GENERATION OF THE TRIPHASIC ELECTROMYOGRAPHIC PATTERN ACCOMPANYING TARGETED BALLISTIC MOVEMENTS

by

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Abstract

Muscles involved in rapid, targeted movements about a single-joint often display a triphasic (agonist – antagonist – agonist) electromyographic (EMG) pattern. Early work using movement perturbations suggested that for short movements, the entire EMG pattern was prepared and initiated in advance (Wadman et al., 1979), whereas more recent TMS evidence indicates that the ANT may be programmed separately (MacKinnon & Rothwell, 2000) with execution of the bursts occurring serially (Irlbacher et al., 2006). The purpose of this thesis was to investigate the generation of triphasic EMG bursts for movements of different amplitudes. In Experiment 1, participants performed rapid elbow extension movements to 20° and 60° targets and on some trials, a startling acoustic stimulus (SAS), which is thought to trigger prepared motor commands at short latency, was delivered at the onset of AG1. For short movements, this perturbation elicited ANT and AG2 early, suggesting the agonist and antagonist bursts may have been programmed separately and were sufficiently prepared (for SAS trigger) at the time of AG1 onset. In contrast, the same manipulation did not disrupt EMG timing parameters for the long movement, where the ANT and AG2 bursts normally occurred later. The inability to trigger these bursts separate from AG1 raised the possibility that for a longer movement, ANT and AG2 were not fully prepared in advance of movement onset. In Experiment 2, generation of the ANT (and AG2) bursts underlying a longer movement was examined in more detail. We presented a SAS later in movement, relative to each participant’s expected ANT onset (70 ms before). This perturbation produced an early onset of both the ANT and AG2 bursts. Similar to recent TMS findings, our findings suggest that the triphasic pattern is executed serially. By manipulating movement amplitude, and thus specific EMG timing characteristics, we have shown the trigger
signal for initiating the ANT burst occurs not in direct relation to the AG1 burst, but rather in close temporal proximity to the expected onset of ANT.
Preface

This thesis is concerned with the findings from two experiments conducted by the Motor Control and Learning Laboratory at the University of British Columbia. The experiments were conducted under the supervision of Dr. Ian M. Franks, Dr. Romeo Chua, and Dr. Dana Maslovat, as well as in collaboration with Dr. Anthony N. Carlsen from the University of Ottawa.

I was responsible for the majority of the experimental design, data collection, data marking, data analysis, and drafting of the manuscript. The principle programmer for the experiments was Dr. Dana Maslovat.

Ethics approval was obtained from the University of British Columbia Behavioural Research Ethics Board. Certificate Number: H09-00632.

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Dedication

to my family, Rick, Linda, and Kim
1 Chapter: General Introduction

Electrical activity of muscles for a single-joint movement was first described almost 90 years ago by Kurt Wacholder (Wachholder & Altenburger, 1926), a German physiologist, and his assistant, Hans Altenburger (for a translation and interpretation see Sternad & Corcos, 2001). Slow movement was characterized by continuous activity in the participating muscles, whereas rapid movement displayed a stereotypical phasic pattern with two agonist bursts separated by a single antagonist burst. Since its discovery using fast single-joint isotonic movements, the triphasic electromyographic (EMG) pattern has been investigated extensively in both healthy and clinical populations (for a review see Berardelli et al., 1996). It has also been observed in isometric actions (e.g. Corcos, Agarwal, Flaherty, & Gottlieb, 1990; Gordon & Ghez, 1984), and in muscles involved with multi-joint movements (e.g. Wadman, Denier van der Gon, & Derksen, 1980).

Ballistic self-terminated movements begin with a burst of activity in the agonist muscle (AG1), functioning to accelerate the limb toward the target. AG1 is sometimes followed shortly (~10 ms after onset) by an initial period of co-activation in the antagonist muscle, which may aid in terminating acceleration (Cooke & Brown, 1990; Hallett & Marsden, 1979). Near the end of AG1, a distinct burst in the antagonist muscle (ANT) appears. The ANT generates a braking effort to stop movement near the target, but often the force generated is large enough to cause a slight reversal in displacement. Any terminal oscillations are counteracted by a final burst in the agonist muscle (AG2), usually occurring around peak displacement.

Despite a considerable number of experiments that have investigated this triphasic pattern of muscle activity, the processes and structures involved in its production are not well understood (Hallett, 2012). Early work argued that the pattern is generated by a combination of
central programming and myotatic reflexes (see discussion in Hallett, Shahani, & Young, 1975), but there is also evidence to suggest that the majority of the triphasic pattern is produced centrally. As an example, all three bursts are still present in deafferented patients (Cooke, Brown, Forget, & Lamarre, 1985; Hallett et al., 1975; Rothwell et al., 1982), and AG2 is preserved after blockade of the motor nerve innervating the ANT muscle (Garland, Angel, & Moore, 1972).

Further support for central rather than reflexive control of single-joint movement comes from experiments showing that the instructional set provided to the participant affects the triphasic pattern. For example, when the task does not require voluntary braking of movement, ANT activity is largely reduced (Gottlieb, 2001; Mustard & Lee, 1987; Waters & Strick, 1981). Similarly, when the instruction involved stopping a passively extended limb at a target, ANT and AG2 bursts are preserved, in the absence of AG1 (Chow et al., 2003).

