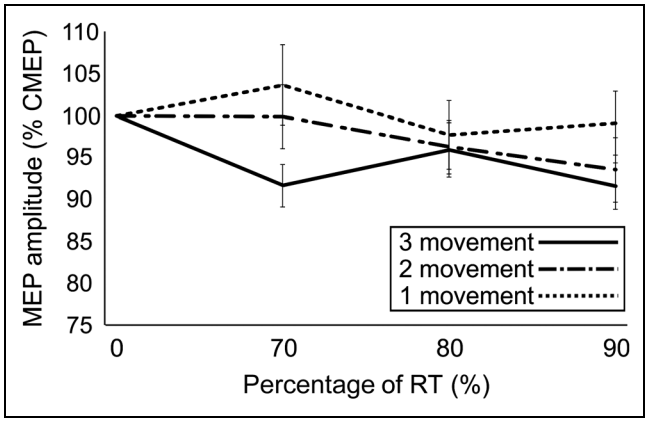

Figure 6. Mean MEP amplitude expressed as a percentage of mean CMEP amplitude. No differences were found for any comparisons. Error bars represent *SEM*.

revealed that motoneuronal excitability increased between all time points (all ps $< .041$).

The analysis of the MEP normalized to 0% premotor RT (Figure 5) did not reveal a main effect of Complexity, $F(2, 34) = 0.514$, $p = .603$, $\eta_p^2 = .029$, nor did it reveal an interaction, $F(6, 102) = 0.224$, $p = .968$, $\eta_p^2 = .013$; however, there was a main effect of Time, $F(2.02, 34.4) = 15.1$, $p < .001$, $\eta_p^2 = .470$. The post hoc analysis revealed that corticospinal excitability at 80% RT was greater than at 0% RT and greater at 90% compared with 0%, 70%, and 80% RT. When the absolute MEP amplitudes (Table 1) were analyzed to mimic the statistical approach of previous studies, there was a main effect of Complexity, $F(2, 34) = 4.69$, $p = .016$, $\eta_p^2 = .216$, and Time, $F(1.83, 31.0) = 12.6$, $p < .001$, $\eta_p^2 = .426$, but no interaction, $F(6, 102) = 0.420$, $p = .864$, $\eta_p^2 = .024$. The post hoc analysis indicated that corticospinal excitability was greater in the two- than one-movement condition ($M = 0.062$ mV, 95% CI [0.00, 0.125], $p = .051$) and in the two-movement compared with three-movement condition ($M = 0.057$ mV, 95% CI [0.002, 0.112], $p = .042$).

When normalized to the CMEP collected under the same conditions, the MEP had no main effect of Complexity, $F(2, 34) = 1.66$, $p = .205$, $\eta_p^2 = .089$, or Time, $F(3, 51) = 1.90$, $p = .141$, $\eta_p^2 = .101$, as well as no interaction, $F(6, 102) = 1.32$, $p = .254$, $\eta_p^2 = .072$ (Figure 6). Hence, although absolute MEPs showed a main effect of Complexity, this analysis suggests that corticospinal excitability alterations due to complexity were not mediated primarily at the cortical level.

DISCUSSION

The aims of this study were to establish a time course for motoneuronal excitability before the onset of movement and to determine if movement complexity affects motoneuronal and/or cortical excitability in a simple RT task. The first major finding of this study was an increase in the excitability of motoneurons before the onset of voluntary EMG (Figure 4). This preparatory increase in excitability occurred with minimal RMS EMG before the imperative stimulus under any condition (Figure 2). This indicated the triceps brachii muscle was largely relaxed before both TMS or TS. Furthermore, the analysis revealed that, at baseline (0% premotor RT), movement complexity had no impact on MEP or CMEP size (Table 1). The increase in motoneuronal excitability conflicts with the only previous study to assess spinal excitability before movement (MacKinnon & Rothwell, 2000). This disparity is likely due to two factors. The first is the technique used to measure spinal excitability. In the previous study, the H-reflex was used; however, the H-reflex, but not the CMEP, could be influenced by presynaptic inhibition (Hultborn, Meunier, Pierrot-Deseilligny, & Shindo, 1987) during preparatory processes. Thus, any increases in motoneuronal excitability may have been masked by presynaptic inhibition. The second factor is that corticospinal excitability only increased ~10 msec before EMG onset in the previous study, a finding that was at odds to the existing literature that showed increases in corticospinal excitability ~80–100 msec before EMG onset (Chen et al., 1998; Hoshiyama et al., 1996; Tarkka et al., 1995; Pascual-Leone, Brasil-Neto, et al., 1992; Pascual-Leone, Valls-Solé, et al., 1992; Tomberg & Caramia, 1991). As demonstrated in Figures 5 and 6, our data indicate that motor pathway excitability was increased at 70%–80% RT, a finding more in keeping with the existing literature.

Indeed, when pooled across complexities, the CMEP amplitude at the earliest interval tested (70% premotor RT) was 10% greater than the value at 0% premotor RT (Figure 4). To translate this relative time of 70% RT to an absolute time before movement onset, we used the following equation for each participant at each level of complexity: time before movement = [mean premotor RT − (mean premotor RT × 0.7)]. Group means for the one-, two-, and three-target conditions were 70 ± 8, 77 ± 10, and 78 ± 10 msec, respectively. Hence, the data suggest that motoneuronal excitability is increased at least 75 msec before movement onset in a simple RT task. As we did not test earlier intervals, it is not possible to state when the increase in motoneuronal excitability first occurred. This increase in motoneuronal excitability at ~75 msec before movement is at least 50% earlier than the ~50 msec estimated by facilitation of the H-reflex during preparation for an isometric contraction (Day et al., 1983; Pierrot-Deseilligny et al., 1971; Gottlieb et al., 1970). Importantly, these studies were not performed in a simple RT paradigm. This is a major disadvantage in the assessment of preparatory processes because a simple RT paradigm is the only one that allows participants to prepare fully for upcoming movements. Besides the difference in task (RT vs. non-RT), the discrepency from previous studies may also be due to contraction type (dynamic vs. static).

