EXAMINING CORTICAL INVOLVEMENT IN THE STARTREACT EFFECT USING TRANSCRANIAL MAGNETIC STIMULATION

by

Andrew James Stevenson

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Abstract

The goal of this thesis was to examine the subcortical storage and triggering hypothesis proposed by Valls-Solé et al. (1999) and Carlsen et al. (2004b). This hypothesis suggests movements that can be prepared in advance of an imperative stimulus are stored and triggered from subcortical areas without cortical involvement by a startling acoustic stimulus (SAS). The rapid release of prepared movements by a SAS has been termed the StartReact effect, and premotor reaction times (PMTs) <70 ms are often observed. We used transcranial magnetic stimulation (TMS) to probe cortical involvement in the StartReact effect.

In Experiment 1 we examined whether a TMS-induced cortical silent period could delay the release of movement by a SAS. Thirteen participants performed 20° wrist extension movements as fast as possible in response to either a control tone (82.3 dB) or a SAS (123.2 dB). During selected control and startle trials, suprathreshold TMS was delivered to the contralateral primary motor cortex (M1) 50 or 70 ms prior to mean PMT. Startle PMTs were faster than control PMTs, while TMS significantly delayed movement onset in control and startle trials compared to No TMS or Sham TMS conditions. Additionally, TMS facilitated the size of the first agonist burst (AG1) in both control and startle trials.

Experiment 1 provided evidence for the involvement of M1 in the StartReact effect, however, it was possible that suprathreshold TMS disrupted subcortical startle reflex pathways. Experiment 2 utilized subthreshold TMS to M1, which has been shown to reduce ongoing EMG activity during a voluntary contraction by activating inhibitory cortical interneurons and not descending motor pathways (Davey, et al., 1994). TMS was delivered to the contralateral M1 during AG1 activity in random control and startle trials. Despite all seven participants displaying TMS-induced suppression in isometric wrist extension, EMG suppression was observed in only one participant in control and startle trials. Data are discussed with respect to a recent model of preparation and initiation proposed by Carlsen et al. (in press).

Preface

This thesis details the experimental findings of studies conducted in the Motor Control and Learning Laboratory at the University of British Columbia. All work was conducted under the supervision of Dr. Ian M. Franks and in collaboration with my Thesis Committee, including Dr. Dana Maslovat, Dr. Romeo Chua, and Dr. Jean-Sébastien Blouin.

For both experiments, I was responsible for the majority of the experimental design, data collection, data analysis and manuscript preparation. Dr. Dana Maslovat and Dr. Romeo Chua were the principle programmers for both of the experiments. Assistance with physical set-up of the hardware and technical support was provided by Dr. Dana Maslovat (both experiments) and Paul Nagelkerke (Experiment 1).

Ethical approval was obtained for both studies from the Behavioural Research Ethics Board at the University of British Columbia. Certificate Number: #H10-01354

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List of Abbreviations

AG1: initial agonist burst

AG2: second agonist burst

ANOVA: analysis of variance

ANT: antagonist burst

ECR: extensor carpi radialis

EMG: electromyography

FCR: flexor carpi radialis

HSD: honestly significant difference

IS: imperative stimulus

LL: lateral lemniscus

M1: primary motor cortex

MEP: motor evoked potential

MSO: maximum stimulator output

nRPC: nucleus reticularis pontis caudalis

OOc: orbicularis oculi

PMT: premotor reaction time

PPI: prepulse inhibition

Q100: integrated EMG over first 100 ms of muscle activity

Q20-50: integrated EMG between 20-50 ms of muscle activity after EMG onset

Q30: integrated EMG over first 30 ms of muscle activity

RT: reaction time

SAS: startling acoustic stimulus

SCM: sternocleidomastoid

SEM: standard error of mean

SP: silent period

StartReact: rapid release of a preplanned movement by a startling acoustic stimulus

TMS: transcranial magnetic stimulation

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It gives me great pleasure to acknowledge a number of individuals, without whom the completion of this thesis would not have been possible. First and foremost, I offer my sincerest gratitude to my supervisor, Dr. Ian Franks. His constant advice, encouragement, and opportunities to learn and ask questions along every step of the way have been truly invaluable.

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to my fiancée, Karina,

and my family, Ross, Lynn & Matt

1 Chapter: General Introduction

Movement preparation and initiation has received a great deal of attention in motor control literature (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004b; Day et al., 1989; Henry & Rogers, 1960; Rosenbaum, 1980; Valls-Solé, Rothwell, Goulart, & Cossu, 1999; Wadman, van der Gon, Geuze, & Mol, 1979). Researchers have used the metaphor of a motor program to help describe the processes involved in the preparation and execution of motor acts. Wadman et al. (1979) tested the concept of a motor program whereby fast elbow extension movements were unexpectedly blocked. When movements were not blocked a characteristic triphasic EMG pattern emerged (Hallett, Shahani, & Young, 1975) where the triceps (agonist), biceps (antagonist) and triceps accelerated, decelerated and then clamped the forearm in place. Interestingly, when the movement was blocked, EMG data for the first 100 ms after the initial agonist onset was almost identical to that of the unblocked trial for both the triceps and the biceps. However, after 100 ms, the EMG pattern was modified by proprioceptive feedback. These results led Wadman et al. (1979) to suggest that movements could be prepared in advance of a "go" signal and fast movements can be pre-programmed from central areas at least for the first 100 ms.

More recently, a methodology for examining the nature of these pre-programmed responses without problems associated with proprioceptive feedback has utilized the startle reflex (for recent reviews see Carlsen, Maslovat, & Franks, in press; Carlsen, Maslovat, Lam, Chua, & Franks, 2011; Valls-Solé, Kumru, & Kofler, 2008). Using a wrist movement in response to a visual imperative stimulus (IS), Valls-Solé et al. (1999; 1995) sometimes presented a very loud (>130 dB) startling acoustic stimulus (SAS) at the same time as the IS. These "startle" trials produced extremely fast premotor reaction times (PMT; time from the onset of the IS to the onset of the voluntary muscle activity) without changing the whole triphasic EMG pattern or the movement kinematics.

The time course of such reactions following a SAS (PMTs < 70 ms; Carlsen, et al., 2004b; Valls-Solé, et al., 1999) was hypothesized to be too fast to invoke cortical activity. It has been shown that it takes 35 ms for the stimulus to reach the auditory cortex (Erwin &

Buchwald, 1986) and then 20-30 ms for a signal to be transmitted to the peripheral muscles (Rothwell, 1997), leaving very little time, if any, for cortical processing. PMTs for startle trials contrast to normal PMTs of around 180 ms in response to visual stimuli and PMTs of 140 ms in response to acoustic stimuli (Brebner & Welford, 1980). These conduction time findings led to the subcortical triggering hypothesis (Carlsen, et al., 2004b; Valls-Solé, et al., 1999), which suggests that, at least for simple movements, sufficient detail of the movement is prepared and stored in advance, and then triggered by the startling stimulus from subcortical areas with limited involvement of the cortex. The reticular formation is an area in the brainstem common to both the startle reflex and voluntary movement pathways (Rothwell, MacKinnon, & Valls-Solé, 2002; Yeomans & Frankland, 1995). The giant neurons of the pontine reticular system (RPC) have been implicated as important contributors to the startle response, and are activated at very short latencies following a startling stimulus (Yeomans & Frankland, 1995). Additionally, the reticular system consists of many connections between various cortical and spinal systems, including cortical inputs, interneurons, and motor outputs (Hallett, 2002). Because cell assemblies have been hypothesized as a neural mechanism for motor programs (Wickens, Hyland, & Anson, 1994), Carlsen et al. (2004b) suggested that cell assemblies located in the reticular formation might provide a mechanism for subcortical program storage.

Carlsen et al. (2004a) provided further evidence for the subcortical triggering hypothesis by examining the effects of a SAS in both simple and choice reaction time (RT) paradigms. In a choice RT task, response selection and response programming must occur during the PMT interval, whereas in a simple RT task, response programming can occur before the IS and response selection is not required. If the appropriate response has to be selected from several alternatives (i.e., choice RT), the response cannot be programmed in advance. Additionally, neurophysiological studies have provided evidence for cortical involvement in the execution of movements requiring a choice of action (see Schluter, Krams, Rushworth, & Passingham, 2001; Schluter, Rushworth, Passingham, & Mills, 1998). As found previously, in the simple RT condition the prepared voluntary response was elicited at very short latencies following the startle. However, because choice RT involved cortical processing and the correct response could not be prepared in advance, startle did not facilitate the voluntary response

and gave rise to more movement production errors. The authors argued that, since movements requiring ongoing cortical processing following the IS were not facilitated by a SAS, it was unlikely that the startle facilitation of prepared movements resulted from a general increase in neural activation of the system.

Another possible explanation for what has been termed the StartReact effect (Valls-Solé, et al., 1999) is that an increase in stimulus intensity might cause this drastic decrease in RT (see Piéron, 1919, cited in Woodworth, 1938, p. 318). Carlsen et al. (2007) tested this hypothesis by varying the intensity of the IS between 83 and 123 dB and measuring the orbicularis oculi (OOc) and sternocleidomastoid (SCM) muscles as indicators that a startle response had been elicited in the participants. They found that when an OOc response was observed in the absence of SCM activity, PMT decreased as the IS became more intense. However, when SCM activity was observed, there was a dramatic reduction in PMT regardless of the intensity of the IS. This provided evidence that PMTs associated with a startle response were separate from stimulus intensity facilitated responses, and that this startle facilitation was more highly associated with SCM EMG activity rather than OOc (see Carlsen, et al., 2011).

Carlsen et al. (2009) conducted one further test of the subcortical triggering hypothesis. They examined the difference in PMT following the presentation of a SAS between muscles involving heavy dependency on corticomotoneuronal connections (e.g., intrinsic hand; Baker & Riddle, 2007) and muscles with innervations including extensive reticulospinal pathways. Participants performed either ballistic index finger abduction or arm extension movements in a startle paradigm where the SAS was set to an intensity that would elicit a startle response (SCM activity) on around 50% of startle trials. In the arm extension task, the presence of a startle response led to significantly shorter PMTs compared to the effect of increasing the stimulus intensity alone (Carlsen, et al., 2007). In the finger abduction task, however, there was no additional decrease in PMT when a startle response was observed above the effect of increasing the intensity of the IS. These results also supported the subcortical triggering hypothesis in the rapid release of a prepared movement by a SAS because only movements that involved muscles more strongly innervated by subcortical pathways were subject to this rapid release.

The primary goal of this thesis was to examine the subcortical storage and triggering hypothesis put forward by Valls-Solé et al. (1999) and Carlsen et al. (2004b). Experiment 1 directly examined the involvement of the primary motor cortex (M1) in the mediation of the StartReact effect by examining whether a cortical silent period (SP) elicited by suprathreshold transcranial magnetic stimulation (TMS) could delay the release of movement by a SAS. Experiment 2 further examined the involvement of M1 in the StartReact effect by investigating whether the activation of inhibitory interneurons within M1 by subthreshold TMS could cause suppression in the initial agonist EMG burst following the rapid release of movement by a SAS.

2   Chapter: Experiment 1 – Suprathreshold TMS

2.1   Introduction

While there has been growing evidence for subcortical storage and triggering, one methodology that has the potential to directly examine the involvement of M1 in the rapid triggering of a movement by a SAS is TMS. Recently, Alibiglou et al. (2009) examined the subcortical triggering hypothesis by investigating whether a single, suprathreshold pulse of TMS delivered to M1 could influence the StartReact effect. These authors found that the presentation of a SAS alone at -200 ms (200 ms before the IS in a fixed-foreperiod simple RT task) resulted in the early release of movement an average of 71.7 ms after the stimulus. Furthermore, the application of TMS prior to control voluntary movements resulted in a significant delay in PMTs. Importantly, they also found that the application of TMS prior to SAS-induced movements resulted in a significant delay in the early release of movement. In addition, the onset of SCM activity following a SAS, used as the indicator of startle, was not affected by TMS. The authors concluded that M1 does mediate the StartReact effect, while the TMS pulse did not influence the subcortical startle reflex pathways.

The premise of the methodology used by Alibiglou et al. (2009) was that the delay in SAS-induced movements and in voluntary reaction time due to TMS to M1 is caused by cortical mechanisms. There is a large amount of evidence to suggest that this is a cortical effect. One of the first studies to show that brain stimulation to M1 could affect its neuronal processes was by Day et al. (1989). They delivered either electrical or magnetic suprathreshold stimulation over the contralateral M1 during the premotor RT period in a simple RT wrist movement task. Electrical or magnetic stimuli to the contralateral M1 delayed the execution of the movement for up to 150 ms, without affecting the pattern of the agonist and antagonist EMG bursts. Additionally, the delay increased when stimulus intensity increased and with stimuli that were applied nearer to the usual time of onset of the voluntary reaction. Day et al. (1989) suggested that "the brain stimulus delayed movement by inhibiting a group of strategically placed neurons in the brain (probably in the motor cortex) which made them

unresponsive for a brief period to the command signals they receive which initiate the motor program of agonist and antagonist muscle activity" (p. 649).

In addition to transcranial stimulation during the premotor RT period lengthening RT (Burle, Bonnet, & Vidal, 2002; Day, et al., 1989; Romaiguère, Possamaï, & Hasbroucq, 1997; Schluter, Rushworth, Mills, & Passingham, 1999; Schluter, et al., 1998; Ziemann, Tergau, Netz, & Hömberg, 1997), Hashimoto et al. (2004) also found that there was an increase in MEPs (a direct measure of cortical excitability; Reis et al., 2008) and integrated response EMG. Whereas Day et al. (1989) showed that peripheral electrical stimulation to the muscles decreased the size of response EMG activity, Hashimoto et al. (2004) argued that suprathreshold TMS of M1 centrally disturbed motor commands (motor programs) due to an increase in the integrated EMG caused by suprathreshold TMS.

It has been suggested that the effects of TMS on RT are positively correlated with those seen in the duration of the cortical silent period (SP; Ziemann, et al., 1997). A SP is defined as the suppression in the ongoing EMG activity following a MEP caused by a suprathreshold TMS pulse applied to M1 during voluntary muscle contraction of the contralateral target muscle, and can last up to 300 ms (Inghilleri, Berardelli, Cruccu, & Manfredi, 1993). Ziemann et al. (1997) found that there is a positive correlation between the duration of the SP and the maximum TMS-induced delay in RT. The first 50 ms of the SP is thought to depend on spinal inhibitory mechanisms such as recurrent inhibition and after-hyperpolarization of spinal motoneurons, while the later part of the SP (> 50 ms) is produced mainly by cortical inhibitory mechanisms, such as activating cortical inhibitory interneurons, and an interruption of voluntary cortical drive (Chen, Lozano, & Ashby, 1999; Fuhr, Agostino, & Hallett, 1991; Inghilleri, et al., 1993; Ni, Gunraj, & Chen, 2007; Ziemann, Netz, Szelényi, & Hömberg, 1993). For example, Ziemann et al. (1993) tested the excitability of spinal motoneurons during the SP using the Hoffmann (H) reflex. They found that the amplitude of the H wave was decreased for the first 50 ms of the SP but was no longer suppressed in the later part of the SP. Ziemann et al. (1997) also studied F waves (Espiritu, Lin, & Burke, 2003) in their agonist muscle during the TMS-induced RT delay to probe the spinal motoneuron pool excitability as a possible source of the delay in RT. In trials without TMS

they found that mean F wave amplitudes were increasingly facilitated from 60 ms prior to response onset. When TMS was applied 50 ms prior to the individual expected voluntary response, a very similar pre-movement facilitation occurred during the period of RT delay and the F waves were consistently larger in amplitude. Therefore, they concluded that inhibition of the spinal motoneuron pool is unlikely to play a major role in delaying the RT produced by TMS over the motor cortex.

The goal of Experiment 1 was to extend the work of Alibiglou et al. (2009) and examine the subcortical triggering of prepared movements by a SAS using TMS to probe involvement of the contralateral M1. We adapted the paradigm used by Day et al. (1989) and Hashimoto et al. (2004) to that used by Alibiglou et al. (2009) and delivered suprathreshold TMS to the contralateral M1 *during* the PMT period in a simple RT task with either a control tone (82.3 dB) or a startling acoustic stimulus (123.2 dB) as the IS. It was expected that suprathreshold TMS would delay PMT in control trials and also increase the size of the first agonist EMG burst. Our primary interest, however, was the effect of TMS on startle trials, as the subcortical triggering hypothesis would predict that TMS would have no effect on the StartReact effect.

2.2   Methods

2.2.1   Participants

Eighteen participants with no obvious upper body abnormalities or sensory or motor dysfunctions volunteered to participate in this study. All participants gave written informed consent, and the study was conducted in accordance with the ethical guidelines set by the University of British Columbia. Participants were right handed based on a laterality quotient greater than .60 on the Edinburgh Handedness Inventory (Oldfield, 1971). However, only data from thirteen participants (6 male, 7 female; age 22 ± 3 years) were employed in the final analysis. Five participants did not show activation in the sternocleidomastoid (SCM) muscle during any startle trials (which is thought to be the most reliable indicator of a startle

response), and thus were excluded from the analysis (see Carlsen, et al., 2011 for more detail regarding the exclusion criteria for participants).