While it is generally accepted that the triphasic pattern is produced centrally, considerable debate surrounds how the central nervous system plans single-joint movements. Because various EMG parameters co-vary with movement kinematics, much of the argument has centered on whether movement is planned in terms of desired kinematic characteristics (e.g. Brown & Cooke, 1990; Cooke & Brown, 1990), shifts in limb equilibrium position (e.g. Feldman & Levin, 1995), or directly in terms of muscle activation patterns (e.g. Gottlieb, 1998). Although the issue remains unresolved, Gottlieb and colleagues have described consistent “strategies” by which the excitatory input onto the motoneuron pools of antagonist muscle pairs may be controlled (Corcos, Gottlieb, & Agarwal, 1989; Gottlieb, Corcos, & Agarwal, 1989a, 1989b). “Fast and accurate” movements (or in the absence of instructional constraint; Gottlieb, Corcos, Agarwal, & Latash, 1990) to targets of varying distance are modified by adjusting only the durations and latencies of these excitatory pulses. Referred to as the “speed-insensitive”
strategy, the observed effect on agonist EMG activity is a varied duration but constant initial rise rate of the AG1 burst. Antagonist EMG is similarly modified, with the ANT burst having a constant initial rise, but varied latency and duration (Gottlieb et al., 1989a; Gottlieb, Latash, Corcos, Liubinskas, & Agarwal, 1992). Under this strategy, EMG patterns change in a stereotypical manner, with movements of progressively larger amplitude performed with a longer AG1 burst and a delayed ANT onset. The second strategy, termed “speed-sensitive” is employed for movements with either an explicit timing/velocity goal or an implicit accuracy constraint (Corcos et al., 1989; Gottlieb et al., 1989b). Different movement speeds are attained by modifying intensity of the excitatory pulses. This is reflected in the EMG pattern by a constant duration, but larger initial rise rate of agonist activity for faster movements. Antagonist latency is proportional to total movement time, but the initial rise rate also increases with movement speed. As EMG bursts performed under this strategy (in the case of slower movements) can appear of smaller amplitude (and thus more difficult to determine onset/offset times), or disappear altogether (in the case of AG2), the present investigations were restricted to movements performed under the speed-insensitive strategy.

While a large body of the research literature on the triphasic EMG pattern has focused on how the pattern changes with various task parameters (e.g. distance, speed, or movement time), less is known about how the three bursts are programmed and initiated. One technique that has been used to investigate this issue is the paradigm known as movement blocking (Latash & Gottlieb, 1991; Wadman, Dernier van der Gon, Geuze, & Mol, 1979). In these experiments, participants perform rapid elbow movements to targets of varying distance. On certain trials, the intended movement is unexpectedly obstructed (blocked). EMG patterns from blocked trials (where movement did not occur) are compared to those obtained from unblocked trials. Both
Latash and Gottlieb (1991) and Wadman et al. (1979) reported that a triphasic pattern was still present on blocked trials and that the first 100 ms of EMG were unchanged, as if movement had actually occurred. Wadman et al. (1979) concluded (for short movements) that the entire triphasic EMG pattern was prepared in advance as a unit and once initiated was carried out largely uninfluenced by proprioceptive feedback for at least the first 100 ms. However in the programming of larger amplitude movements, where ANT onset normally occurred later than 100 ms after AG1, it was reported that this burst was absent on blocked trials (Chua, Nagelkerke, Mussell, Cader, & Franks, 2000; Wadman et al., 1979). Two possibilities were suggested to explain this effect: Either (1) the ANT burst was prepared in advance (with AG1) but there was a enough time for feedback processes to intervene and terminate the remainder of action before ANT initiation, or (2) the ANT was not prepared in advance but instead was programmed and initiated online (on movement trials) while the limb was already in motion.

Considerable evidence now suggests that the AG1 burst is generated by primary motor cortex. Both single cell studies in behaving primates and transcranial magnetic stimulation (TMS) studies in humans have shown increased motor cortical excitability prior to the onset of agonist EMG activity (Chen, Yaseen, Cohen, & Hallett, 1998; Lamarre, Busby, & Spidalieri, 1983; Lamarre, Spidalieri, & Lund, 1981; MacKinnon & Rothwell, 2000). In contrast, the role of motor cortex in the generation of the ANT is not known. For instance, recordings from motor cortical neurons that fired in association with agonist activity had a decreased firing rate when that same muscle functioned as the antagonist (Lamarre et al., 1983; Lamarre et al., 1981). Similarly, MacKinnon and Rothwell (2000) found that motor cortex excitability increased just prior to EMG onset when a wrist flexor acted as AG1, but when the same muscle functioned as the ANT (wrist extension), changes in excitability were absent. These authors concluded that the
ANT burst was likely prepared separately and initiated subcortically. In a follow-up study, Irlbacher, Voss, Meyer, and Rothwell (2006) investigated the effects of carefully timed (TMS induced) cortical inhibition during production of the triphasic pattern. When the cortical silent period occurred within the first 30-40 ms of AG1, onset of the ANT was delayed, but relative timing between ANT and AG2 was preserved. If the silent period occurred during the first 30-40 ms of the ANT, AG2 was delayed. However, when the silent period was timed to occur outside of the critical first 40 ms of a muscle burst, the subsequent bursts remained unaffected. It was proposed that tonic cortical outflow was needed to maintain excitability of a non-cortical system (e.g. cerebellum) which ultimately produced the ANT. Therefore each muscle burst of the triphasic pattern appeared to be executed serially, with primary motor cortex generating the trigger signal for a burst 30-40 ms into the previous burst. It should be noted that these conclusions were based from wrist movements of relatively short amplitude. As the ANT and AG2 bursts normally occur later for longer movements, it is unclear whether this critical 30-40 ms trigger window holds for movements of all amplitudes.