The second purpose of this study was to determine if motoneuronal excitability, cortical excitability, or both

contribute to task-dependent increases in corticospinal excitability during preparation for complex movements. Previous TMS studies have demonstrated that corticospinal excitability (i.e., the MEP) increases as task complexity increases during preparation for both static (Roosink & Zijdewind, 2010; Abbruzzese et al., 1996; Flament et al., 1993) and dynamic (Kennefick et al., 2019) movements; however, no previous studies have examined the influence of motoneuronal excitability on this response. As seen in Figure 4, this is the first study to show that motoneuronal excitability increases with complexity during the premotor period, indicated by larger CMEP in the two-movement condition compared with the one-movement condition ($p = .037$) and in the three-movement condition compared with one-movement condition ($p = .038$). The CMEP is influenced by descending drive from the cortex (e.g., McNeil, Martin, Gandevia, & Taylor, 2011; Martin et al., 2006) so the increase in CMEP size with movement complexity is undoubtedly affected by change at a higher center (or centers). However, as the TS to elicit a CMEP is delivered below the level of the cortex, CMEP size does not reflect excitability of the cortical neurons that contribute to the MEP. Hence, the failure of the normalized MEP to show a modulation with complexity or time (Figure 6) indicates that the excitability of these cortical neurons is not affected strongly by the movement preparation phase of this simple RT task. This is not to suggest that cortical regions are unaffected by movement complexity, but it reinforces the idea that the cortical neurons activated by TMS appear to include a population distinct from those used to control voluntary motor output (McNeil et al., 2011). Furthermore, it cannot be excluded that the nonfocal nature of the round TMS coil may have influenced the MEP data via activation of brain areas projecting to the primary motor cortex.

The corticospinal tract is responsible for a broad cortical modulation of motoneuronal output. Specifically, this tract is heavily implicated in the control of afferent inputs, spinal reflexes, and motoneuronal activity (Lemon & Griffiths, 2005). In the current study, we have shown that CMEP amplitude increases in concert with movement complexity. Although the CMEP represents the excitability of the corticospinal tract at the motoneuronal level, it is important to consider that the control of movement is also mediated by other descending tracts such as the tectospinal, rubrospinal, reticulospinal, and vestibulospinal descending tracts (Waldman, 2009), which the stimulation techniques used in the current study cannot probe. The vestibulospinal tract can function to maintain accuracy in complex voluntary tasks, such as reaching, by encoding self-motion relating to the head in space. Recently, there has been a growing body of evidence implicating the vestibulomotor system in the online control of arm movement, regardless of whether participants were seated (Smith, Allsop, Mistry, & Reynolds, 2017; Smith & Reynolds, 2017; Moreau-Debord, Martin, Landry, & Green, 2014; Mars, Archambault, & Feldman, 2003; Bresciani, Blouin, Popov, Sarlegna, et al., 2002) or standing (Bresciani, Blouin, Popov, Bourdin, et al., 2002). This ultimately provides essential information to the CNS for movement and balance control (Cullen, 2012) via input to the vestibular nuclei originating from cortical, cerebellar, and other brainstem areas (Cullen, 2016). Furthermore, there is evidence to suggest that spinal motoneurons have direct or indirect (via the motor cortex) connections to premotor areas capable of influencing movement (Dum & Strick, 2002). As such, although motoneuronal excitability increased as a function of movement complexity, it is possible that subcortically mediated processes or input from premotor areas could influence CMEP (or MEP) size. The experimental design of this study precludes investigation of these possibilities so additional studies are needed to explore how complexity influences the role of other brain areas in movement preparation.

In conclusion, a purpose of this study was to establish the time course of motoneuronal excitability before the onset of movement in a simple RT task. A second purpose was to describe the task-dependent effect of increases in movement complexity on motoneuronal and cortical excitability. The current study addressed methodological limitations in previous studies to suggest that, in the preparation phase before a simple RT movement, motoneuronal excitability increases at least 50% earlier than previously measured. We also observed a significant increase in CMEP amplitude with increasing movement complexity. When the MEP amplitude was normalized to the CMEP amplitude, the data suggested that enhanced motoneuronal excitability is the principal mechanism for the previously observed increases in MEP size with movement complexity. Further research should explore the possibility that movement complexity-based increases in corticospinal excitability involve a contribution from subcortical (e.g., the brainstem) or premotor areas above the level of the motoneurons.

Author Contributions

M. K., P. V. D., and C. J. M. contributed to the conception and design of the experiment. M. K., J. S. B., and C. J. M. contributed to collection, analysis, and interpretation of data. M. K. drafted the manuscript. All authors revised the manuscript critically for important intellectual content, read, and approved the final submission.

Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council of Canada (DG 435912-2013 and DG 04412-2017) and the Canada Foundation for Innovation/British Columbia Knowledge Development Fund (30979 and 32260).

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