2.2.2 Experimental Task and Acoustic Stimuli

Participants were required to respond as quickly as possible to each presentation of an auditory stimulus by initiating a ballistic right wrist extension movement. The participants sat in a height-adjustable chair facing a table, with their right arm secured in a semi-prone position with the palm facing inward to a custom-made aluminum wrist manipulandum that moved in the transverse plane with an axis of rotation at the wrist joint. The arm portion of the manipulandum was oriented at an angle of 15º outward from the body midline, as this has been found to be a more comfortable position than an orientation parallel to the body midline. The wrist starting position was neutral (neither flexion nor extension) and was indicated by tactile feedback (using a magnet as a noncontact detent). The imperative stimulus (IS) followed the warning tone and could either consist of a control stimulus (82.3 ± 0.9 dB, 100 ms, 1000 Hz) or startling stimulus (123.2 ± 0.5 dB, 40 ms, 1000 Hz, <1 ms rise time). All auditory signals were generated by a customized computer program and were amplified and presented via a loudspeaker placed directly behind the head of the participant. The acoustic stimulus intensities were measured using a sound level meter (Cirrus Research model CR: 252B) set to measure impulse at a distance of 30 cm from the loudspeaker (approximately the distance to the ears of the participant).

The experimental task performed was an active right wrist extension as fast as possible to a target region located at 20º of angular displacement from the starting position. Participants were informed that they would first hear a warning tone indicating the start of a trial. Following this warning tone, the IS was presented with a variable foreperiod between 1500 and 2500 ms. The warning tone (100 ms, 1000 Hz, 80 dB) was generated by the computer using a 16 bit sound card (Creative SoundBlaster 16®) and standard computer speakers (Juster® sp-691n). Instructions were to move "as fast and as accurately as possible" from the starting position and stop on the target. Similar to previous experiments in our laboratory

(e.g., Carlsen, et al., 2004b) a monetary bonus of 10 cents (Canadian) was offered per trial for beating a prescribed minimum RT.

2.2.3   Instrumentation

Electromyographic (EMG) data were collected from the right wrist prime movers: the extensor carpi radialis longus (ECR) and the flexor carpi radialis (FCR), as well as bilaterally from the startle indicator SCM. The recording sites were prepared and cleansed to decrease electrical impedance, and bipolar preamplified Ag/AgCl surface EMG electrodes were attached in the middle of the muscle bellies parallel to the line of force to the muscles. These electrodes were connected via shielded cabling to an external amplifier system (model 544, Therapeutics Unlimited). A grounding electrode was placed on the participants' left radial styloid process. Wrist angular displacement data were collected using a potentiometer attached to the pivot point of the manipulandum. All raw data were digitally sampled for 2 s at 1 kHz (PCI-6023E, National Instruments) using a personal computer running a customized program written with LabVIEW software (National Instruments). Data collection was automatically initiated 500 ms before the IS.

2.2.4   Transcranial Magnetic Stimulation

Single-pulse TMS was delivered using a Magstim 200 stimulator (Magstim Company, Dyfed, UK, maximum output intensity 2.0 T) through a figure-eight coil (each external diameter 7.0 cm). The coil was handheld and oriented tangentially to the scalp and perpendicular to the central sulcus so that the induced current flow was in a posterior to anterior direction (Werhahn et al., 1994). The optimal 'hot spot' for eliciting MEPs in the right ECR (agonist muscle for the response) was determined by stimulating over the contralateral M1. Because voluntary muscle contraction has been found to enhance corticospinal excitability, the participants were required to be relaxed with no muscular contraction and to fixate their eyes directly ahead (Hess, Mills, & Murray, 1987; Thompson et al., 1991) while the 'hot spot' was found. Once a 'hot spot' was identified, the site was stimulated four times in order to confirm whether it was an optimal 'hot spot' where

maximum MEPs could be evoked. This site was marked to ensure consistent coil placement. Rest motor threshold in the ECR was then determined to the nearest 1% of maximum stimulator output (MSO) where a minimum 50 μV MEP was evoked in 5 of 10 consecutive trials (Rossini et al., 1994). To set the experimental intensity of the TMS, participants maintained an isometric contraction in the ECR of 10% maximum voluntary contraction. The intensity of the magnetic stimulus was adjusted to produce a SP, defined as the time from the start of the MEP to the return of background EMG, in the ongoing voluntary EMG of approximately 150 ms (Ridding and Taylor, 2001; Smith et al., 2007).

2.2.5   Experimental Procedure

After all of the participant's questions were answered and written informed consent was obtained, participants were seated 1.0 m from a 21-in. LCD monitor which displayed the RT from the previous trial, any monetary bonus gained, and the cumulative total of bonuses. Next, the participant's right arm was strapped into the manipulandum and EMG sensors attached. Once TMS stimulation intensities were found, participants were first exposed to one startle trial while at rest, followed by one trial each of isometric maximum voluntary contraction for wrist extension and flexion (each lasting 2 s). To ensure consistent and fast reaction times, participants then practiced the task for 50 trials. The criteria for consistent RTs was a stable mean RT and a SD < 15 ms for the previous 10 trials. More trials were given if the criteria were not met. Once RTs were consistent, participants performed a block of 25 trials consisting of 20 control tone (82.3 dB) trials and 5 startle (123.2 dB) trials. Mean PMT, defined as the time from the onset of the IS to the onset of ECR EMG activity, was calculated separately for control trials and startle trials in this block after each trial. The timing of TMS delivery was based on individual participants' mean control and startle PMTs (see below).

At the conclusion of the experiment, the participants' arm was removed from the manipulandum, EMG sensors were removed, and the participants were debriefed.

2.2.6   Experimental Design

The experiment included seven conditions. Because TMS emits an audible and vibratory click, a Sham condition was also used where the TMS coil was held perpendicular to the scalp. In this condition, the click of the TMS was heard and vibration felt but no neural tissue was stimulated. The seven conditions were:

A)   Control Trials (40 trials, Figure 2.1 A): The IS for these trials was a control (82.3 dB) tone and there was no TMS stimulation.

B)   Control + TMS (50 trials, Figure 2.1 B): The IS for these trials was a control tone, and TMS was delivered 50 (25 trials) or 70 (25 trials) ms before each participant's mean control PMT.

C)   Control + Sham TMS (30 trials, Figure 2.1 C): The TMS coil was held perpendicular to the scalp at the same site of stimulation and was delivered 50 (15 trials) or 70 (15 trials) ms before each participant's mean control PMT. This ensured that no neural tissue was stimulated but the participant could hear the sound and feel the vibration emitted by TMS.

D)   SAS (8 trials, Figure 2.1 D): The IS for these trials was a SAS, and there was no TMS stimulation.

E)   SAS + TMS (10 trials, Figure 2.1 E): The IS for these trials was a SAS, and TMS was delivered 50 (5 trials) or 70 (5 trials) ms before each participant's mean control PMT.

F)   SAS + Sham TMS (6 trials, Figure 2.1 F): The IS for these trials was a SAS, and the TMS coil was held perpendicular to the scalp (see Condition 3). TMS was delivered 50 (3 trials) or 70 (3 trials) ms before each participant's mean control PMT.

G)   Catch (16 trials, Figure 2.1 G): Trials with no IS or TMS stimulation, two in each block.
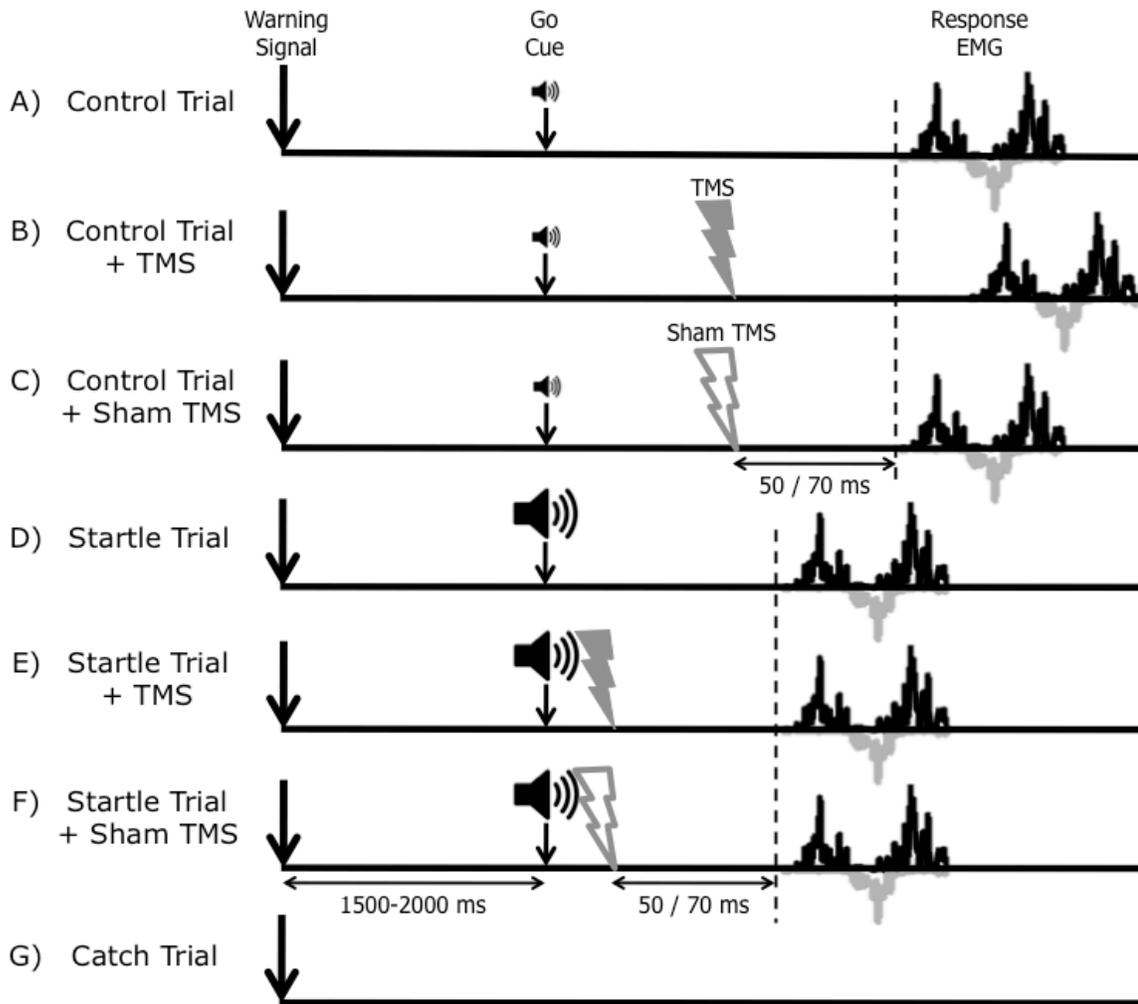
Figure 2.1   Timing of critical events in the experimental conditions. Arrows indicate the timing of warning and imperative stimuli (there was a random foreperiod between warning and imperative stimuli). The small speakers represent control (82.3 dB) trials and the large speakers represent startle (123.2 dB) trials. The filled lightning represents the timing of direct TMS stimulation and the open lightning represents Sham TMS conditions. The triphasic response EMG pattern depicted in each condition is the same EMG from a single representative (control) trial, with the black line showing ECR activity (positively rectified) and the grey line showing FCR activity (negatively rectified). The timing of the triphasic EMG in this diagram shows where the response was hypothesized to occur in each condition. [This figure was adapted from Alibiglou et al. (2009)].

160 movement trials were performed in total, separated across eight experimental blocks of 20 trials. Three blocks were Sham TMS blocks with the coil held perpendicular to the scalp. The customized computer program responsible for data collection determined Sham TMS blocks randomly. Each block contained five control trials, five control + TMS (TMS present or Sham) trials at each timing, one SAS trial, one SAS + TMS (TMS present or Sham) trial at each timing, and two catch trials. The order was pseudo-randomized such that SAS conditions could not be the first in a block or occur twice in a row. The approximate trial-to-trial interval was 10 s, although this varied because of the random foreperiod.

2.2.7   Data Reduction

Analysis was restricted to the testing trials only (practice trials were not analyzed). A total of 100 of the 2080 trials were discarded (4.8%). Reasons for discarding trials included displacement reaction times less than 80 ms (i.e., anticipation, 9 trials) or in excess of 400 ms (i.e., inattentive participants, 40 trials), incorrect movements (30 trials), and startle trials in which no detectable startle response (SCM activity) was observed (21 trials) (see Carlsen, et al., 2011).

EMG data were analyzed off-line via a custom LabVIEW program. EMG signals were filtered using a dual-pass elliptical filter with a low-pass frequency setting of 25 Hz. Movement onset was determined as the first change in angular displacement by 0.2 degrees following the go signal.

Surface EMG burst onsets were defined as the point at which the EMG first began a sustained rise above baseline levels. The location of this point was determined by first displaying the EMG pattern on a computer monitor with a superimposed line indicating the point at which rectified, filtered EMG activity first increased on the EMG trace by more than 2 standard deviations based on the 100 ms immediately preceding the IS. This method allowed for the correction of potential errors caused by the algorithm. PMT was defined as EMG onset in the ECR muscle for the wrist extension task. PMT was the main measure of RT for this study because it represents an estimate of the total central processing time. EMG

offsets were marked in a similar fashion, using mean EMG activity following the end of the movement as a baseline level to account for any residual time between bursts. These were also verified and manually adjusted, with the activity between EMG onset and EMG offset defined as a distinct burst.

Integrated EMG values for the first agonist (ECR) EMG burst were computed by numerically integrating the normalized and rectified EMG for each participant. First the EMG was rectified, amplitude and baseline normalized, and onset normalized (aligned to a common onset). These values were numerically integrated (ms time base) over the duration of the EMG burst (from onset to offset) from the common onset (0 ms). A subset of the first 30 ms (Q30) was also taken consisting of 0-30 ms to capture the initial burst. The Q30 was also calculated for the SCM muscle EMG within SAS trials.

2.2.8   Statistical Analysis

All dependent measures were analyzed with separate within-subjects analyses of variance (ANOVAs) for the TMS present condition and Sham TMS condition because the No TMS timing acts as a control for both conditions. To compare EMG patterns, burst onsets and durations were calculated. The onset of the first agonist burst (AG1, ECR) was measured from the time of the onset of the "go" stimulus and represented premotor reaction time (PMT). Onset of the antagonist (ANT, FCR) and second agonist burst (AG2, ECR), were calculated as the time from the onset of the AG1, as this allowed for determination of the relative timing of the triphasic EMG pattern. Muscle onsets, burst durations, and integrated AG1 EMG (Q30s and Q100s) values were analyzed independently using a two (acoustic stimulus; control, startle) by three (TMS timing; No TMS, PMT -50 ms, PMT -70 ms) within-subjects ANOVA. SCM onsets, durations and Q30 values were analyzed using one-way (TMS timing; No TMS, PMT -50 ms, PMT -70 ms) within-subjects ANOVAs. Greenhouse-Geisser corrected degrees of freedom were used to correct for violations of the assumption of sphericity. Differences with a probability of < 0.05 were considered significant. Partial eta squared (η_p^2) values are reported as a measure of effect size. Tukey's

honestly significant difference (HSD) post hoc tests were administered to determine the locus of the differences.

2.3   Results

A summary of the results for all dependent measures, including means and standard deviations, are provided in Table 1. The main results of Experiment 1 were that TMS delayed PMT and modulated the size of the first agonist burst in both control and startle trials, while TMS did not modulate the startle response (SCM activity) in startle trials.