The purpose of the current thesis was to determine if a critical time window exists in which the antagonist muscle response can be elicited early as this would provide additional support for serial execution of the components of the triphasic pattern. We used a startling acoustic stimulus (SAS) to elicit the early response and used different amplitude movements to determine if the time course of this critical window was affected by movement distance. Previous literature has shown that replacing an imperative stimulus with a loud sound (> 120 dB) in a simple reaction time (RT) paradigm results in both the elicitation of the startle response and the early initiation (< 65 ms to EMG onset) of pre-programmed motor commands (known as the StartReact effect; Valldeoriola et al., 1998). Despite occurring at short latency, the kinematics
and EMG activation patterns of the prepared voluntary movement appeared mostly unaltered from control trials (for reviews see Carlsen, Maslovat, & Franks, 2012; Rothwell, 2006; Valls-Solé, Kumru, & Kofler, 2008). To explain this StartReact effect, Alibiglou and MacKinnon (2012) and Carlsen et al. (2012) postulated that activation from structures involved in the startle reflex may have acted as an early trigger for the prepared movement. These authors argued that startle-related neural activity ascending from the reticular formation acted as a short latency trigger for the cortically stored prepared movement, by increasing neural activation above a trigger threshold. Because the initiation of each triphasic burst is thought to depend on output from primary motor cortex (Irlbacher et al., 2006) and the triggering effect of startle is thought to act on motor cortex (Alibiglou & MacKinnon, 2012; Carlsen et al., 2012), we reasoned that carefully timed SAS pulses might probe generation of the components of the triphasic pattern. Early elicitation of the ANT would suggest that this burst is generated separately (from AG1), and that structures involved in its control are activated to a sufficient level (for early trigger) at the time of SAS delivery.
Chapter: Experiment 1 – Agonist Startle

2.1 Introduction

The majority of previous StartReact effect experiments have presented the SAS either simultaneous with, or prior to the imperative stimulus (e.g. Alibiglou & MacKinnon, 2012; Carlsen, Chua, Inglis, Sanderson, & Franks, 2009; Carlsen, Dakin, Chua, & Franks, 2007; Maslovat, Hodges, Chua, & Franks, 2011; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999). A few studies have however employed the SAS later, after the imperative signal was presented (Castellote, Queralt, & Valls-Solé, 2012; Kumru & Valls-Solé, 2006; MacKinnon et al., 2007; Queralt, Valls-Solé, & Castellote, 2008). These latter studies delivered the SAS at preset intervals following the imperative stimulus (c.f. Castellote et al., 2012), and reported no change to inter-burst timing. Because startle has not been shown to affect an action after it has been initiated, it was concluded that once commenced, a pre-programmed ballistic movement was resistant to perturbations caused by the SAS (Queralt et al., 2008). The methodology used in these previous studies did not take into account trial-to-trial response onset (reaction time) variability, and thus the SAS was likely delivered at different time points relative to response onset. As will be argued below, independent elicitation of a muscle burst requires precise SAS timing. Methods need to be taken which overcome trial-to-trial onset differences, which can be larger than the triggering effects observed in our experiments.

Participants in Experiment 1 performed ballistic, elbow extension movements to short (20°) and long (60°) targets. On random trials, we delivered a SAS at AG1 onset, in order to examine preparation of the ANT and AG2 muscle bursts. If the entire triphasic pattern is prepared in advance and executed as a unit, the SAS would not be expected to have any effects on the timing of the ongoing movement. Alternatively, if the ANT is programmed separately
from AG1, and the output of each burst is dependent upon cortical activity during production of
the previous muscle burst, we hypothesized that an appropriately timed SAS would prematurely
trigger the ANT (and AG2 bursts). By using movements of different amplitudes, we examined
how the interval between AG1 and ANT might affect preparation of the ANT burst. Irrespective
of amplitude, if ANT is already prepared at AG1 onset, we expected an early ANT initiation for
both movements. Alternatively, if the timing of ANT preparation changes with movement
amplitude, we expected a differential effect of the SAS, depending on movement amplitude.
2.2 Methods

2.2.1 Participants

Fifteen right handed participants, free of any upper body abnormalities (including sensory or motor dysfunctions) and hearing issues participated in Experiment 1 after providing informed written consent. However only data from 10 participants (7 males, 3 females; mean age of 24 ± 4 years) were used in the final analysis (4 participants did not display a consistent startle reflex and 1 participant failed to exhibit the StartReact effect; see Carlsen, Maslovat, Lam, Chua, & Franks, 2011 for detection and classification guidelines). The experiment was approved by the University of British Columbia ethics committee and conducted in accordance with the ethical guidelines set forth by the Declaration of Helsinki.

2.2.2 Apparatus

Participants sat in a height adjustable chair facing a 22 inch colour monitor (Acer X223W, 75-Hz refresh) resting on a table. The right arm of each participant was placed in a lightweight manipulandum attached to the table on the right side of the monitor. The manipulandum’s axis of rotation was positioned such that it aligned with the participants’ right elbow joint and restricted movement to flexion and extension in the horizontal plane. The arm was secured with Velcro straps and hand placed in a semi-supinated position to grasp onto a vertical metal rod at the end of the manipulandum. The start position of the arm was located such that a 30° extension movement resulted in the arm being straight in front of the participant (i.e. perpendicular to the monitor) and was defined as 0°. Targets were placed on the table top at 20° and 60° of extension from the home position and remained visible throughout the duration of the experiment.
2.2.3 Task and Stimuli

All trials began with a warning tone (80.0 ± 2.0 dB, 100 ms, 100 Hz) presented simultaneously with a visual precue in the form of text displayed on the monitor. The text specified the appropriate movement amplitude and read either “Short (20˚)” or “Long (60˚)”. Presentation of a visual imperative stimulus (IS; 12 cm diameter green circle) followed the warning tone by a randomly generated foreperiod of 2500 to 3500 ms. Participants were instructed to “look straight ahead at the monitor” and “respond to the IS by extending their right arm to the appropriate target as fast and as accurately as possible.” To encourage advance preparation of an accurate movement, participants were rewarded $0.05 (CDN) per trial for a fast reaction and $0.05 for an accurate peak displacement (± 3 degrees from the appropriate target). Displacement and RT feedback were displayed on the monitor for 3 seconds following each trial. On selected trials, an auditory signal was delivered either simultaneous with the IS or time-locked to AG1 onset. In this latter condition the signal was triggered at the point where rectified raw EMG activity first increased to more than 5 standard deviations (SD) above baseline (mean of 100 ms of EMG activity preceding the imperative stimulus). The auditory signals (1000 Hz, 40 ms duration, < 1 ms rise time) were either a control acoustic stimulus (80.0 ± 0.3 dB) or a startling acoustic stimulus (124.0 ± 0.3 dB). All auditory signals were generated by a customized computer program, amplified, and presented behind the head of the participant. Sound intensity was verified using a sound level meter (Quest Electronics Model 2700, impulse setting, A-weighted scale) placed 30 cm from the loudspeaker (same distance to the ears of the participant).

Participants performed in a single testing session which lasted approximately 1 hour. After electrode placement, each participant performed 5-10 self-paced trials to each target and the experimenter ensured presence of the triphasic EMG pattern. Confident with electrode
placement, participants then performed a maximal voluntary contraction (at 30° of elbow flexion) of the triceps brachii (agonist) and biceps brachii (antagonist). Next, participants performed 9 practice trials and a block of 38 testing trials to one target, followed by 9 practice trials and a block of 38 testing trials to the other target (order counterbalanced, 5 minute rest period in between). In the practice conditions, presentation of trials was random and included 3 VIS (visual IS only) trials, 3 AUD GO trials (auditory control (80.0 dB) stimulus presented simultaneously with the IS), and 3 AUD AG1 trials (80.0 dB stimulus presented at AG1 onset). During test trials each block included 10 VIS trials, 10 AUD GO trials, 10 AUD AG1 trials, 4 SAS GO (auditory startle (124.0 dB) stimulus presented simultaneously with the IS) trials, and 4 SAS AG1 (124.0 dB stimulus presented at AG1 onset) trials. Trial presentation order was pseudo-randomized, with the stipulation that the SAS trials did not occur on subsequent trials or in the first 3 trials of each block.

2.2.4 Recording Equipment

Preamplified surface electrodes were used to collect EMG data from the following four muscles: right lateral head of the triceps brachii (agonist), right long head of the biceps brachii (antagonist), and right and left sternocleidomastoid (SCM; startle indicator). Shielded cabling connected the electrodes to an external amplifier system (Delsys model DS-80) and a ground electrode was attached to the right ulnar styloid process. Recording sites were shaved, scrubbed, and cleansed in order to reduce electrical impedance. Electrodes were oriented parallel to the muscle fibers and attached using double-sided adhesive tape. At the manipulandum’s axis of rotation, a potentiometer (Precision model MD157) was used to measure angular displacement. A customized LabView® (National Instruments) computer program controlled stimulus and feedback presentation. All signals were digitally sampled at 1000 Hz (National Instruments, PCI-
MIO-16E-1) and EMGs were bandpass filtered between 20-450 Hz for 3 seconds beginning 500 ms prior to the IS.

### 2.2.5 Data Reduction

Data analysis was restricted to the testing trials only (practice trials were excluded from analysis). A total of 93 of the 760 trials were omitted (12.2%). Reasons for discarding trials included very long RTs (i.e. inattention, 36 trials), or very short RTs (i.e. anticipation, 17 trials). These trials were determined by calculating the means and SD of agonist onset times for each condition, within each participant. The criterion was set such that trials greater than or less than 2 SD from the respective mean were discarded. We also removed trials with errorful movements (e.g. no movement, an exceptionally long movement time, or non-SAS trials with an initial peak displacement greater than 10° from required target, 8 trials). SAS trials were omitted if no positive startle response (defined as SCM activity within 120 ms of the stimulus, 4 trials) was detected. An additional 28 AUD AG1 trials were removed for incorrect trigger of the auditory stimulus (> 15 ms from AG1 onset). After removal of these trials, the mean stimulus onset was 3.6 (± 3.7) ms after AG1 onset. Because all discarded trials were identified offline during data analysis they could not be repeated during the experiment.

### 2.2.6 Dependent Measures

Surface EMG burst onsets were defined as the point at which the rectified raw EMG first began a sustained rise above baseline levels (the calculated mean of activity for 100 ms preceding the IS on a trial-by-trial basis). The location of this point was determined by displaying the EMG on a computer monitor with a superimposed line indicating the time at which activity increased to more than 5 standard deviations above baseline. Onset was verified by visually locating and adjusting (if necessary) the onset marker to the point at which activity
first began a sustained rise above baseline. This methodology allowed for correction of errors due to the strictness of the algorithm. A similar method was used to mark EMG offset. Premotor RT was defined as the interval from presentation of the imperative signal to onset of agonist activity. AG1 Q30 was defined as the integrated area (mV*ms) of the first 30 ms of rectified raw agonist activity. Although the EMG burst durations and inter-burst intervals were our primary measures of interest, presenting the SAS at AG1 onset for the short (20°) movement often disrupted activity in the agonist muscle. Specifically, AG2 was compressed into the AG1 burst and we could not accurately mark AG1 offset or AG2 onset (for a detailed description see the Results and Discussion sections for Experiment 1). Therefore it was not possible to determine AG1 duration, AG2 duration, AG1-AG2 interval, or the ANT-AG2 interval. For the short movement, we restricted quantitative EMG analysis to the AG1-ANT interval and ANT duration. Similar disruption of agonist activity did not occur for the long movement, therefore we could quantitatively analyze all burst durations and inter-burst intervals.