Table 2.1   **Experimental results for each TMS condition (TMS and Sham TMS), stimulus type and TMS timing, showing means and standard deviations (in brackets). AG1 = initial agonist burst (ECR), ANT = antagonist burst (FCR), AG2 = second agonist burst (ECR).**

| | TMS Condition | | | | | |
| | *Control* | | | *Startle* | | |
Variable	*No TMS*	*TMS -50*	*TMS -70*	*No TMS*	*TMS -50*	*TMS -70*
Premotor RT (ms)	146.7 (26.8)	227.6 (40.4)	205.1 (38.1)	83.5 (12.3)	148.3 (40.7)	127.7 (32.6)
AG1-ANT time (ms)	75.5 (10.4)	53.1 (13.1)	54.0 (7.8)	63.3 (14.3)	49.6 (14.7)	51.1 (9.0)
AG1-AG2 time (ms)	138.9 (31.5)	124.1 (22.5)	128.6 (22.0)	118.2 (32.2)	113.6 (22.5)	117.8 (23.1)
AG1 Duration (ms)	93.1 (23.1)	75.7 (18.7)	81.0 (18.0)	78.7 (31.3)	72.7 (18.1)	79.1 (17.3)
ANT Duration (ms)	75.8 (28.7)	73.0 (27.1)	74.2 (26.6)	75.7 (37.1)	69.2 (26.1)	73.2 (27.8)
AG2 Duration (ms)	98.4 (26.4)	85.4 (24.7)	89.2 (23.8)	85.6 (30.2)	85.1 (26.1)	88.3 (18.1)
AG1 Q30 (mV*ms)	6.5 (2.8)	8.2 (2.8)	9.0 (3.2)	7.3 (3.3)	9.6 (3.4)	9.7 (4.2)
AG1 Q100 (mV*ms)	19.7 (8.1)	22.1 (7.8)	23.6 (7.1)	21.3 (7.6)	27.0 (9.0)	26.6 (9.9)
SCM Onset (ms)				63.3 (16.9)	68.8 (19.6)	59.0 (12.5)
SCM Duration (ms)				40.1 (17.0)	36.8 (9.6)	38.4 (12.5)
SCM Q30 (mV*ms)				0.45 (0.07)	0.43 (0.04)	0.43 (0.06)

| | Sham TMS Condition | | | | | |
| | *Control* | | | *Startle* | | |
Variable	*No TMS*	*TMS -50*	*TMS -70*	*No TMS*	*TMS -50*	*TMS -70*
Premotor RT (ms)	142.0 (22.8)	149.6 (24.9)	137.3 (25.4)	83.9 (16.7)	88.6 (13.5)	83.2 (15.0)
AG1-ANT time (ms)	73.3 (15.2)	68.8 (12.6)	70.6 (15.8)	56.5 (10.6)	60.2 (12.6)	61.3 (17.5)
AG1-AG2 time (ms)	132.0 (26.7)	131.5 (26.1)	137.0 (26.0)	104.1 (28.2)	115.8 (26.5)	122.2 (27.8)
AG1 Duration (ms)	79.4 (12.7)	81.5 (19.0)	83.5 (16.3)	67.7 (22.9)	75.0 (18.7)	74.8 (20.4)
ANT Duration (ms)	74.9 (24.0)	77.2 (31.9)	74.9 (29.9)	71.5 (35.9)	71.0 (30.5)	71.9 (27.0)
AG2 Duration (ms)	102.2 (30.3)	90.5 (20.1)	91.0 (19.4)	91.1 (33.2)	90.9 (35.7)	83.5 (24.6)
AG1 Q30 (mV*ms)	6.8 (2.3)	7.9 (2.2)	8.5 (2.5)	8.3 (4.0)	9.1 (4.0)	8.8 (3.5)
AG1 Q100 (mV*ms)	20.7 (8.0)	23.4 (8.1)	22.3 (7.0)	24.6 (10.2)	24.0 (9.2)	23.9 (8.6)
SCM Onset (ms)				58.4 (11.2)	67.9 (17.7)	67.4 (17.4)
SCM Duration (ms)				44.8 (13.7)	40.4 (16.1)	37.9 (16.7)
SCM Q30 (mV*ms)				0.43 (0.04)	0.46 (0.16)	0.42 (0.06)

2.3.1 Transcranial Magnetic Stimulation Parameters

The mean TMS intensity used across participants was 67 ± 2% of MSO (157 ± 16% of rest motor threshold). The mean timing of TMS -50 was 98 ± 28 ms in control trials and 41 ± 19 ms in startle trials, while the mean timing of TMS -70 was 78 ± 28 ms in control trials and 21 ± 19 ms in startle trials.

2.3.2 Premotor Reaction Time

Mean PMT values for control and startle trials are shown in Figure 2.2. As predicted, PMTs for startle trials were faster than control trials for both the Sham and TMS present conditions, and PMTs for control trials were delayed following suprathreshold TMS. However, contrary to the subcortical triggering hypothesis, startle trials were also delayed following suprathreshold TMS. The Sham TMS had no effect on control or startle PMTs. These results were confirmed by a number of main effects. The main effects for stimulus type (startle vs. control) in the TMS present condition, $F(1,12) = 97.07, p < .001, \varepsilon = 1, \eta_p^2 = .89$, and Sham TMS condition, $F(1,12) = 107.64, p < .001, \varepsilon = 1, \eta_p^2 = .90$, revealed that PMTs for startle trials (TMS condition $M = 118$ ms, Sham TMS condition $M = 86$ ms) were faster than for control trials (TMS present condition, $M = 182$ ms; Sham TMS condition, $M = 136$ ms). There was also a significant main effect for TMS timing in the TMS present condition, $F(2,24) = 48.77, p < .001, \varepsilon = 1, \eta_p^2 = .80$. Post-hoc analyses revealed that TMS delivered 50 ms ($M = 178$ ms) and 70 ms ($M = 159$ ms) prior to mean PMT in startle and control trials significantly delayed PMT compared to trials without TMS ($M = 112$ ms), while TMS delivered 50 ms prior to mean EMG onset produced a significantly longer delay than TMS delivered 70 ms prior. There was no significant interaction between stimulus type (startle vs. control) and TMS timing (No TMS vs. -50 ms vs. -70 ms), $F(2,24) = 3.03, p = .067, \varepsilon = .53, \eta_p^2 = .20$, indicating that TMS delayed PMT similarly for both control and startle trials. For the Sham TMS condition, there was no main effect for TMS timing, nor was there a stimulus type by TMS timing interaction effect, indicating that Sham TMS had no influence on PMTs (No TMS, $M = 110$ ms; TMS -50, $M = 115$ ms; TMS -70, $M = 108$ ms).
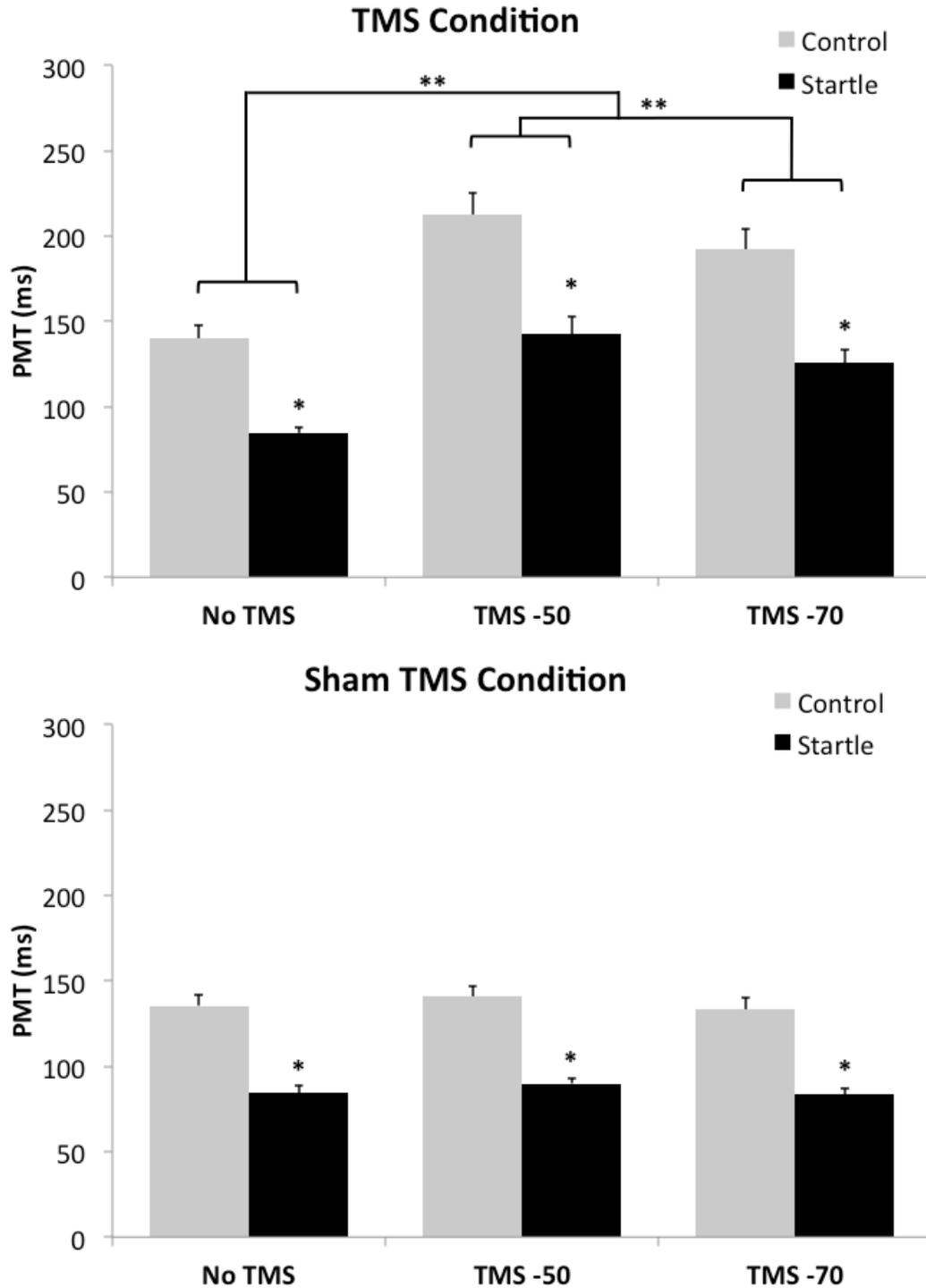
Figure 2.2   Mean premotor RT values (SEM) for control and startle trials separated by TMS timing and TMS condition (top panel = TMS present, bottom panel = Sham TMS). Note the increase in PMT following TMS for both control and startle trials in the TMS present condition (top panel). * denotes an effect of the IS, ** denotes an effect of TMS.

2.3.3 Triphasic EMG Characteristics

Mean AG1 integrated EMG (Q30 and Q100) values for control and startle trials in the TMS present condition are shown in Figure 2.3. For AG1 Q30 in the TMS present condition there was a main effect of stimulus type, $F(1,12) = 22.18$, $p = .005$, $\varepsilon = .99$, $\eta_p^2 = .65$, indicating that the size of the first agonist burst was facilitated during startle trials ($M = 8.2$ mV*ms) compared to control trials ($M = 7.2$ mV*ms). Independent of this modulation, there was also a main effect of TMS timing, $F(2,24) = 11.47$, $p < .001$, $\varepsilon = .99$, $\eta_p^2 = .49$. Post-hoc analyses revealed that TMS delivered 50 ($M = 8.3$ mV*ms) and 70 ms ($M = 8.6$ mV*ms) prior to mean PMT in startle and control trials significantly facilitated the first agonist burst compared to trials without TMS ($M = 6.2$ mV*ms). There was no difference between TMS delivered at either -50 ms and TMS delivered at -70 ms. Similar results were found when analyzing Q100 values for the initial agonist burst in the TMS present condition. There was a significant main effect of stimulus type, $F(1,12) = 12.71$, $p = .004$, $\varepsilon = .91$, $\eta_p^2 = .51$, indicating that the size of the first agonist burst was facilitated during startle trials ($M = 22.4$ mV*ms) compared to control trials ($M = 19.5$ mV*ms). Again, independent of this modulation, there was also a main effect of TMS timing, $F(2,24) = 9.42$, $p = .001$, $\varepsilon = .96$, $\eta_p^2 = .44$. Post-hoc analyses revealed that TMS delivered 50 ms ($M = 21.9$ mV*ms) and 70 ms ($M = 22.4$ mV*ms) prior to mean PMT in startle and control trials significantly facilitated the first agonist burst compared to trials without TMS ($M = 18.4$ mV*ms). There was no difference between TMS delivered at either 50 or 70 ms prior to mean PMT.

For the Sham TMS condition, there was a main effect of stimulus type in the Sham TMS condition for AG1 Q30, $F(1,12) = 4.88$, $p = .047$, $\varepsilon = .53$, $\eta_p^2 = .29$, and Q100, $F(1,12) = 5.80$, $p = .033$, $\varepsilon = .60$, $\eta_p^2 = .33$, indicating that the size of the first agonist burst was facilitated during startle trials (Q30, $M = 8.1$ mV*ms; Q100, $M = 22.0$ mV*ms) compared to control trials (Q30, $M = 6.8$ mV*ms; Q100, $M = 19.3$ mV*ms). As expected, the analysis of AG1 Q30 and Q100 values in the Sham TMS condition showed no main effect for TMS timing (Q30, $p = .187$; Q100, $p = .644$), or any significant interaction effects.
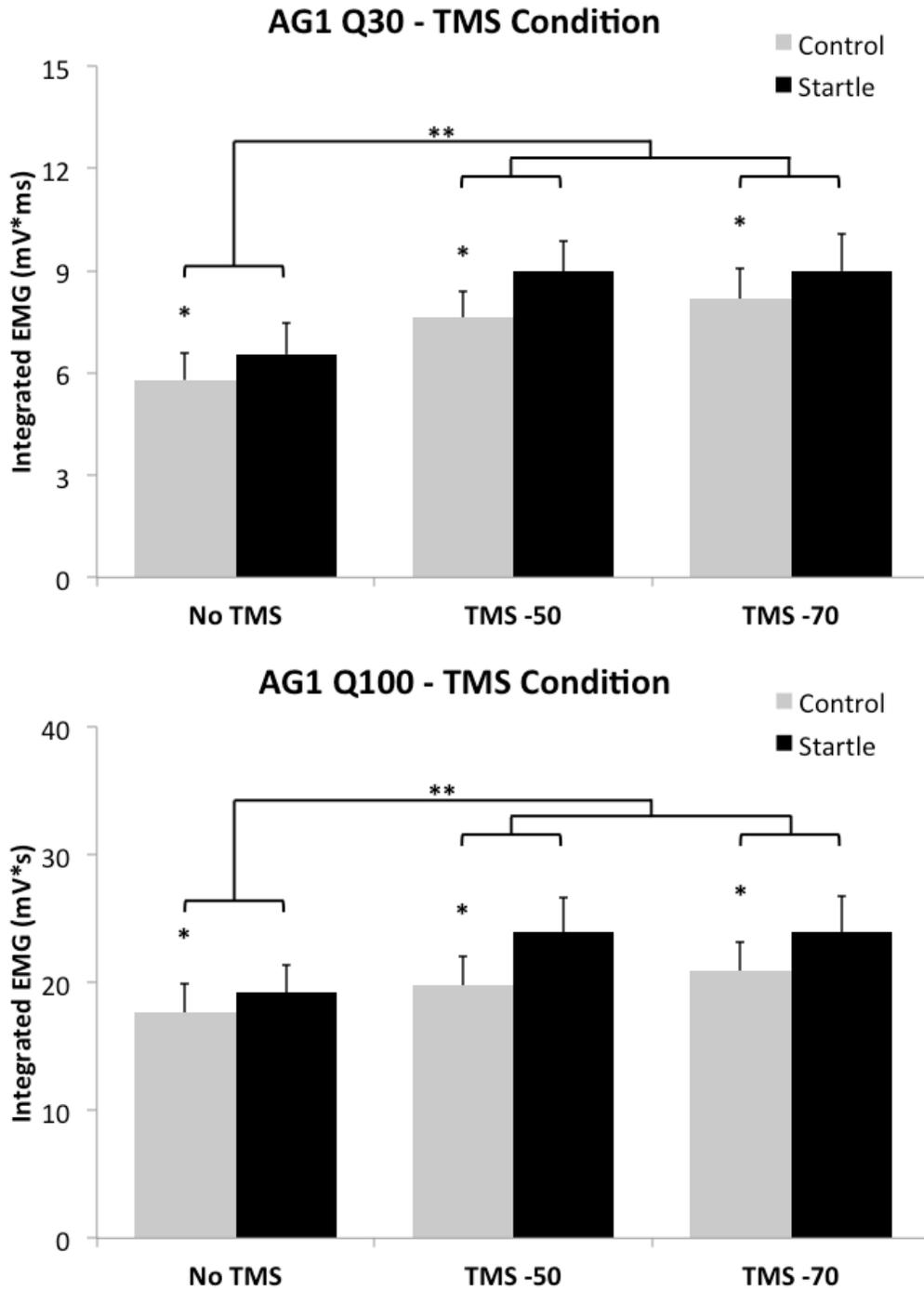
Figure 2.3   Mean AG1 integrated EMG (Q30 = top panel, Q100 = bottom panel) values (SEM) for control and startle trials separated by TMS timing in the TMS present condition. Note the increase in integrated EMG in the TMS -50 and TMS -70 conditions for both control and startle trials. * denotes an effect of the IS, ** denotes an effect of TMS.