In terms of kinematic measures, we chose to analyze peak displacement and time-to-peak displacement, relative to displacement onset. Displacement onset was defined as the first point of change that was greater than 0.2° of angular displacement from the starting position following IS presentation. Peak displacement was determined as the first zero crossing of velocity following displacement onset and time-to-peak displacement was defined as the interval from displacement onset to peak displacement.

2.2.7 Statistical Analysis

To ensure delivery of an 80.0 dB stimulus at AG1 onset had no effect on kinematics or patterns of muscle activation, we ran preliminary One-Way Repeated Measure ANOVAs on all dependent measures (within each movement amplitude) comparing the control conditions (VIS,
AUD GO, and AUD AG1). With the exception of premotor RT, we found no significant differences amongst the 3 control conditions (all p values > .05). Confident that delivery of an 80.0 dB tone at AG1 onset did not disrupt EMG or kinematics, all subsequent analyses involving the “control” condition will refer to 80.0 dB presentation at the IS (AUD GO).

Premotor RT was tested using a 2 (Movement Amplitude: short, long) × 3 (Stimulus Timing: control, SAS GO, SAS AG1) Repeated-Measures ANOVA. The significant main effect for Timing was interpreted with Tukey’s Honestly Significant Difference (HSD) test. A partial eta squared ($\eta^2_p$) value was reported to convey effect size. To ensure our manipulation of movement amplitude (short, long) induced the desired change on the triphasic pattern, we also ran a preliminary analysis on selected variables (AG1 Q30, AG1 Duration, and the AG1-ANT Interval) for the control trials using paired sample t-tests.

For the main statistical analysis we were not directly interested in how the dependent measures changed between movement amplitudes; therefore variables were analyzed independently for the short and long movements. A priori Dunnett contrasts (see Glass & Hopkins, 1996, p.g. 451) were used to compare the two SAS conditions (GO and AG1) to the control condition. The $\alpha$ level for the entire experiment was set at .05.

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1 Premotor RT was expected to be shorter when an auditory tone was presented with the visual IS. This effect can be attributed to inter-sensory facilitation (e.g. Nickerson, 1973; Reynolds & Day, 2007).
2.3 Results

2.3.1 Premotor Reaction Time

The StartReact effect was confirmed by the ANOVA on premotor RTs. A main effect for stimulus timing \( F(2, 18) = 146.26, p < .01, \eta^2_p = .94 \) was found and as expected, a Tukey HSD test showed that delivering a SAS with the IS elicited the response at short latency \((M = 84.8 \text{ ms})\), compared to control \((M = 118.6 \text{ ms})\). The post-hoc test also showed that control trials (i.e. visual stimulus + AUD GO) had a shorter premotor RT than those where a visual IS was followed by a SAS delivered at AG1 onset \((M = 169.1 \text{ ms})\).¹

2.3.2 Speed-Insensitive Strategy

To ensure our manipulation of movement amplitude induced the desired EMG changes, preliminary \( t \)-tests confirmed that duration of the AG1 burst increased \([t(9) = 6.50, p < .01; 85.9 \text{ vs. } 115.1 \text{ ms}]\) and onset of the ANT burst occurred later \([t(9) = 7.62, p < .01; 69.8 \text{ vs. } 109.1 \text{ ms}]\) as larger movements were performed. Analysis of AG1 Q30 revealed the initial rise of the AG1 burst was not modulated to reach targets of different distance \((p > .10)\). Confident that the participants employed the speed-insensitive strategy, we examined preparation of the triphasic pattern for movements to the two different target amplitudes.
<table>
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<th>Long (60°)</th>
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<td>SAS-AG1</td>
<td>SAS-IS</td>
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<td>Premotor RT (ms)</td>
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<td>86.2 (11.2)*</td>
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<td>AG1 Duration (ms)</td>
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<td>Peak Displacement (Degrees)</td>
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<td>26.8 (4.0)*</td>
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<tr>
<td>Time-to-Peak Displacement (ms)</td>
<td>165.2 (34.5)</td>
<td>121.1 (17.6)*</td>
</tr>
</tbody>
</table>

**Table 2.1.** Experiment 1 Results for the different stimulus conditions within both movement amplitudes. Values are means and standard deviations (in parentheses). AG1, first agonist burst (triceps brachii); ANT, antagonist burst (biceps brachii); AG2, second agonist burst.
Figure 2.1. Boxplots of mean triphasic EMG configurations for the short (20’) movement. Box length represents duration of each EMG burst. Error bars correspond to standard error of burst onsets and offsets. To account for reaction time differences between conditions, all data were time-locked to AG1 onset. Asterisks denote significant differences in inter-burst timing compared to control (Dunnett’s test p < .05). Top panel corresponds to the control condition (80 dB presented at IS). Middle panel illustrates when SAS was delivered at AG1 onset. Note the early onset of ANT and AG2 (i.e. the dashed white line corresponds to the approximate location of AG2 onset). Bottom panel represents SAS GO condition. Note that although premotor RT was significantly shortened, no significant change to inter-burst time was observed in this latter condition.
2.3.3 Short Movement Summary