EMG boxplots are shown in Figure 2.4 with the relative timing of the triphasic burst for each condition (including SCM activation for startle trials) for the TMS (top panels) and Sham TMS (bottom panels) conditions. These represent grand means for startle and control trials for each TMS timing in each TMS condition, and are normalized with respect to the "go" signal (and thus show PMT differences as well). The pattern of EMG activity produced was investigated by examining the relative onset of the ANT and AG2 bursts, and the three muscle burst durations. Overall the results indicated that the TMS and SAS resulted in a compression of the triphasic EMG pattern. Muscle burst durations remained the same across conditions. However, relative ANT and AG2 onset were facilitated by the SAS and TMS.
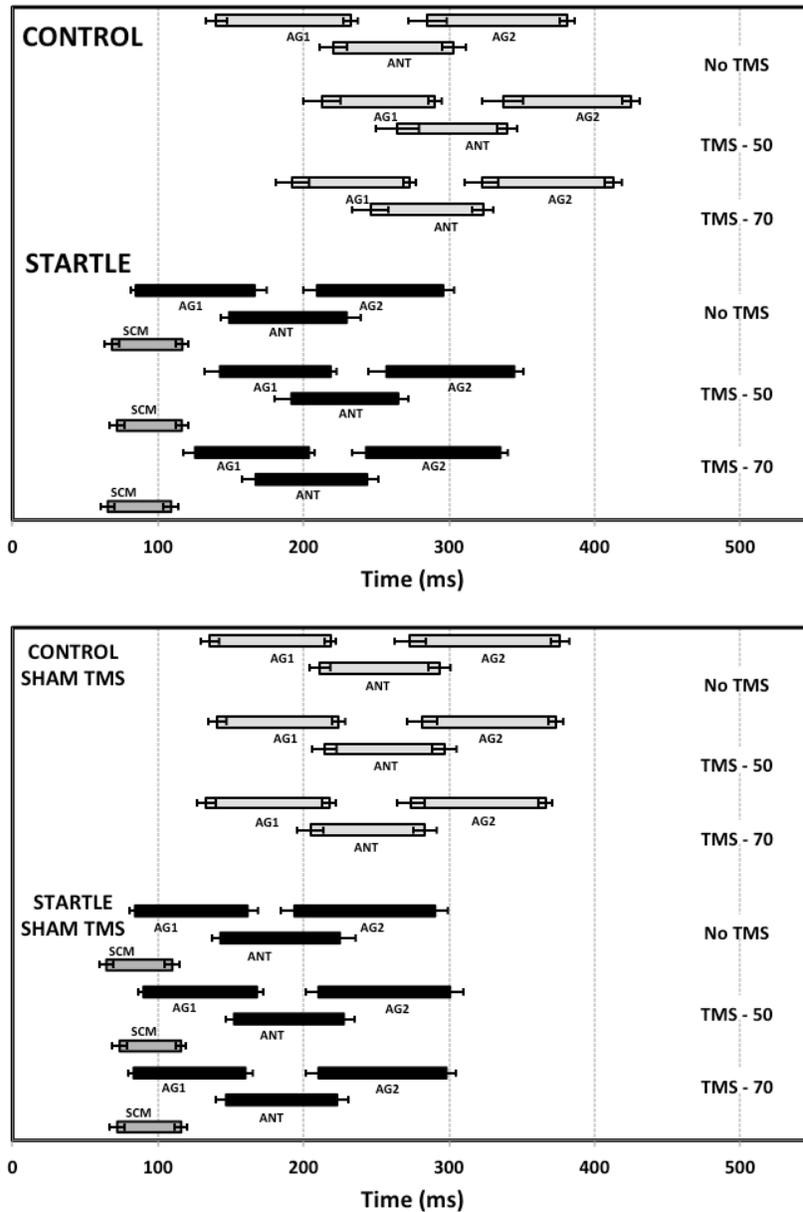
Figure 2.4   Plots of triphasic EMG configurations (first agonist burst, antagonist burst, second agonist burst) during control and startle trials for each TMS timing in the TMS present condition (top panel) and Sham TMS condition (bottom panel). Boxes represent EMG burst durations with mean (SEM) onsets and offsets with respect to time. AG1 represents the initial agonist (ECR) burst, ANT represents the antagonist (FCR), AG2 represents the second agonist burst, and SCM represents the startle indicator (sternocleidomastoid). Note the delay in EMG onset due to TMS in both control and startle trials in the TMS present condition (top panel), while EMG onset was unaffected by Sham TMS (bottom panel).

The analysis of duration for all three muscle bursts in both TMS present and Sham TMS conditions showed no main effects or interaction effects, indicating that EMG burst duration was not different across TMS timing or stimulus conditions. Analysis of ANT onset in the TMS present condition revealed a significant main effect of TMS timing, $F(2,24) = 15.95$, $p < .001$, $\varepsilon = 1$, $\eta_p^2 = .57$, with the onset of the ANT in the no TMS timing ($M = 65$ ms) occurring later than the ANT onset in the TMS -50 ($M = 52$ ms) and TMS -70 ($M = 52$ ms) conditions. In addition, a significant main effect of stimulus type was found in the TMS present condition, $F(1,12) = 5.01$, $p = .037$, $\varepsilon = 58$, $\eta_p^2 = .32$, with the onset of the ANT occurring later following control ($M = 59$ ms) compared to startle trials ($M = 54$ ms). There was also a significant main effect of stimulus type in the Sham TMS condition, $F(2,24) = 14.79$, $p = .002$, $\varepsilon = .94$, $\eta_p^2 = .55$, with ANT onset in the control trials ($M = 72$ ms) occurring later than in the startle trials ($M = 61$ ms). Analysis of AG2 onset in the TMS present condition revealed a significant main effect of TMS timing, $F(2,24) = 6.59$, $p = .005$, $\varepsilon = .87$, $\eta_p^2 = .36$, with the onset of the ANT in the no TMS timing ($M = 136$ ms) occurring later than the ANT onset in the TMS -50 ($M = 120$ ms) and TMS -70 ($M = 122$ ms) conditions. In addition, a significant main effect of stimulus type was found in the TMS present condition, $F(1,12) = 26.63$, $p < .001$, $\varepsilon = 1$, $\eta_p^2 = .69$, with AG2 onset in the control trials ($M = 132$ ms) occurring later than in the startle trials ($M = 120$ ms). There was also a significant main effect of stimulus type for AG2 onset in the Sham TMS condition, $F(1,12) = 66.89$, $p < .001$, $\varepsilon = 1$, $\eta_p^2 = .85$, with AG2 onset in the control trials ($M = 138$ ms) occurring later than in the startle trials ($M = 116$ ms). There were no other significant main or interaction effects.

2.3.4   Activation of SCM during Startle Trials

SCM onsets and durations for startle trials are shown in the EMG boxplots in Figure 2.4. The analyses of SCM EMG onset and duration did not reveal any significant differences due to TMS timing for either TMS present (onset, $p = .198$; duration, $p = .258$) or Sham TMS (onset, $p = .075$; duration, $p = .608$) conditions following startle trials. In addition, the analyses of SCM integrated EMG (Q30) did not reveal any differences due to TMS timing for either TMS present ($p = .129$) or Sham TMS ($p = .543$) conditions following startle trials.

This indicates that TMS or Sham TMS did not influence SCM onset, duration, or the amplitude of SCM following the presentation of a SAS.

2.4   Discussion

The objective of Experiment 1 was to examine the subcortical storage and triggering hypothesis of prepared movements by a SAS (Carlsen, et al., 2004b; Valls-Solé, et al., 1999). We accomplished this by using suprathreshold TMS to probe the involvement of the contralateral primary motor cortex in the production of a startle triggered movement. The subcortical triggering hypothesis suggests that sufficient details of a movement can be stored and triggered from subcortical structures (e.g. reticular formation) without cortical involvement. Therefore, it was predicted that a TMS-induced cortical silent period would have no effect on the rapid release of a preplanned movement in startle (123.2 dB) trials, whereas in control (82.3 dB) trials, PMT would be delayed and the size of the first agonist EMG burst would be modulated by the TMS (Day, et al., 1989; Hashimoto, et al., 2004). Contrary to expectations (although consistent with the work of Alibiglou, et al., 2009), the results of the present study supported cortical (M1) involvement in the StartReact effect. We found that TMS delayed PMTs and facilitated the size of the initial agonist burst in both control and startle trials.

The major finding in Experiment 1 was that suprathreshold TMS delivered to the contralateral M1 50 and 70 ms prior to the expected EMG response onset delayed PMT for control and startle trials, compared to No TMS and Sham TMS conditions (see Figure 2.2). As expected, the SAS triggered the movement faster than control trials for all conditions. There was no interaction between TMS timing and the IS, which suggested that TMS delayed the startle trials to the same degree as control trials. However, because the subcortical triggering hypothesis postulates that there is minimal cortical involvement in the StartReact effect, it would have been expected that a TMS-induced cortical SP would have little or no impact on the early release of prepared movements by a SAS. Additionally, consistent with previous research (Day, et al., 1989; Hashimoto, et al., 2004; Romaiguère, et al., 1997), we found that the closer the stimulation to the actual overt response in both control

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and startle trials, the longer the delay in RT. Further support that the TMS-induced cortical SP delayed RT was provided by the Sham TMS condition, where no neural tissue was stimulated. During the Sham TMS condition the audible click and vibration emitted by the TMS coil had no impact on PMT, regardless of stimulation timing (see Figure 2.2).

In addition to TMS delaying the onset of movement, the size of the initial agonist burst in both control and startle trials was facilitated compared to No TMS or Sham TMS conditions (see Figure 2.3). Hashimoto et al. (2004) also found a facilitation of integrated response EMG following suprathreshold TMS delivered in the premotor RT period at timings where PMT was delayed. They argued that, because peripheral electrical stimulation to the muscles decreased the size of response EMG (Day, et al., 1989), suprathreshold TMS of M1 centrally disrupts motor commands. The facilitation in AG1 EMG in both control and startle trials in the present study implicates the involvement of M1 in the initiation of movements in both conditions. The TMS-induced facilitation was independent of the observed facilitation in AG1 EMG following startle compared to control trials, which has been reported in other recent studies involving the use of a SAS (Carlsen, et al., 2004a; Maslovat, Carlsen, Chua, & Franks, 2009; Maslovat, Carlsen, Ishimoto, Chua, & Franks, 2008). These authors attributed an increase in AG1 burst size to the increased neural activation associated with being startled.

The triphasic EMG pattern from the ballistic 20º wrist extension movement varied across conditions (see Figure 2.4). While the duration of the EMG bursts remained consistent across all Sham and TMS present conditions, the onset of the ANT and AG2 bursts (relative to AG1) were facilitated by TMS in both control and startle trials compared to trials without TMS. Additionally, relative ANT and AG2 onsets in both Sham TMS and TMS present conditions occurred earlier in startle as compared to control trials. Although movements have typically been shown to be the same following startle trials (Carlsen, et al., 2004b), a reduction in ANT and AG2 onset latencies relative to AG1 onset has also been found in recent startle studies (Maslovat, Carlsen, et al., 2009; Maslovat, Hodges, Chua, & Franks, in press). Irlbacher et al. (2006) recently suggested that the triphasic EMG pattern is not triggered as a single entity, but each subsequent burst has its own trigger (possibly cortical)

that occurs about 30-40 ms after the start of the preceding burst. The decrease in ANT and AG2 burst onsets may be due to the increase in the observed size of the AG1 burst. However, participants attained the same endpoint in the movement despite the facilitation in AG1 size. Therefore, because of an increase in size of the first 30 ms of AG1, the following bursts may have been triggered earlier in order to break (ANT) and clamp (AG2) the wrist movement, thus reaching the intended target (Irlbacher, et al., 2006). The implication is that the triphasic EMG pattern is possibly not prepared completely in advance, but may be modifiable based on how the movement itself is performed.

One possible alternative explanation for the delay in movement onset and modulation of the initial agonist burst in startle trials is that the TMS could have directly influenced the subcortical (reticulospinal) pathways, which have been hypothesized to mediate the rapid release of movement by a SAS (Carlsen, et al., 2004b; Valls-Solé, et al., 1999). Recent evidence suggests that suprathreshold TMS may evoke responses in the brainstem. For example, Fisher et al. (2010) found that at high TMS intensities to M1, responses were frequently observed by single unit recordings in the reticular formation of anaesthetized macaques monkeys at a latency of 2 ms. Although this possibility exists, it seems unlikely due to the present data showing that the startle response was unaffected by TMS. The characteristics of SCM muscle activity (onset, duration and magnitude), used as an indicator as to whether or not participants were startled (Carlsen, et al., 2011), were no different when TMS was present compared to No TMS and Sham TMS conditions. This result would indicate that the subcortical (reticular formation) startle pathways were not influenced by the TMS. Alibiglou et al. (2009) also found that the onset of SCM activity following a SAS was not affected by TMS, whereas movement onset was delayed. The authors also concluded that the StartReact effect is mediated, in part, by cortical pathways and that these pathways differ from the pathways that mediate the startle reflex. Furthermore, prepulse inhibition (PPI) studies have provided evidence that the mechanisms for the startle response and the StartReact effect are physiologically distinct (Maslovat, Kennedy, Conway, Franks, & Chua, 2009; Valls-Solé, Kofler, Kumru, Castellote, & Sanegre, 2005). Valls-Solé et al. (2005) showed that the startle response (EMG in OOc and SCM) can be significantly suppressed when the SAS is preceded by a low intensity electrical stimulus to the fingers (PPI), but this

PPI did not affect the reaction time for the prepared response (i.e. the early release still occurred). In addition, Maslovat et al. (2009) found that the startle response (SCM activity) was suppressed by an auditory prepulse of 84 dB 100 ms prior to the IS, while the rapid release of movement was maintained.

In summary, M1 may be involved in the rapid release of prepared movements by a SAS – as supported by the suprathreshold TMS-induced modulation of the StartReact effect. This is in contrast to the subcortical storage and triggering hypothesis put forward by Valls-Solé et al. (1999) and Carlsen et al. (2004b), which suggests that movements that can be prepared in advance of an IS can be stored and triggered by a SAS from subcortical (i.e. brainstem) structures. The introduction of TMS to the contralateral M1 during the reaction time period delayed PMT and modulated the size of the initial agonist burst in both control and startle trials. In conjunction with recent evidence from Alibiglou et al. (2009), the present work provides further evidence to demonstrate that movements released early by a SAS are potentially initiated from motor cortical areas, and that this initiation may be mediated via an involuntary subcortical trigger.

3   Chapter: Experiment 2 – Subthreshold TMS

3.1   Introduction

In Experiment 1 we examined the subcortical storage and triggering hypothesis of prepared movements by a SAS (Carlsen, et al., 2004b; Valls-Solé, et al., 1999). Contrary to what the subcortical triggering hypothesis would predict, the cortical silent period delayed PMT and modulated the size of the initial agonist burst in both control and startle trials. Therefore, the findings of Experiment 1 provided evidence that the rapid release of movement by a SAS may be mediated in part by a pathway via M1. Recent evidence, however, suggests that suprathreshold TMS may evoke responses in the reticular formation (Fisher, et al., 2010). The reticular formation has previously been suggested to mediate the rapid release of movement by a SAS (Carlsen, et al., 2004b; Valls-Solé, et al., 1999). It is therefore possible that the delay in movement onset and modulation of the initial agonist burst observed in Experiment 1 was due to activity induced in the brainstem rather than M1. The rationale for this possibility comes from a recent study showing that neurons in the reticular formation may respond at very short latencies following a single-pulse of suprathreshold TMS (Fisher, et al., 2010). These authors found that at high TMS intensities to M1, responses were frequently observed by single unit recordings in the reticular formation of anaesthetized macaques monkeys at a latency of 2 ms.

To further examine the involvement of M1 in the StartReact effect, Experiment 2 utilized subthreshold TMS. Subthreshold TMS has been shown to reduce the ongoing EMG activity during a voluntary contraction (Butler, Larsen, Gandevia, & Petersen, 2007; Davey, Romaiguère, Maskill, & Ellaway, 1994) and during walking (Petersen et al., 2001). Using surface EMG, Davey et al. (1994) observed a suppression of voluntary muscle activity in upper limb muscles at a latency of about 30 ms after the subthreshold TMS was delivered. The duration of this suppression lasted 8-26 ms. In addition, Butler et al. (2007) reported suppression in single motor unit firing following subthreshold TMS stimulation. While it is believed that the MEP elicited by suprathreshold TMS is due to the direct or transsynaptic excitation of corticospinal neurons, the SP (EMG suppression) reflects the recruitment of

inhibitory cortical interneurons (see Reis, et al., 2008). The SP can be obtained in the absence of a MEP by stimulation below motor threshold (Classen & Benecke, 1995; Davey, et al., 1994). Therefore, because inhibitory cortical interneurons can be activated without directly stimulating descending pathways, subthreshold TMS can provide a means of examining cortical involvement in the StartReact effect without the possibility of affecting the subcortical startle response pathways.

In Experiment 2, subthreshold TMS was delivered to the contralateral M1 during AG1 activity in a simple RT task with either a control tone (82.2 dB) or a SAS (123.2 dB) as the IS. It was expected that subthreshold TMS would cause a suppression of the AG1 EMG burst 30 ms following stimulation in control trials. Based on the results of Experiment 1, where evidence was shown for M1 involvement in the rapid release of preplanned movements following a SAS, it was also expected that the AG1 EMG burst in startle trials would show suppression due to subthreshold TMS.

3.2 Methods

3.2.1 Participants

Seven participants (4 male, 3 female; age 24 ± 3 years) with no obvious upper body abnormalities or sensory or motor dysfunctions volunteered to participate in this study. All participants gave written informed consent, and the study was conducted in accordance with the ethical guidelines set by the University of British Columbia. Participants were right handed based on a laterality quotient greater than .60 on the Edinburgh Handedness Inventory (Oldfield, 1971).

3.2.2 Experimental Task and Acoustic Stimuli

Participants were required to respond as quickly as possible to each presentation of an auditory stimulus by initiating a ballistic right wrist extension movement. The participants sat in a height-adjustable chair facing a table, with their right arm secured in a semi-prone

position with the palm facing inward to a custom-made aluminum wrist manipulandum that moved in the transverse plane with an axis of rotation at the wrist joint. The arm portion of the manipulandum was oriented at an angle of 15º outward from the body midline, as this has been found to be a more comfortable position than an orientation parallel to the body midline. The wrist starting position was neutral (neither flexion nor extension) and was indicated by tactile feedback (using a magnet as a noncontact detent). The imperative stimulus (IS) followed the warning tone and could either consist of a control stimulus (82.2 ± 0.6 dB, 100 ms, 1000 Hz) or startling stimulus (123.2 ± 0.4 dB, 40 ms, 1000 Hz, <1 ms rise time). All auditory signals were be generated by a customized computer program and were amplified and presented via a loudspeaker placed directly behind the head of the participant. The acoustic stimulus intensities were measured using a sound level meter (Cirrus Research model CR:252B) set to measure impulse at a distance of 30 cm from the loudspeaker (approximately the distance to the ears of the participant).

The experimental task performed was an active right wrist extension as fast as possible to a target region located at 20º of angular displacement from the starting position. Participants were informed that they would first hear a warning tone indicating the start of a trial. Following this warning tone, the IS was presented with a variable foreperiod between 1500 and 2500 ms. The warning tone (100 ms, 1000 Hz, 80 dB) was generated by the computer using a 16 bit sound card (Creative SoundBlaster 16®) and standard computer speakers (Juster® sp-691n). Instructions were to move "as fast and as accurately as possible" from the starting position and stop on the target. Similar to previous experiments in our laboratory (e.g., Carlsen et al., 2004b) a monetary bonus of 10 cents (Canadian) was offered per trial for beating a prescribed minimum RT.

3.2.3   Instrumentation

Electromyographic (EMG) data were collected from the right wrist prime movers: the extensor carpi radialis longus (ECR) and the flexor carpi radialis (FCR), as well as bilaterally from the startle indicator SCM. The recording sites were prepared and cleansed to decrease electrical impedance, and bipolar preamplified Ag/AgCl surface EMG electrodes were

attached in the middle of the muscle bellies parallel to the line of force to the muscles. These electrodes were connected via shielded cabling to an external amplifier system (Delsys Model DS-80). A grounding electrode was placed on the participants' left radial styloid process. Wrist angular displacement data were collected using a potentiometer attached to the pivot point of the manipulandum. All raw data were digitally sampled for 2 s at 1 kHz (PCI-6023E, National Instruments) using a personal computer running a customized program written with LabVIEW software (National Instruments). Data collection was automatically initiated 500 ms before the IS.

3.2.4   Transcranial Magnetic Stimulation

Single-pulse TMS was delivered using a Magstim 200 stimulator (Magstim Company, Dyfed, UK, maximum output intensity 2.0 T) through a figure-eight coil (each external diameter 7.0 cm). The coil was handheld and oriented tangentially to the scalp and perpendicular to the central sulcus so that the induced current flow was in a posterior to anterior direction (Werhahn, et al., 1994). The optimal 'hot spot' for eliciting MEPs in the right ECR (agonist muscle for the response) was determined by stimulating over the contralateral M1. Because voluntary muscle contraction has been found to enhance corticospinal excitability, the participants were required to be relaxed with no muscular contraction and to fixate their eyes directly ahead (Hess, et al., 1987; Thompson, et al., 1991) while the 'hot spot' was found. Once a 'hot spot' was identified, the site was stimulated four times in order to confirm whether it was an optimal 'hot spot' where maximum MEPs could be evoked. This site was marked to ensure consistent coil placement. Rest motor threshold in the ECR was then determined to the nearest 1% of maximum stimulator output (MSO) where a minimum 50 µV MEP was evoked in 5 of 10 consecutive trials (Rossini, et al., 1994).

In order to set the subthreshold stimulation intensity, the stimulator was first set at 70% of rest motor threshold. While participants actively extended their wrist isometric ally (around 10-15% MVC), 20-40 pulses of TMS were delivered to the stimulation 'hot spot'. Unrectified and full-wave rectified EMG signals were averaged with reference to trigger stimuli using custom LabVIEW software. If, after 40 stimuli, an excitatory response (MEP)

was not observable in the averaged full-wave rectified EMG, the stimulus was deemed to be subthreshold. If there was an observable excitatory response, the stimulation intensity was decreased and another 20-40 stimuli recorded. If there was no excitatory response and no observable suppression, the stimulation intensity was increased and another 20-40 stimuli recorded. Participants were given a minimum rest period of one minute between changes in stimulation intensity (or as needed). The stimulation intensity was set where there was observable suppression in the averaged, full-wave rectified EMG without any excitatory response (Davey et al., 1994; Butler et al., 2007).

During the experiment, delivery of a single-pulse of subthreshold TMS was triggered by the onset of the initial agonist (AG1) EMG activity. This was the point at which rectified, filtered EMG activity first increased on the EMG trace by more than 5 standard deviations based on the 100 ms immediately preceding the IS.

3.2.5 Experimental Procedure

After all of the participant's questions were answered and written informed consent was obtained, participants were seated 1.0 m from a 21-in. LCD monitor which displayed the RT from the previous trial, any monetary bonus gained, and the cumulative total of bonuses. Next, the participant's right arm was strapped into the manipulandum and EMG sensors attached. Once TMS stimulation intensities are found, participants were first exposed to one startle trial while at rest, followed by one trial each of isometric maximum voluntary contraction for wrist extension and flexion (each lasting 2 s). To ensure consistent and fast reaction times, participants then practiced the task for 25 trials. The criteria for consistent RTs was a stable mean RT and a SD < 15 ms for the previous 10 trials. More trials were given if the criteria were not met.

The experiment included four conditions:

A) Control Trials (100 trials, Figure 3.1 A): The IS for these trials was a control (82.2 dB) tone and there was no TMS stimulation.

B) Control + TMS (100 trials, Figure 3.1 B): The IS for these trials was a control tone, and subthreshold TMS was triggered by AG1 onset (as described above).

C) SAS only (5 trials, Figure 3.1 C): The IS for these trials was a SAS, and there was no TMS stimulation.

D) SAS + TMS (35 trials, Figure 3.1 D): The IS for these trials was a SAS, and subthreshold TMS was triggered by AG1 onset (as described above).
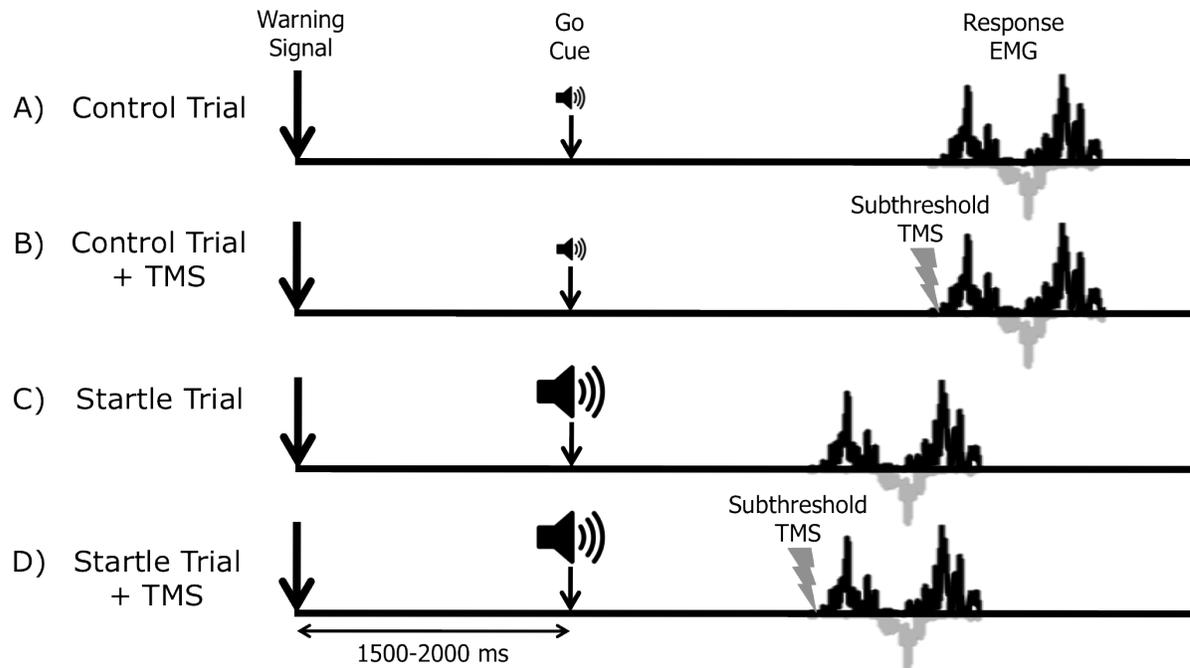
Figure 3.1   Timing of critical events in the experimental conditions. Arrows indicate the timing of warning and imperative stimuli (there was a random foreperiod between warning and imperative stimuli). The small speakers represent control (82.2 dB) trials and the large speakers represent startle (123.2 dB) trials. The filled lightning represents the timing of subthreshold TMS stimulation (triggered by agonist response EMG). The triphasic response EMG pattern depicted in each condition is the same EMG from a single representative (control) trial, with the black line showing ECR activity (positively rectified) and the grey line showing FCR activity (negatively rectified). The timing of the triphasic EMG in this diagram shows where the response was hypothesized to occur in each condition. [This figure was adapted from Alibiglou et al. (2009)].

240 movement trials were performed in total, separated across ten experimental blocks of 24 trials. Each block contained ten control trials, ten control + TMS trials and four SAS trials (SAS + TMS or SAS only), with the five SAS only trials randomly distributed across blocks. The order was pseudo-randomized such that SAS conditions could not be the first in a block or occur twice in a row. The approximate trials-to-trial interval was 10 s, although this varied because of the random foreperiod.

At the conclusion of the experiment, the participants' arm was removed from the manipulandum, EMG sensors were removed, and the participants were debriefed.

3.2.6   Data Reduction

Analysis was restricted to the testing trials only (practice trials were not analyzed). A total of 172 of the 1680 trials were discarded (10.2%). Reasons for discarding trials included displacement reaction times less than 80 ms (i.e., anticipation, 5 trials) or in excess of 400 ms (i.e., inattentive participants, 7 trials), incorrect movements (34 trials), TMS errors (i.e., occurring at the wrong time due to the algorithm used, 119 trials), and startle trials in which no detectable startle response (SCM activity) was observed (7 trials) (see Carlsen et al., 2011).

EMG data were analyzed off-line via a custom Lab View program. EMG signals were filtered using a dual-pass elliptical filter with a low-pass frequency setting of 25 Hz. Movement onset was determined as the first change in angular displacement by 0.2 degrees following the go signal.

Surface EMG burst onsets were defined as the point at which the EMG first began a sustained rise above baseline levels. The location of this point was determined by first displaying the EMG pattern on a computer monitor with a superimposed line indicating the point at which rectified, filtered EMG activity first increased on the EMG trace by more than 2 standard deviations based on the 100 ms immediately preceding the IS. This method allowed for the correction of potential errors caused by the algorithm. PMT was defined as

EMG onset in the ECR muscle for the wrist extension task. PMT was the main measure of RT for this study because it represents an estimate of the total central processing time. EMG offsets were marked in a similar fashion, using mean EMG activity following the end of the movement as a baseline level to account for any residual time between bursts. These were also verified and manually adjusted, with the activity between EMG onset and EMG offset defined as a distinct burst.

Integrated EMG values for the first agonist (ECR) EMG burst were computed by numerically integrating the normalized and rectified EMG for each participant. First the EMG was rectified, amplitude and baseline normalized, and onset normalized (aligned to a common onset). These values were numerically integrated (ms time base) over the first 100 ms of the EMG burst (Q100) from the common onset (0 ms). A subset of 30 ms (Q20-50) was also taken consisting of 20-50 ms to capture the initial burst where any inhibition caused by the TMS. The initial 30 ms (Q30) was also integrated for the SCM muscle EMG within SAS trials. For each condition within each participant, full-wave rectified agonist EMG signals was averaged with reference to trigger stimuli (or AG1 onset for No TMS conditions) to examine any inhibition of the EMG burst caused by the TMS.

3.2.7 Statistical Analysis

All dependent measures were analyzed with within-subjects analyses of variance (ANOVAs). To compare EMG patterns, burst onsets and durations were calculated. The onset of the first agonist burst (AG1, ECR) was measured from the time of the onset of the "go" stimulus and represents PMT. Onset of the antagonist (ANT, FCR) and second agonist burst (AG2, ECR), were calculated as the time from the onset of the AG1, as this allowed for determination of the relative timing of the triphasic EMG pattern. Muscle onsets, burst durations, and integrated AG1 EMG (Q20-50s and Q100s) values were analyzed independently using a two (acoustic stimulus; control, startle) by two (TMS; No TMS, TMS) within-subjects ANOVA. SCM onsets, durations and Q30 values were analyzed using paired samples t-tests (No TMS vs. TMS). Differences with a probability of < 0.05 were considered significant. Partial eta squared (η_p^2) values were reported as a measure of effect size.

3.3 Results

A summary of the results for all dependent measures, including means and standard deviations, are provided in Table 3.1. The main results of Experiment 2 were that, despite all participants exhibiting EMG suppression in an isometric wrist extension task, a TMS-induced reduction in AG1 EMG was observed in only one of seven participants in control and startle trials.

Table 3.1   Experimental results for each TMS condition (No TMS and TMS) and stimulus type, showing means and standard deviations (brackets).

AG1 = initial agonist burst (ECR), ANT = antagonist burst (FCR), AG2 = second agonist burst (ECR).

Variable	Control		Startle	
	No TMS	TMS	No TMS	TMS
Premotor RT (ms)	135.6 (34.8)	144.3 (32.6)	82.2 (16.9)	82.4 (10.7)
AG1-ANT time (ms)	67.0 (11.1)	62.7 (16.7)	57.9 (19.8)	62.3 (16.6)
AG1-AG2 time (ms)	112.9 (14.5)	112.0 (18.3)	109.3 (24.0)	110.9 (20.0)
AG1 Duration (ms)	74.3 (10.1)	73.8 (13.7)	72.6 (21.1)	74.9 (18.2)
ANT Duration (ms)	65.6 (12.7)	71.2 (15.0)	64.9 (8.3)	69.3 (9.3)
AG2 Duration (ms)	79.6 (6.4)	83.4 (12.5)	73.2 (20.5)	77.2 (7.8)
AG1 Q20-50 (mV*ms)	4.5 (1.6)	4.9 (2.4)	5.4 (2.1)	5.8 (2.7)
AG1 Q100 (mV*ms)	11.8 (4.5)	12.3 (4.7)	15.2 (7.4)	15.4 (6.6)
SCM Onset (ms)			65.3 (15.8)	66.0 (16.1)
SCM Duration (ms)			57.6 (11.8)	53.9 (11.9)
SCM Q30 (mV*ms)			0.54 (0.19)	0.53 (0.19)

3.3.1   Transcranial Magnetic Stimulation Parameters

Figure 3.2 shows the results of all seven participants for setting the TMS intensity used for Experiment 2 (s01 and s03 are the same participant tested on different occasions). The stimulation intensity was subthreshold for excitation of the right ECR muscle, but produced a reduction in the voluntary EMG between 20 and 40 ms after stimulation onset in all participants, replicating the findings of Davey et al. (1994). The mean subthreshold TMS intensity used across all participants was $24 \pm 3\%$ of MSO ($55 \pm 5\%$ of rest motor threshold).

The mean timing of subthreshold TMS delivery was 147.7 ± 32.3 ms in control trials and 85.6 ± 11.1 ms in startle trials. This was 3.4 ± 1.2 ms after mean PMT in control trials and 3.2 ± 1.2 ms after mean PMT in startle trials.
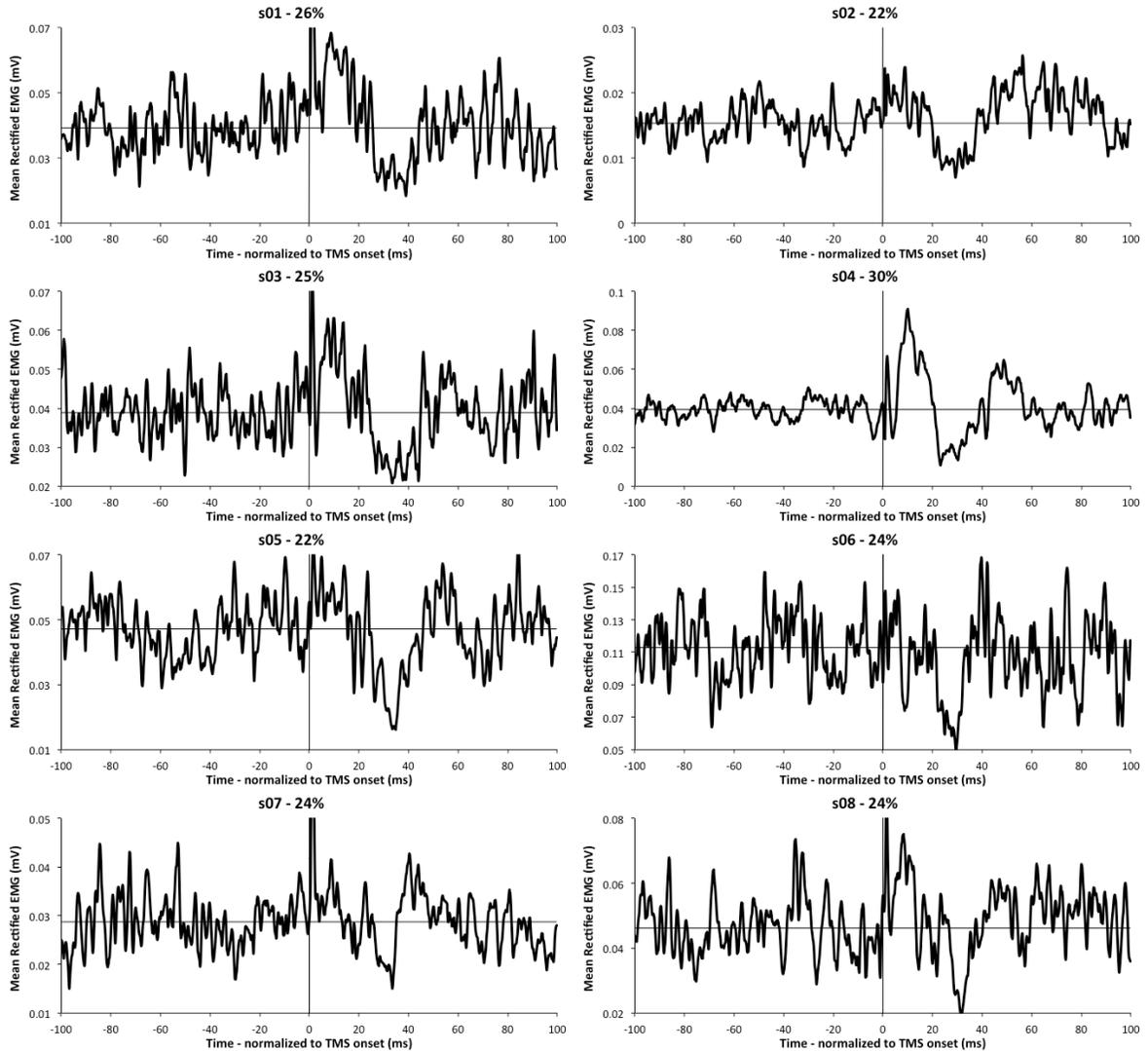
Figure 3.2   Mean rectified EMG activity in the right ECR for each participant during isometric wrist extension. EMG data are normalized to TMS onset. The percentage value represents TMS stimulation intensity as a percentage of maximum stimulator output. The horizontal black line represents the mean background EMG activity for the 100 ms preceding TMS onset. Note the decrease in EMG activity between 20-40 ms following TMS onset in each participant.