Presenting a SAS at AG1 onset for the short movement disrupted the triphasic pattern and associated kinematic profile (see Figure 2.1 for group mean EMG timing data in boxplot form as well as Figure 2.2 for averaged data from an exemplar participant). The braking action provided by the ANT muscle burst and the clamping force generated by the AG2 burst were both triggered early (Figure 2.2, panel B, grey line). The two agonist bursts appeared temporally compressed making it difficult to determine where AG1 ended and AG2 began (see approximate location in Figure 2.1). In addition to EMG modification, kinematic characteristics were also altered. Although participants reached the correct target, the early brake provided by the perturbed ANT resulted in an increased time to reach peak displacement (see Figure 2.3, top panel, grey line).
Figure 2.2. Average displacement and EMG data (low-pass Butterworth filtered at 150 Hz) from an exemplar participant for 4 movements under each condition to the 20° target. Horizontal axis normalized to displacement onset (time 0). Vertical axis normalized to percent of maximum voluntary contraction (MVC). Startle trials represent the 4 startling acoustic stimulus (SAS: 124 dB) trials for each respective condition. The 4 control trials were chosen (from a total 10 trials) to most closely represent the mean intra- and inter-burst intervals for this exemplar participant. A. SAS presented with the imperative stimulus (SAS GO, grey) compared to the control stimulus (80 dB) presented with the imperative stimulus (black). Note the similar burst durations and inter-burst intervals. See also how peak displacement was reached earlier despite a slight overshoot of the target. B. SAS presented at AG1 onset (grey) compared to the control stimulus presented with the imperative stimulus (black). Speaker symbol denotes SAS delivery at AG1 onset. Note how the ANT burst was elicited early when the SAS was delivered at AG1 onset. Activity in the agonist was less clear, but it appeared AG2 was also triggered early, merging into the end of AG1. See how peak displacement was reached later for startle trials.
Figure 2.3. Group ensemble averaged displacement data for each of the respective conditions for Experiment 1. A. Short movement (20° target). Note how startling acoustic stimulus (SAS) delivery with the imperative signal (SAS GO, grey dashed line) resulted in a shorter time-to-peak displacement, and a significant overshoot of the target. Also see how SAS delivery at AG1 onset (grey solid line) increased time-to-peak displacement, compared with control (black). B. Long movement (60° target). All stimulus conditions reached the correct target, but both SAS conditions reduced time-to-peak displacement when compared with control.
2.3.4 Short Movement Electromyography

A priori Dunnett contrasts were conducted on all quantifiable dependent measures to determine whether delivery of a SAS at AG1 onset (see Figure 2.2B) or at the IS (see Figure 2.2A) had any effect when compared with 80.0 dB delivery at the IS (control). Analysis of the AG1-ANT interval showed delivery of a SAS at AG1 onset significantly shortened this interval \( (p < .01; M = 49.8 \text{ ms}) \), compared to control, but no significant difference existed between control \( (M = 70.7 \text{ ms}) \) and SAS GO \( (p > .10; M = 69.8 \text{ ms}) \). Despite an early elicitation, SAS delivery at AG1 onset did not significantly modify duration of the ANT \( (p > .10) \) compared with control. Similarly, delivering a SAS at the IS did not significantly affect ANT duration \( (p > .10) \).

2.3.5 Short Movement Kinematics

Planned contrasts on peak displacement and time-to-peak displacement showed that delivering a SAS with the IS resulted in a significant overshoot of the target \( (p < .01; M = 26.8^{\circ}) \) and a decreased time to reach peak displacement \( (p < .05; M = 121.1 \text{ ms}) \) compared to control (peak displacement \( M = 22.3^{\circ} \); time-to-peak displacement \( M = 147.2 \text{ ms} \)). In comparison, delivery of a SAS at AG1 onset, did not significantly modify peak displacement \( (M = 21.8^{\circ}) \), but did result in an increased time-to-peak displacement \( (M = 165.2 \text{ ms}) \), compared to control (Figure 2.3A: Note how the SAS GO condition overshot the 20° target, but reached peak displacement earlier than control. Also note how the SAS AG1 condition reached peak displacement later than control).

2.3.6 Long Movement Summary

In contrast to the effects of SAS presentation at AG1 onset for the short (20°) movement, the same manipulation had minimal impact upon EMG timing for the 60° (long) movement (see Figure 2.4 for group mean EMG timing data in boxplot form as well as Figure 2.5 for averaged...
data from an exemplar participant). Kinematic features underwent minor modification on SAS trials with movements on these trials reaching peak displacement faster (see Figure 2.3B).
Figure 2.4. Boxplots of mean triphasic EMG configurations for the long (60°) movement in Experiment 1. Box length represents duration of each EMG burst. Error bars correspond to standard error of burst onsets and offsets. To account for reaction time differences between conditions, all data were time-locked to AG1 onset. Top panel corresponds to the control condition (80 dB presented at IS), SAS delivery at AG1 onset is illustrated in middle panel. Bottom panel represents SAS GO condition. Note that there were no significant changes in inter-burst intervals compared to control for both SAS conditions.
Figure 2.5. Average displacement and EMG data (low-pass Butterworth filtered at 150 Hz) from an exemplar subject for 4 movements to the 60˚ target. Horizontal axis normalized to displacement onset (time 0). Vertical axis normalized to percent of maximum voluntary contraction (MVC). Startle trials represent the 4 startling acoustic stimulus (SAS: 124 dB) trials for each respective condition. The 4 control trials were chosen (from a total 10 trials) to most closely represent the mean intra- and inter-burst intervals for this participant. A. SAS presented with the imperative stimulus (SAS GO, grey) compared to the control stimulus (80 dB) presented with the imperative stimulus (black). Note the similar burst durations and inter-burst intervals. See also how peak displacement was reached earlier. B. SAS presented at AG1 onset (grey) compared to the control stimulus presented with the imperative stimulus (black). Speaker symbol denotes SAS delivery at AG1 onset. Note the timing between bursts appear identical.
2.3.7 **Long Movement Electromyography**

Similar to the short movement analysis, pre-planned Dunnett contrasts were conducted on all dependent measures to determine whether delivery of a SAS at AG1 onset or at the IS had any effect when compared with control. Analysis of AG1 duration revealed that this burst was longer when a SAS was presented at AG1 onset ($p < .02; M = 127.8$ ms) but no significant difference was found between SAS GO ($p > .10; M = 109.3$ ms) and control ($M = 115.1$ ms). ANT duration was found to be shorter for the SAS GO condition ($p < .05; M = 72.0$ ms), but no significant difference was found between SAS AG1 ($p > .10; M = 80.1$ ms) and control ($M = 83.6$ ms). Amongst the remaining EMG timing variables and importantly, the AG1-ANT interval, we found no significant differences (all $p$ values $> .10$), suggesting delivery of a SAS at AG1 onset or at the IS had minimal impact on the timing structure of the triphasic EMG pattern responsible for the control of a long movement.