3.3.2   Premotor Reaction Time

Mean PMT values for control and startle trials are shown in Figure 3.3. As predicted, PMTs for startle trials were faster than control trials in both TMS and No TMS conditions, and PMTs were not influenced by TMS stimulation. These results were confirmed by a main effect for stimulus type (startle vs. control), $F(1,6) = 20.67$, $p = .004$, $\varepsilon = .96$, $\eta_p^2 = .78$, revealing that PMTs for startle trials ($M = 82$ ms) were faster than for control trials ($M = 140$ ms). There was no significant main effect for TMS (No TMS vs. TMS), $F(1,6) = 3.60$, $p = .106$, $\varepsilon = .36$, $\eta_p^2 = .38$, or significant interaction effect, $F(1,6) = 1.67$, $p = .244$, $\varepsilon = .19$, $\eta_p^2 = .22$, indicating that TMS did not influence PMT.

Premotor Reaction Time
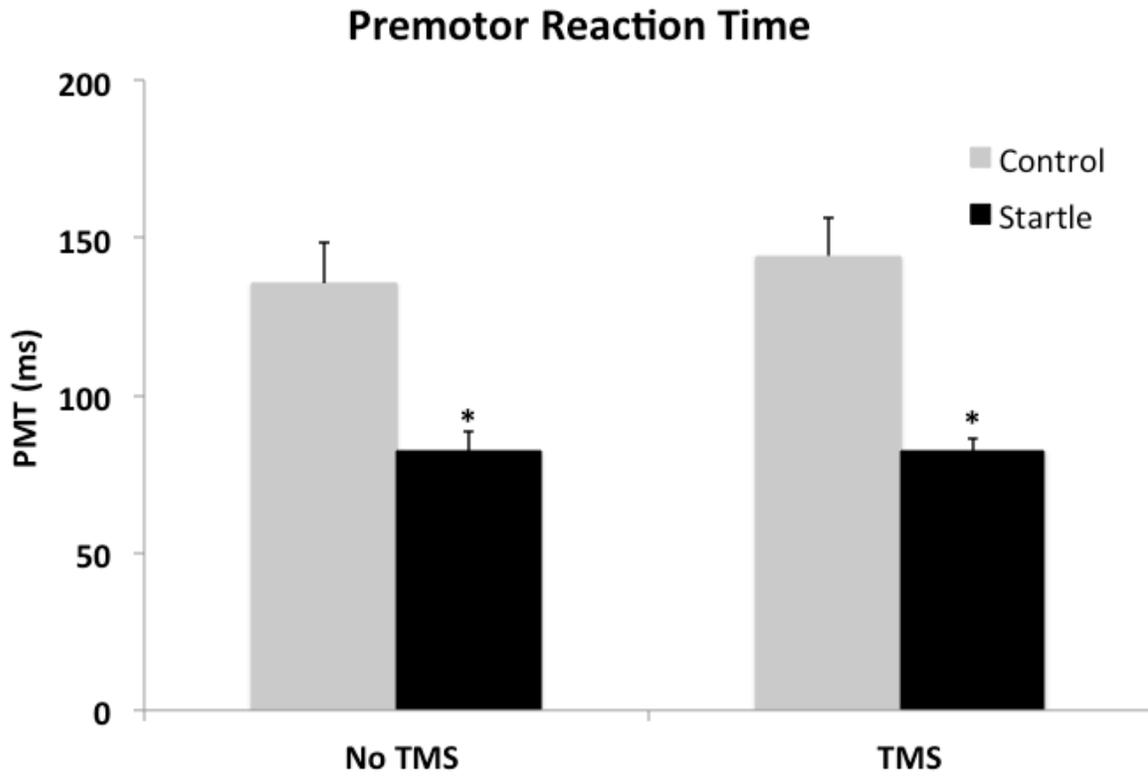
Figure 3.3   Mean premotor RT values (SEM) for control and startle trials separated by TMS condition. Note the decrease in PMT for startle trials in both the No TMS and TMS conditions, and that there was no difference for either control or startle trials across TMS condition. * denotes an effect of the IS.

3.3.3   Effect of Subthreshold TMS on Agonist EMG

Figures 3.4 and 3.5 show the mean rectified agonist (ECR muscle) EMG for three representative participants for both control and startle trials with and without TMS. All TMS trials were normalized to TMS onset (time 0), while all No TMS trials were normalized to PMT and adjusted for the slight delay in TMS triggering (3.4 ms in control trials and 3.2 ms in startle trials.

s01 and s03 in Figure 3.4 was the same participant. This participant was first tested with a TMS intensity of 26% MSO (s01) under the full experimental procedure. Due to a large number of MEPs appearing in control trials (see Figure 3.4 A where a distinct MEP can be observed beginning after 20 ms), this participant was re-tested without SAS trials with a stimulus intensity of 25% MSO (s03). Under both TMS intensities this participant showed a distinct reduction in ongoing EMG activity between 20-40 ms following TMS onset during the isometric wrist extension task for setting experimental stimulus intensity (Figure 3.2). Figure 3.4 C shows a distinct reduction in mean agonist EMG activity between 20-40 ms following TMS delivery in control trials without any excitation (MEP). Despite the MEPs appearing in control trials for s01, there was no distinct MEP observed in startle trials (Figure 3.4 B). Although less clear, there was evidence of a reduction in mean agonist EMG activity between 20-40 ms after TMS delivery in SAS trials (Figure 3.4 B).

The remaining six out of seven participants produced results similar to the two participants depicted in Figure 3.5. Contrary to our predictions for both control and startle trials, TMS did not elicit a reduction in mean agonist EMG between 20-40 ms following TMS onset compared with No TMS trials in these participants. This was despite the clear reduction in voluntary EMG between 20-40 ms following TMS stimulation that was observed in each participant during the isometric wrist extension task (Figure 3.2). No evidence of MEPs (excitation) was observed in any of these six participants in either control or startle trials, indicting that the TMS intensities used were subthreshold for each participant.
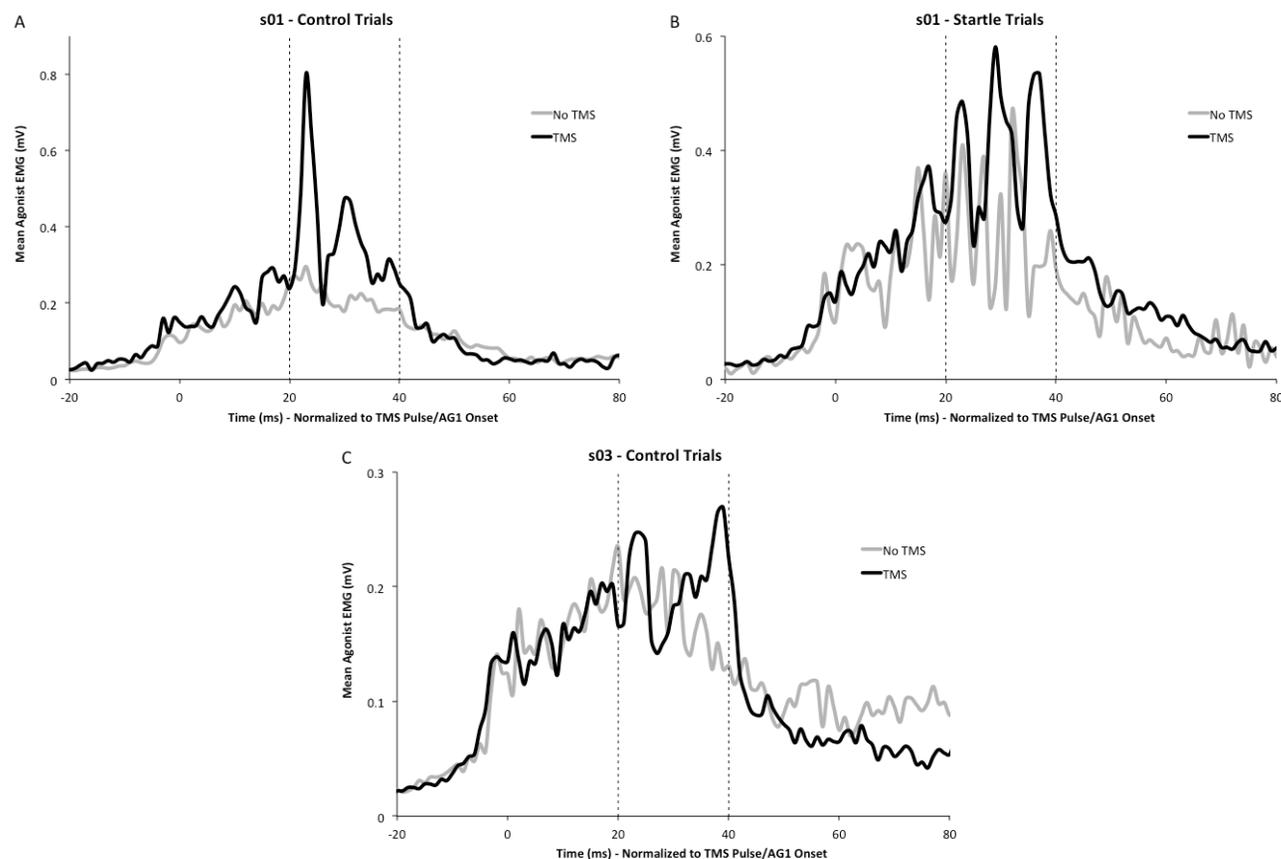
Figure 3.4   Mean rectified agonist (ECR muscle) EMG traces for s01 and s03 (the same participant) for both control (panels A and C) and startle (panel B) trials. s03 was tested without startle trials and with a slightly lower TMS intensity (25% maximum stimulator output vs. 26% for s01). The black line represents trials with subthreshold TMS while the grey line represents trials with no TMS stimulation. All TMS trials are normalized to TMS onset (time 0), while all No TMS trials are normalized to PMT onset and adjusted for the slight delay in TMS triggering (3.4 ms in control trials and 3.2 ms in startle trials). The dashed vertical lines represent the time where EMG suppression was expected to occur. Note in the top right and bottom panels the trend towards EMG suppression in the TMS condition. Also note in the top left panel the presence of a MEP (excitation) beginning around 20 ms after TMS stimulation.
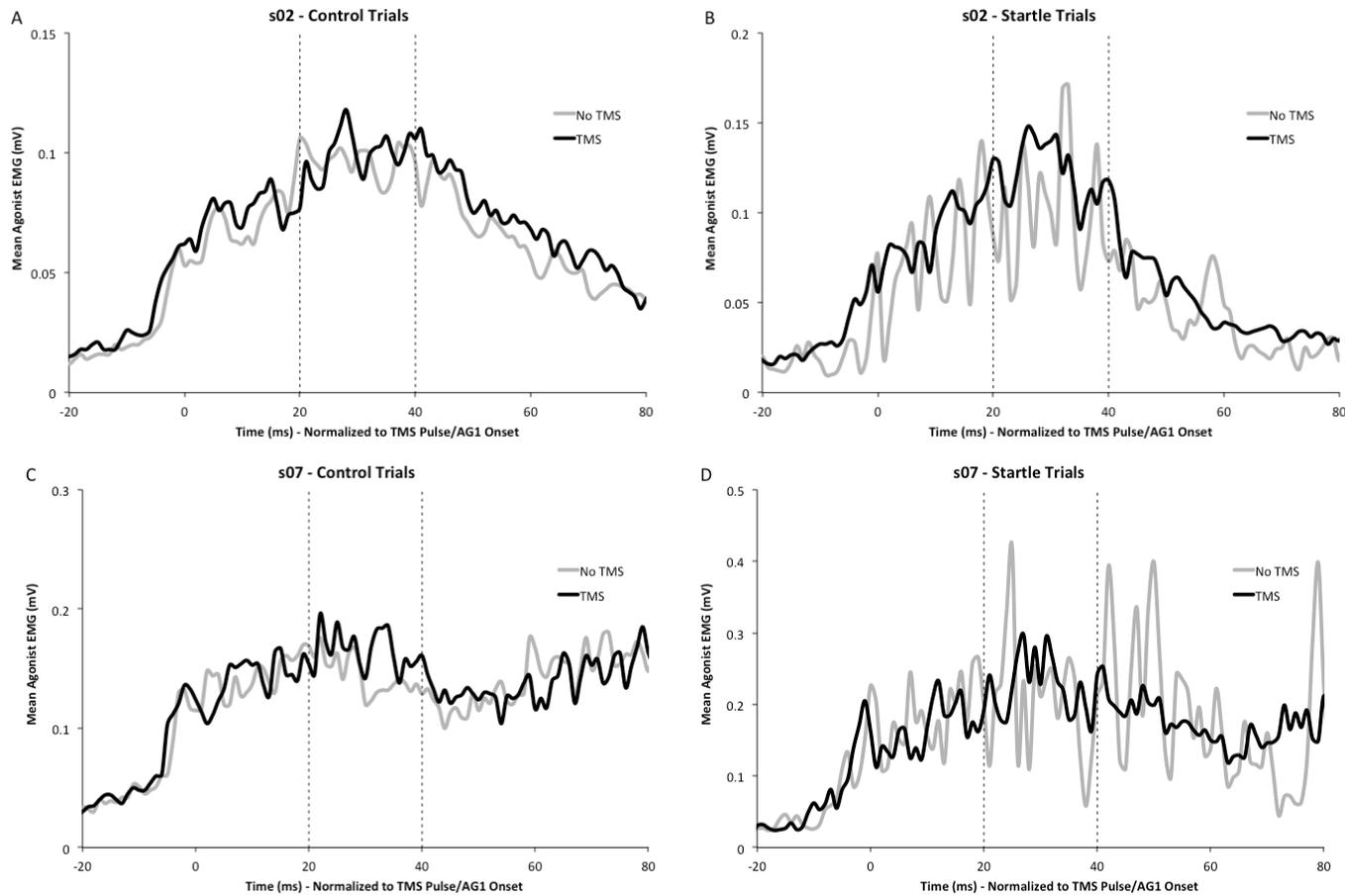
Figure 3.5 **Mean rectified agonist (ECR muscle) EMG traces for s02 (panels A and B) and s07 (panels C and D; two representative participants) for both control (panels A and C) and startle (panels B and D) trials. The black line represents trials with subthreshold TMS while the grey line represents trials with no TMS stimulation. All TMS trials are normalized to TMS onset (time 0), while all No TMS trials are normalized to PMT onset and adjusted for the slight delay in TMS triggering (3.4 ms in control trials and 3.2 ms in startle trials). The dashed vertical lines represent the time where EMG suppression was expected to occur. Note a lack of EMG suppression in the TMS trials for both stimulus conditions in both participants. This pattern of results was observed in 6 out of 7 participants.**

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3.3.4 Triphasic EMG Characteristics

Mean AG1 integrated EMG (Q20-50 and Q100) values for control and startle trials are shown in Figure 3.6. To examine any EMG suppression in the initial agonist burst caused by the TMS, the integrated EMG activity was calculated for each condition between 20 and 50 ms after AG1 burst onset (Q20-50). There were no significant main or interaction effects for AG1 Q20-50 (all p's > .15). This indicates there was no overall suppression caused by TMS in the initial agonist burst between 20 and 50 ms after burst onset. For AG1 Q100 there was a main effect of stimulus type, $F(1,6) = 10.11$, $p = .019$, $\varepsilon = .76$, $\eta_p^2 = .63$, indicating that the size of the first agonist burst was facilitated during startle trials ($M = 15.3$ mV*ms) compared to control trials ($M = 12.0$ mV*ms). There was no significant main effect for TMS (No TMS vs. TMS), $F(1,6) = .30$, $p = .605$, $\varepsilon = .08$, $\eta_p^2 = .05$, or significant interaction effect, $F(1,6) = .36$, $p = .568$, $\varepsilon = .08$, $\eta_p^2 = .06$, indicating that TMS did not influence the first 100 ms of integrated EMG activity for the initial agonist burst.