2.3.8 **Long Movement Kinematics**

Analysis of peak displacement revealed no significant differences between the SAS conditions and control. In contrast, time-to-peak displacement was significantly shorter than control ($M = 241.7$ ms) for both SAS delivery times (SAS AG1 $M = 205.4$ ms, $p < .05$; SAS GO $M = 183.5$ ms, $p < .01$) (Figure 2.3B, grey solid and dashed lines)
2.4 Discussion

The primary objective of Experiment 1 was to determine whether a critical time window existed in which we could elicit an early response in the antagonist muscle, providing support for serial execution of the triphasic EMG burst components. We found evidence for this critical time period for the short movement occurring near AG1 onset but this did not generalize to the long movement. Our findings suggest the ANT burst is generated separately (from AG1) and the time course of its programming may occur differently for movements of different amplitudes.

Similar to previous work (see reviews by Carlsen et al., 2012; Rothwell, 2006; Valls-Solé et al., 2008), a StartReact effect was observed when a SAS was presented with the IS; premotor RT was reduced from ~120 ms to ~85 ms whilst maintaining characteristics of the control movements. By time-locking delivery of a SAS to AG1 onset, we specifically aimed to examine generation of the triphasic EMG pattern underlying movement control. For the short movement (20° elbow extension), where the ANT burst normally occurred ~70 ms after AG1 onset, delivering a SAS at AG1 onset elicited the ANT approximately 20 ms earlier (see Figure 2.2B). Despite an earlier onset, the duration of this burst was not modified from control trials. Based on these results, the pattern of muscle activation may not be structured as a single entity, but rather the ANT may be programmed separately (from AG1) with initiation of the three bursts occurring serially. These results are consistent with the work by Irlbacher et al. (2006) who hypothesized the first 30-40 ms of one muscle burst is critical to determine onset time of the subsequent burst. By presenting the SAS at AG1 onset, we appeared to have influenced the critical ANT trigger window (for the 20° movement), as evidenced by the early ANT burst. Although the present experiment was not designed to directly examine AG2 generation (which would have involved delivering a SAS during early ANT activity), our agonist muscle findings are still supportive of
the serial model. By advancing the ANT burst, we reason the AG2 trigger signal was also advanced. Our results indicated that the relative timing between ANT and AG2 was preserved, but because AG2 was elicited early relative to AG1, the two agonist bursts compressed together (see Figure 2.2B and also Figure 2.1).

Kinematic data for the 20° movement were also disrupted by delivering the SAS at AG1 onset (see Figure 2.3A). Although participants attained an accurate peak displacement, the early braking force provided by the perturbed (i.e. early) ANT resulted in an increased amount of time needed to reach peak displacement. While AG2 typically acts to reduce terminal oscillations (Berardelli et al., 1996), in the present study, the function of the early AG2 appeared to accommodate for the premature ANT burst. Because the decelerating force provided by the ANT was elicited early, AG2 counteracted the brake, allowing motion of the limb to continue in the direction of elbow extension, thus completing the task of moving towards the target, albeit at a slower time course.

As suggested by Irlbacher et al. (2006), and supported by the results of our short movement condition, early activity of a muscle burst plays an important role in determining onset time of the subsequent burst. By manipulating movement amplitude, and thus prolonging onset of the ANT (to ~110 ms after AG1 onset) (e.g. Gottlieb et al., 1989a), we were able to assess whether the preparation time course of the ANT (and AG2) bursts occurred similarly for movements of varying amplitudes. In contrast to the effect observed for the short movement, the SAS did not elicit the ANT or AG2 bursts for the long movement (see Figure 2.5B and also Figure 2.4). Because previous StartReact work has shown a SAS will not trigger motor commands assembled after the stimulus (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004); it appeared that for the long movement, the ANT burst was not programmed (to a sufficient level
for SAS trigger) at the time of AG1 onset. We have therefore provided evidence that the time
course of preparation processes involved in generating the ANT (and AG2) burst(s) occurred
differently for short and long movements.
3 Chapter: Experiment 2 – Antagonist Startle

3.1 Introduction

Although the short movement results of Experiment 1 are consistent with and supportive of the work by Irlbacher et al. (2006), they did not generalize to the long movement, suggesting the critical time window for the next muscle burst may be dependent on the relative EMG timing between bursts. Therefore in Experiment 2 we furthered our investigation of serial triphasic execution by altering the time of SAS delivery in long movements. Participants performed ballistic elbow extension movements only to a 60° target. To examine whether the critical window determining onset of antagonist activity is related to the expected onset of the ANT, we delivered the SAS at a set interval (70 ms) prior to each participant’s anticipated ANT onset. The interval of 70 ms was chosen primarily because it was a similar interval that was successful in probing ANT readiness for the short movement in Experiment 1. It is interesting to note that for long movement control trials in the first experiment, we found large between-participant variability in the AG1-ANT interval (SD of ~20 ms). Variability on the short trials was much smaller (SD of ~7 ms). To control for this variability and also test whether the pertinent variable in which a SAS could independently trigger antagonist activity is related to expected onset of the ANT, a SAS was delivered relative to each participant’s average ANT latency.
3.2 Methods

3.2.1 Participants

Fourteen right handed participants, free of any upper body abnormalities (including sensory or motor dysfunctions) and hearing issues participated in Experiment 2 after providing informed written consent. Five of the participants were recruited from Experiment 1 and 9 were novel to studies in our laboratory. Only data from 11 participants (8 males, 3 females; mean age of 25 ± 5 years) were used in final analysis (3 participants did not display a consistent startle reflex, see Carlsen et al., 2011 for detection and classification guidelines). The experiment was conducted in accordance with the ethical guidelines set forth by the Declaration of Helsinki and approved by the University of British Columbia ethics board.