EMG boxplots are shown in Figure 3.7 with the relative timing of the triphasic burst for each condition (including SCM activation for startle trials). These represent grand means for startle and control trials for each TMS condition, and are normalized with respect to the "go" signal (and thus show PMT differences as well). The pattern of EMG activity produced was investigated by examining the relative onset of the ANT and AG2 bursts, and the three muscle burst durations. The analysis of duration for all three muscle bursts showed no main effects or interaction effects, indicating that EMG burst durations were not different across TMS or stimulus conditions (all p's > .10). In addition, the analysis of relative ANT and AG2 onsets showed no main effects or interaction effects, indicating that relative ANT and AG2 onsets were also not different across TMS or stimulus conditions (all p's > .10). Overall the results indicated that the triphasic EMG burst pattern was preserved and not modified by TMS and stimulus conditions.
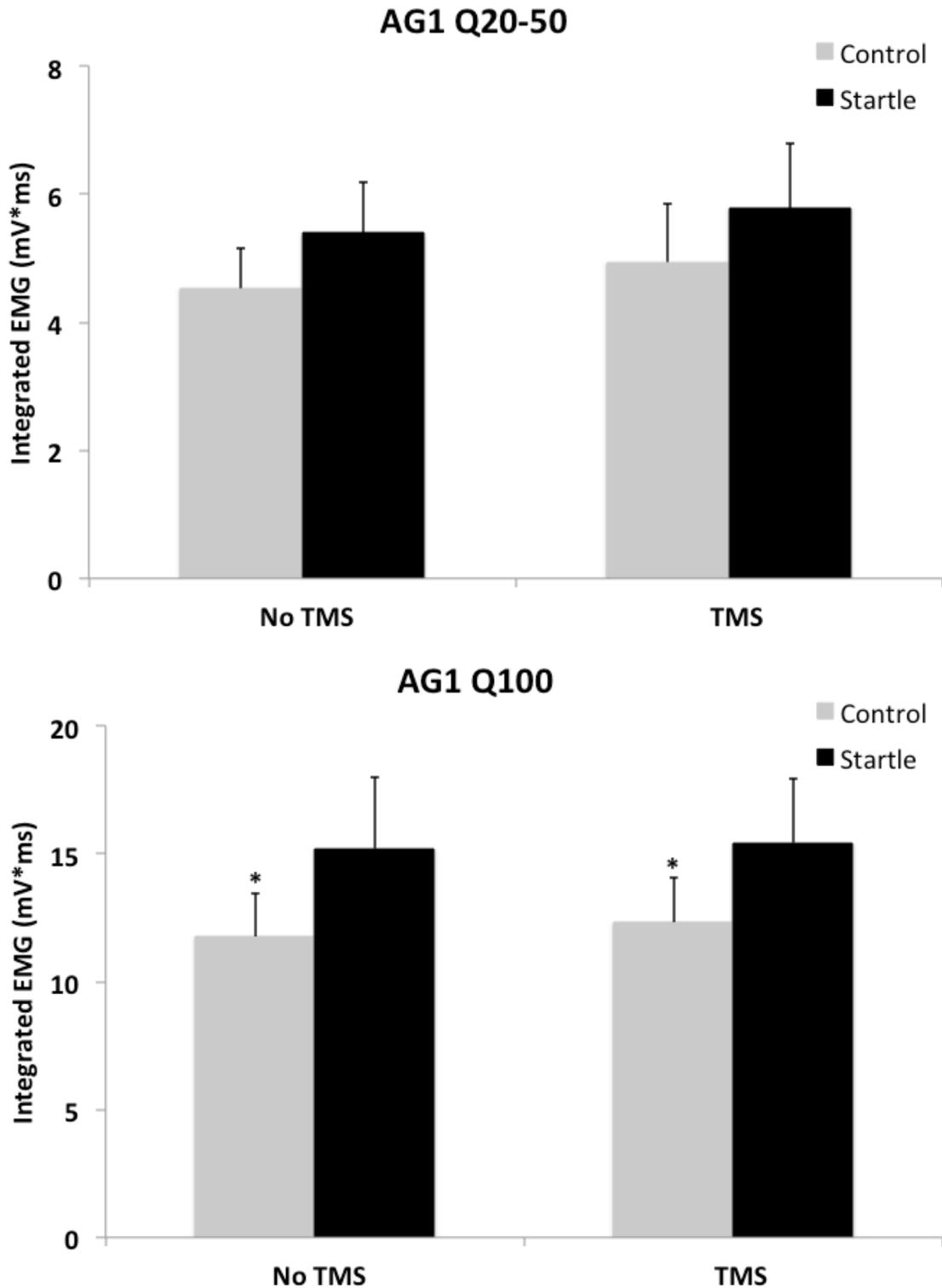
Figure 3.6 **Mean AG1 integrated EMG (Q20-50 = top panel, Q100 = bottom panel) values (SEM) for control and startle trials separated by TMS condition. Note the increase in Q100 integrated EMG for startle trials. There were no significant main or interaction effects for Q20-50. * denotes an effect of the IS.**
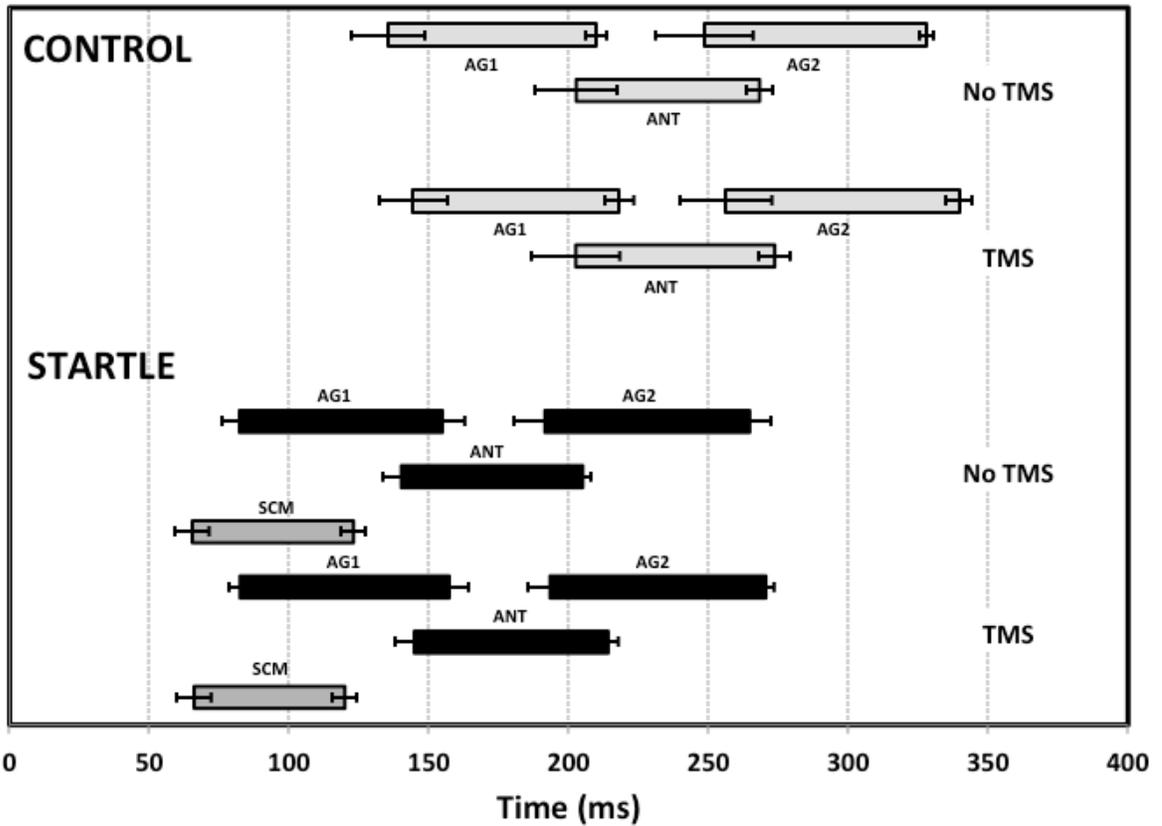
Figure 3.7 **Plots of triphasic EMG configurations (first agonist burst, antagonist burst, second agonist burst) during control and startle trials for each TMS condition. Boxes represent EMG burst durations with mean (SEM) onsets and offsets with respect to time. AG1 represents the initial agonist (ECR) burst, ANT represents the antagonist (FCR), AG2 represents the second agonist burst, and SCM represents the startle indicator (sternocleidomastoid). Note the facilitation of premotor RT in the startle trials, while the triphasic EMG configuration and SCM was unaffected by the subthreshold TMS.**

3.3.5 Activation of SCM during Startle Trials

SCM onsets and durations for startle trials are shown in the EMG boxplots in Figure 3.5. The analyses of SCM onset, duration and integrated EMG (Q30) did not reveal any significant differences due to TMS following startle trials (onset, $p = .659$; duration, $p = .159$; Q30, $p = .665$). This indicates that TMS did not influence SCM onset, duration, or the amplitude of SCM following the presentation of a SAS.

3.4 Discussion

The objective of this study was to extend the findings of Experiment 1 that provided evidence to demonstrate possible cortical (M1) involvement in the rapid release of prepared movements by a SAS. To further examine the involvement of M1 in mediating the StartReact effect we adapted a paradigm first used by Davey et al. (1994), who showed that subthreshold TMS could cause suppression in the ongoing EMG activity during a voluntary contraction. Subthreshold TMS delivery to the contralateral M1 was triggered by initial agonist EMG onset in a simple RT paradigm where the IS was either a control tone or SAS. Based on the results of Experiment 1, it was hypothesized that subthreshold TMS would cause a reduction in the initial agonist EMG burst approximately 30 ms following stimulus onset in both control and startle trials compared to trials without TMS. As predicted, the SAS triggered the prepared 20° wrist extension movement at a much faster latency than control trials across all participants (see Figure 3.3). Contrary to expectations, however, six of the seven participants tested showed no observable agonist EMG suppression following subthreshold TMS in either control or startle trials during the RT time task. This was despite each participant showing a distinct reduction in voluntary EMG activity following subthreshold TMS during the isometric wrist extension task used to set stimulation intensity for the RT task, which replicated the findings of Davey et al. (1994).

The major finding in the present study was that, despite showing voluntary EMG suppression in an isometric wrist extension task caused by subthreshold TMS, only one out of seven participants exhibited any significant amount of suppression in agonist EMG during a simple

RT task with either a control tone or SAS as the IS. This result, however, does not preclude M1 involvement in the StartReact effect in favour of the subcortical storage and triggering hypothesis (Carlsen, et al., 2004b; Valls-Solé, et al., 1999). Indeed, because a lack of EMG suppression was also found in control trials, this suggests that the technique adapted from Davey et al. (1994) during voluntary isometric muscle contractions does not appear to be sensitive enough when applied to ballistic upper limb RT movements. Davey et al. (1994) found that, in a tonically contracting muscle (around 10% MVC), EMG suppression could be elicited by subthreshold TMS without any prior excitatory response. This finding was replicated in all of the participants in the present study (see Figure 3.2). The subthreshold TMS intensity found to elicit EMG suppression in the isometrically contracting ECR was used during the simple RT task. However, due to a number of potential factors (see below), the same stimulation intensity did not elicit EMG suppression in six out of seven subjects in the simple RT task

One possibility for the lack of EMG suppression in the dynamic RT task was that the threshold for excitation of the inhibitory cortical interneurons by TMS changed between performing the isometric wrist extension task and the RT task. The activation of inhibitory interneurons within the cortex by TMS has been proposed to be the cause of voluntary muscle activity suppression by reducing corticospinal output to that muscle (Davey, et al., 1994). The threshold of inhibitory cortical interneurons may have changed to such an extent that the same TMS intensity eliciting suppression in the isometric wrist extension task was insufficient to activate this inhibitory network during the RT task. The background EMG level for all participants during the isometric task (Figure 3.2) was lower compared with the EMG level produced during the RT paradigm for both control and startle trials (Figures 3.4 and 3.5). A reduction in intracortical inhibition is important in movement execution (Stinear, Coxon, & Byblow, 2009). For example, Reynolds and Ashby (1999) provided evidence that during tonic contractions, intracortical inhibition of the agonist MEP decreased. Reynolds and Ashby (1999) also found that a reduction in intracortical inhibition in M1 precedes the increase in excitability before a voluntary contraction. Therefore, it would be important in future studies to examine the potential differences in TMS threshold intensities when

transferring them from a task involving tonic muscle contractions to one involved with dynamic muscle contractions.

One previous study has employed this subthreshold stimulation technique in a dynamic task rather than in a tonic task. Petersen et al. (2001) provided strong evidence for cortical involvement during human walking by activating intracortical inhibitory circuits with subthreshold TMS. These authors demonstrated that subthreshold TMS stimulation produced a suppression of ongoing EMG activity during walking in both tibialis anterior and soleus muscles. Two major differences between the present study and the study by Petersen et al. (2001) could potentially explain the lack of EMG suppression observed in the dynamic RT task. The primary difference was that Petersen et al. (2001) examined a range of TMS intensities during the dynamic walking task itself, whereas the present study examined the effects of only one TMS intensity during the dynamic RT task. The subthreshold TMS intensity used was the intensity eliciting the most EMG suppression (without any prior facilitation) during the isometric wrist extension task, which was the result of examining a range of TMS intensities. Future studies employing this technique in dynamic tasks should examine a range of subthreshold TMS intensities during the actual task of interest. The second major difference was that Petersen et al. (2001) obtained data from 200 trials per TMS stimulation intensity. The present study included a total of 40 RT trials where the IS was a SAS, of which 35 included subthreshold TMS stimulation. Despite the StartReact effect being robust to the effects of habituation (Carlsen, Chua, Inglis, Sanderson, & Franks, 2003; Carlsen, et al., 2011), 40 startle trials at 124 dB in one experimental session is at the higher end of startle trial numbers previously used in the literature (Carlsen, et al., 2011) due to guidelines issued by the National Institute for Occupational Safety and Health (NIOSH). The NIOSH recommend limiting exposure to 124 dB to less than 3 s total noise dose (see NIOSH, 1998). Given that Davey et al. (1994) found EMG suppression elicited by subthreshold TMS in EMG data averaged over 30-80 trials, and Petersen et al. (2001) showed EMG suppression in EMG data averaged over 200 trials, the present experimental design was limited to one TMS intensity being examined per participant. It may therefore be important to first characterize the effects of a range subthreshold TMS intensities in control

trials before applying the technique to startle trials, where the number of startle trials is potentially limited.

One participant did show some evidence of EMG suppression during both control and startle trials (see Figure 3.4). This participant was tested on two occasions at different TMS intensities (26% for s01, Figures 3.4 A and B; 25% for s03, Figure 3.4 C). Despite a large number of MEPs appearing in control trials at 26% MSO (Figure 3.4 A), this participant exhibited evidence of EMG suppression between 20-40 ms after TMS delivery in startle trials at the same stimulation intensity. In addition, when tested on control trials only at 25% MSO, this participant showed a distinct reduction in initial agonist EMG activity between 20-40 ms after TMS onset (Figure 3.4 C). This finding in a single participant shows that this technique has the potential to provide more understanding of the possible role of M1 in mediating the StartReact effect without the direct activation of descending motor pathways.

In summary, the results of the present study did not provide evidence for the involvement of M1 in the rapid release of movements by a SAS. Subthreshold TMS elicited suppression in ongoing voluntary EMG during an isometric wrist extension task in all participants. However, the same stimulation intensity only elicited signs of agonist EMG suppression in a wrist extension RT task in one out of seven participants following both a control tone or a SAS as the IS. Given that some EMG suppression was observed in both control and startle trials in this participant, it is conceivable that subthreshold TMS could assist in our understanding of whether M1 is involved in the StartReact effect. However, a broader range of stimulus intensities needs to be investigated in order to ensure activation of inhibitory cortical interneurons by subthreshold TMS in this dynamic RT task.

4 Chapter: General Discussion

The overall goal of this thesis was to examine the subcortical storage and triggering hypothesis suggested by Valls-Solé et al. (1999) and Carlsen et al. (2004b). This hypothesis arose from the finding that in a simple RT paradigm, where a known movement can be prepared in advance, RTs were greatly reduced when a SAS replaced the usual 'go' signal. The suggested mechanism for what is known as the StartReact effect was that movements prepared in advance of an IS are stored and triggered from subcortical areas without the usual cortical processing by a SAS. The two experiments in this thesis delivered both suprathreshold and subthreshold TMS to the contralateral M1 in order to directly probe cortical involvement in the StartReact effect.

In Experiment 1 we examined the role of M1 in mediating the rapid release of movement by a SAS by inducing a cortical SP in the contralateral M1 with suprathreshold TMS during the premotor RT period in both control and startle trials. It was predicted that the TMS-induced cortical SP would delay PMT in control trials, and also increase the size of the initial agonist response (as measured by EMG). Based on subcortical storage and triggering, it was expected that the cortical SP would not influence PMT or initial agonist response EMG in startle trials. Contrary to predictions (although consistent with the recent work by Alibiglou, et al., 2009), the results of Experiment 1 supported cortical (M1) involvement in the StartReact effect. We found that the TMS-induced cortical SP delayed PMTs and facilitated the size of the initial agonist EMG burst in both control and startle trials.

While the results of Experiment 1 provided strong support for the involvement of M1 in the StartReact effect, it is also possible that the suprathreshold TMS disrupted subcortical (reticular formation) pathways (as suggested by Fisher, et al., 2010). The reticular formation has previously been suggested to mediate the rapid release of prepared movement by a SAS (Carlsen, et al., 2004b; Valls-Solé, et al., 1999). Experiment 2 therefore utilized subthreshold TMS to the contralateral M1, which has been shown to reduce the ongoing EMG activity during a voluntary contraction by activating inhibitory cortical interneurons (Butler, et al., 2007; Davey, et al., 1994; Petersen, et al., 2001). Based on the results of Experiment 1, it was

hypothesized that subthreshold TMS delivered at AG1 onset would elicit a reduction in AG1 EMG in both control and startle trials. However, while subthreshold TMS elicited a reduction in the ongoing EMG in the isometric task used to set the stimulus intensity in all participants, only one participant exhibited any sign of EMG suppression in control and startle trials. Despite the lack of strong evidence for the mediation of the StartReact effect via M1 from Experiment 2, subthreshold TMS stimulation presents a potential avenue for further exploration of cortical involvement.

The data presented in Experiment 1 supports M1 involvement in the rapid release of prepared movements by a SAS, rather than the original subcortical storage and triggering hypothesis (Carlsen, et al., 2004b; Valls-Solé, et al., 1999). A recent model proposed by Carlsen, et al. (in press) helps to integrate these results into the current literature on movement preparation and initiation. Carlsen et al. (in press) propose in their model that the processes of preparing and initiating a response are distinct and both involve an increase in neural activation levels. It has been shown that movement preparation can occur in advance of the 'go' signal when the response is known in advance (Carlsen, et al., 2004b; Chen & Hallett, 1999; Coxon, Stinear, & Byblow, 2006; Valls-Solé, et al., 1999). Carlsen et al. (in press) suggest that response preparation involves increasing the activation of a neural network (i.e., cortical cell assembly) to a point just below threshold for motor output. A cortical cell assembly is a suggested neural explanation for the concept of a motor program (Wickens, et al., 1994). A cell assembly is defined as enhanced synaptic strength between cortical pyramidal neurons, which are recruited together to determine the configuration of cortical neurons to activate for a particular motor response (Wickens, et al., 1994).

Once the response has been prepared, movement initiation then occurs by providing additional activation to bring the prepared cell assembly above the threshold for motor output (Carlsen, et al., in press). The process of movement initiation has been described as a neural accumulator model (Carpenter & Williams, 1995; Hanes & Schall, 1996; Maslovat, Hodges, Chua, & Franks, 2011). The neural accumulator model explains that differences in initiation time (which contribute to differences in RT) can be explained by either: (a) different levels of neural activation achieved during preparation, (b) differences in the rate of activation

accumulation to reach threshold, or (c) both (Maslovat, et al., 2011). Therefore, as Carlsen et al. (in press) suggest, in a simple RT task such as that used in this thesis, preparation would occur in advance of the 'go' signal, resulting in an increase in activation of a cortical cell assembly (neural network) to a certain level below the threshold for movement execution. The recorded simple PMT would then include the time taken to detect and identify the IS, and increase neural activation above that threshold (Carlsen, et al., in press). Based on the neural accumulator model described above, initiation time would be influenced by both the neural activation level achieved during the preparation process, and also on the rate of activation accumulation during initiation (Carlsen, et al., in press; Maslovat, et al., 2011).

In addition, Carlsen et al. (in press) also proposed a possible neural mechanism for the RT facilitation effect of a SAS that is supported by the data from Experiment 1 in this thesis. Until now the cortically mediated pathways proposed to be involved in the StartReact effect have not yet been well delineated. In Experiment 1, a TMS-evoked cortical SP delayed both control and startle trials. However, startle trials were still initiated faster than control trials following the presentation of a SAS, indicating that startle trials were initiated via a faster mechanism than control trials despite the cortical disruption by TMS. Carlsen et al. (in press) put forward the possibility that an involuntary subcortical trigger caused by increased activation from the startle response could release *cortically* prepared and stored movements (in the form of a cell assembly) at fast latencies without the usual cortical processing.

Figure 4.1 shows the proposed neural pathways for the StartReact effect (Carlsen, et al., in press), in addition to the neural pathways involved in voluntary movement production (control trials), the startle reflex pathway, and the previously hypothesized pathway involved in the original subcortical storage and triggering hypothesis (Carlsen, et al., 2004b; Valls-Solé, et al., 1999). In control trials, an acoustic 'go' signal travels through the auditory nerve to the primary auditory cortex via the ventral cochlear nucleus (Erwin & Buchwald, 1986). From there, additional cortical processing occurs and the cortically prepared movement is initiated from M1 down the corticospinal tract, and the response occurs (see Figure 4.1, black arrows). In this situation it is thought that movement initiation occurs due to *voluntary* increase in activation levels to above threshold, as previously detailed.

In startle trials, activation from a SAS reaches the midbrain reticular formation via the ventral cochlear nucleus and the nuclei of the inferior colliculus (see Figure 4.1, dark grey arrows). The pathway for the subcortical trigger by a SAS could be through the giant neurons of the nucleus reticularis pontis caudalis (nRPC) in the reticular formation of the brainstem (Carlsen, et al., in press). The nRPC receives input from the cochlear nucleus and acts as a control center for the startle response (Yeomans & Frankland, 1995). Descending pathways from the nRPC have been shown to mediate the startle response (Yeomans & Frankland, 1995), while ascending pathways to M1 from the nRPC could be responsible for mediating the StartReact effect. Carlsen et al. (in press) identify the thalamus as an important neural structure that may be involved in both voluntary response initiation and the rapid release of movement by a SAS. The thalamus is hypothesized to provide neural input into M1 resulting in the initiation of prepared movements (Haider, Ganglberger, & Groll-Knapp, 1969). Carlsen et al. (in press) propose that ascending activation from the reticular formation caused by the startle reflex may interact with the motor relay nuclei in the thalamus, causing rapid, *involuntary* triggering of a cortically prepared response (Figure 4.1). This is in contrast to the previous subcortical storage and triggering hypothesis, which suggested that movements are prepared and stored in the midbrain reticular formation and triggered by the startle down the descending reticulospinal pathways (see Figure 4.1; Carlsen, et al., 2004b; Valls-Solé, et al., 1999). Therefore, if a movement is triggered by a SAS via the brainstem and the thalamus but M1 is in an inhibited state due to a cortical SP created by TMS, the trigger must wait for termination of the SP before the movement can be initiated (see Figure 4.1). However, if M1 is not inhibited then cortically prepared and stored movements can be involuntarily triggered at much shorter latencies than the normal voluntary trigger (control trials). Additionally, if a cortical SP is induced prior to response onset in control trials, movement initiation cannot begin until the SP is over, and the movement is delayed.
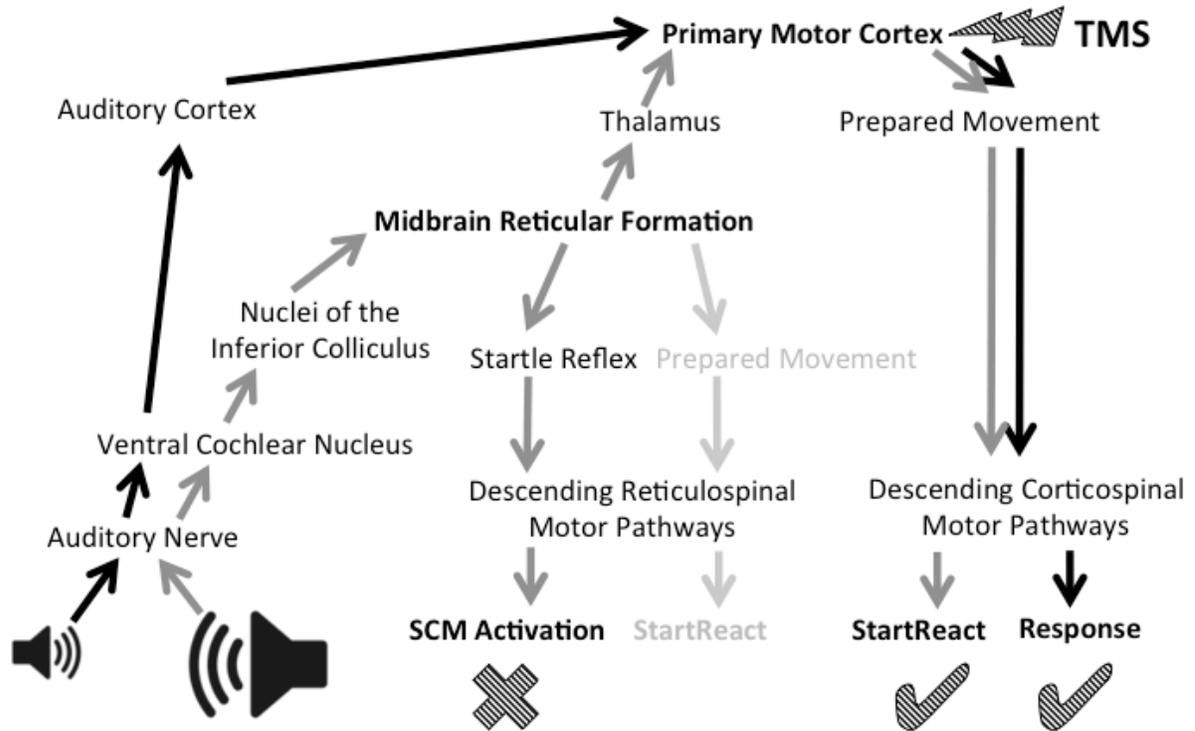
Figure 4.1   **Diagram depicting the neural pathways involved in voluntary movement production (control trials; black arrows), the StartReact effect (based on the neural model proposed by Carlsen, et al., in press) and the startle reflex (dark grey arrows), and the previous subcortical storage and triggering hypothesis of the StartReact effect (light grey arrows). The large speaker represents acoustic input from a startle trial and the small speaker represents acoustic input from a control trial, while the lightning represents a suprathreshold TMS pulse delivered to the contralateral M1 during the premotor RT period. The 'check' symbols indicate where the suprathreshold TMS modulated the StartReact effect and the voluntary response in control trials, while the 'x' symbol indicates where the suprathreshold TMS did not influence the subcortical startle reflex (SCM activation).**

The dissociation of descending and ascending pathways from the nRPC is also supported by the startle response (SCM activation) data from Experiment 1 and from the results of Alibiglou et al. (2009). In both cases, SCM activation following the presentation of a SAS was not influenced by the TMS, but the movement onset was delayed. Because the startle response was unchanged, the subcortical pathways mediating the startle response appeared to be unaffected by TMS delivered to M1 (see Figure 4.1). Studies investigating PPI with the StartReact effect provide further support for the dissociation of descending and ascending pathways projecting from the nRPC. While the StartReact effect was maintained, both a tactile prepulse (Valls-Solé, et al., 2005) and auditory prepulse (Maslovat, Kennedy, et al., 2009) delivered 100 ms prior to the IS diminished the startle response (SCM activity) elicited by the SAS.

Based on the RT results in Experiment 1 and the model proposed by Carlsen et al. (in press), it may be possible to delineate which processes are taking place during the premotor RT interval, and when they occur. Figure 4.2 shows the actual timing of critical events during control and startle trials from Experiment 1, and the approximate timing of important processes during the RT interval. It has been shown that neural conduction time from M1 to peripheral limb muscles is about 25 ms (Pascual-Leone, Valls-Solé, Brasil-Neto, Cohen, & Hallett, 1994; Rothwell, 1997), and this is depicted for all conditions in Figure 4.2. In control trials, it takes about 35 ms for the acoustic 'go' signal to travel through the auditory nerve to the primary auditory cortex via the ventral cochlear nucleus (see Figure 4.1; Erwin & Buchwald, 1986). Based on the work by Hanes and Schall (1996), initiation time in control trials is depicted in Figure 4.2 to be 50 ms. The remaining time in the RT interval for control trials (36 ms) may include additional cortical processing, for example stimulus detection and recognition, and neural conduction to M1. In contrast, however, a SAS takes only 5-7 ms to activate the lateral lemniscus (LL) at the level of the pons (Erwin & Buchwald, 1986; Stelmack, Knott, & Beauchamp, 2003). In addition, conduction time from the LL to the thalamus is 5-10 ms (Stockard, Stockard, & Sharbrough, 1977), and from the thalamus to M1 neural conduction time is only 2-4 ms (Nambu, Yoshida, & Jinnai, 1988; Salami, Itami, Tsumoto, & Kimura, 2003). Conservatively adding the conduction time values for neural activation through the subcortical startle pathways to M1 via the thalamus proposed to

mediate the StartReact effect equates to only 21 ms. Carlsen et al. (in press) suggest that the increased activation from the startle response may release cortically prepared movements involuntarily, so there would be no additional cortical processing in startle trials. The remaining time (36 ms) in startle trials is therefore assumed to be neural initiation time, which is also predicted to be shorter in startle trials compared to control trials due to an increase in accumulation rate of neural activation due to the more intense startling stimulus (Carlsen, et al., in press; Maslovat, et al., 2011).
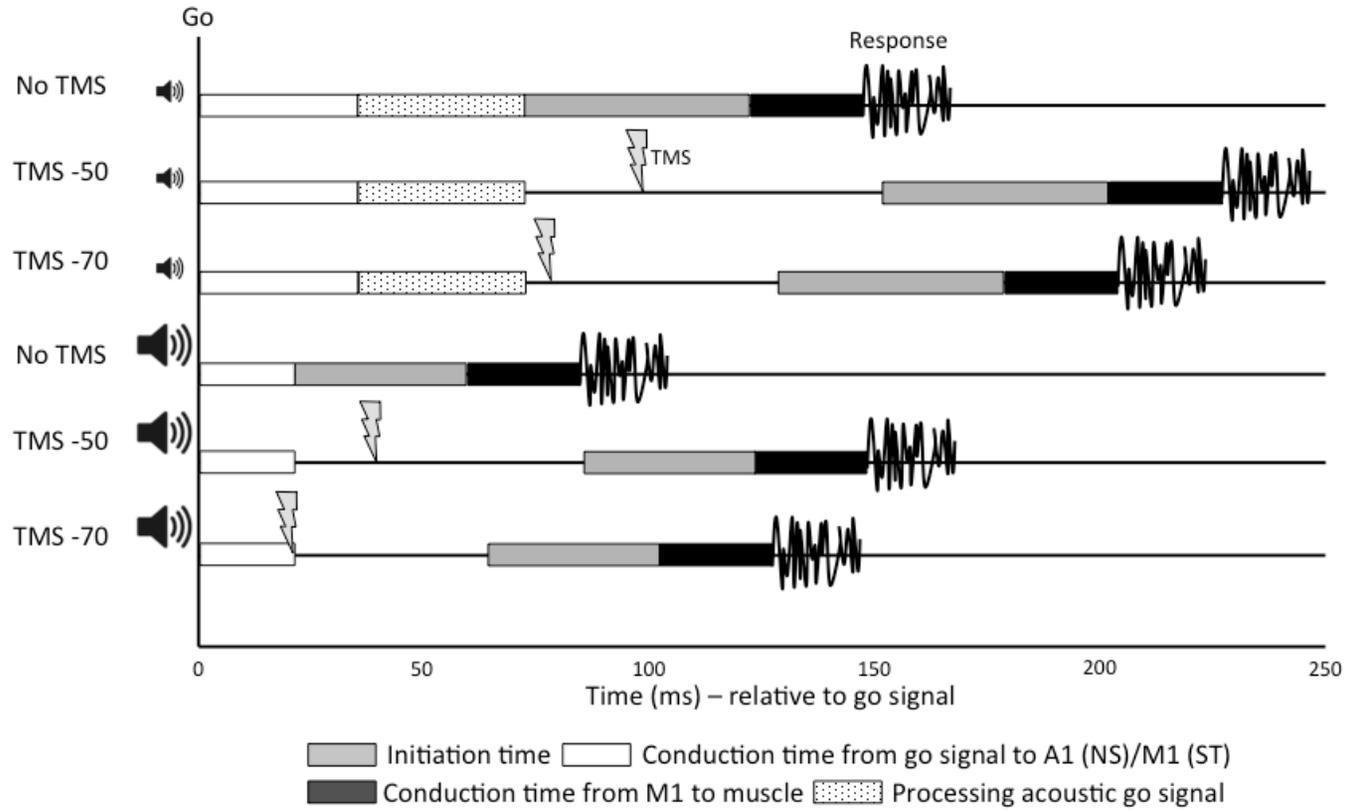
Figure 4.2   Diagram depicting the timing of critical events during control (small speakers) and startle (large speakers) trials in Experiment 1. The EMG represents mean response onset in each experimental condition. The lightning represents the timing of suprathreshold TMS delivery to the contralateral M1 during the premotor RT period in TMS trials. The black bars represent conduction time from M1 to upper limb muscles (25ms, see text for descriptions of all timings). The white bars represent conduction time from the 'go' signal to the primary auditory cortex in control trials (35 ms) and to M1 in startle trials (21 ms). The grey bars in control trials represent initiation time (50 ms). The dotted bars in control trials represents the left over time for additional cortical processing and neural conduction to M1 (36 ms). Assuming no cortical processing in startle trials, the light grey bars represent initiation time (36 ms).

The use of the StartReact effect has become an important methodological tool that allows for the examination of the nature of pre-programmed motor responses. The combination of the startle methodology with neurophysiological techniques such as TMS can provide valuable insights into the neural substrates involved in the preparation and initiation of voluntary movements. The results of this thesis help to provide more details about the neural structures and processes involved in mediating the rapid release of prepared movements by a SAS. This thesis supports the recently proposed neural mechanism for the RT facilitation effect of a SAS by Carlsen et al. (in press), based on their model of movement preparation and initiation. Specifically, the present work provides further evidence that movements released early by a SAS are potentially initiated from motor cortical areas, and that this initiation may occur via an involuntary subcortical trigger.

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