3.2.2 Apparatus

Participants were positioned on a height adjustable chair in front of a 22 inch colour monitor (Acer X223W, 75-Hz refresh) which rested on top of a table. Each participant had the right arm placed in an aluminum manipulandum secured to the table on the right side of the computer monitor. Manipulandum axis of rotation was aligned with each participant’s right elbow joint. Movement was restricted to horizontal plane flexion and extension. The hand was held in a semi-supinated position to grab a vertical metal post at the end of the manipulandum. The start position of the arm was located such that a 30° extension movement resulted in the arm being straight in front of the participant (this was perpendicular to the monitor) and was defined as 0°. Targets were placed on the table top at 20° and 60° of extension from the home position and remained visible throughout the duration of the experiment.
3.2.3 Task and Stimuli

Every trial began with a warning tone (80.0 ± 2.0 dB, 100 ms, 100 Hz) presented simultaneously with a visual precue in the form of text displayed on the monitor. The text specified the appropriate movement amplitude and always read “Long (60°)”. Presentation of a visual imperative stimulus (IS; 12 cm diameter green circle) followed the warning tone by a randomly generated foreperiod of 2500 to 3500 ms. Participants were told to “look straight ahead at the monitor” and “respond to the IS by extending their right arm to the long target as fast and as accurately as possible.” To encourage advance preparation of an accurate movement, participants were rewarded $0.05 (CDN) per trial for a fast reaction and $0.05 for an accurate peak displacement (± 3 degrees from the appropriate target). Displacement and RT feedback were displayed on the monitor for 3 seconds following each trial. On selected trials, an auditory signal was delivered either simultaneous with the IS, time-locked to AG1 onset, or at a set time interval (70 ms) prior to the estimated ANT onset. To determine the estimated ANT onset for the latter condition, a pretest block of movements was performed. After each pretest trial, the experimenter verified (and adjusted if necessary) the point at which rectified raw agonist and antagonist EMG activity increased to more than 5 SD above baseline. The estimated ANT onset was defined as the average AG1-ANT interval attained during the pretest block. During the testing blocks, auditory stimuli were delivered at a specified time after AG1 onset (to occur at 70 ms prior to the estimated ANT onset for each participant). The auditory signals (1000 Hz, 40 ms duration, < 1 ms rise time) were either a control acoustic stimulus (80.0 ± 0.3 dB) or a startling acoustic stimulus (124.0 ± 0.3 dB). All auditory signals were generated by a customized computer program, amplified, and presented behind the head of the participant. Sound intensity
was verified using a sound level meter (Quest Electronics Model 2700, impulse setting, A-weighted scale) placed 30 cm from the loudspeaker (same distance to the ears of the participant).

Participants performed in a single testing session which lasted approximately 1.5 hours. After electrode placement, each participant made 5-10 self-paced trials to each target and the experimenter ensured presence of the triphasic EMG pattern. Confident with electrode placement, participants then performed a maximal voluntary contraction (at 30° of elbow flexion) of the triceps brachii (agonist) and biceps brachii (antagonist). After flexion and extension MVC trials, 1 block of 15 practice trials consisting of the VIS condition was performed. This was followed by a pretest block of 25 VIS trials which was used to determine a mean AG1-ANT time for stimulus delivery in testing blocks. After a 5 minute rest, the testing trials began. Four testing blocks of 38 trials were performed. Each block consisted of 10 VIS trials, 10 AUD GO trials, and either 10 AUD AG1 or AUD ANT-70 (80.0 dB presented 70 ms before expected ANT onset) trials. Testing blocks also contained 4 SAS GO trials, and either 4 SAS AG1 or 4 SAS ANT-70 trials. Two blocks contained the AG1 trials and the other 2 contained ANT-70, an order which we counterbalanced between participants. Trial presentation order within a block was pseudo-randomized, with the stipulation that the SAS trials did not occur on subsequent trials or in the first 3 trials of each block.

3.2.4 Recording Equipment

Preamplified surface electrodes were used to collect EMG data from the following four muscles: right lateral head of the triceps brachii (agonist), right long head of the biceps brachii (antagonist), and right and left sternocleidomastoid (startle indicator). Shielded cabling connected the electrodes to an external amplifier system (Delsys model DS-80) and a ground electrode was attached to the right ulnar styloid process. Recording sites were shaved, scrubbed,
and cleansed in order to reduce electrical impedance. Electrodes were oriented parallel to the muscle fibers and attached using double-sided adhesive tape. At the manipulandum’s axis of rotation, a potentiometer (Precision model MD157) was used to measure angular displacement. A customized LabView® (National Instruments) computer program controlled stimulus and feedback presentation. All signals were digitally sampled at 1000 Hz (National Instruments, PCI-MIO-16E-1) and EMGs were bandpass filtered between 20-450 Hz for 3 seconds beginning 500 ms prior to the IS.

3.2.5 Data Reduction

Data analysis was restricted to the testing trials only (practice trials were excluded from analysis). A total of 117 of the 1672 testing trials were omitted (6.9%). Reasons for discarding trials included very long RTs (65 trials) or very short RTs (13 trials). These trials were determined by calculating the means and SD of agonist onset times for each condition, within each participant. The criterion was set such that trials greater than or less than 2 SD from the respective mean were discarded. We also removed trials with errorful movements (e.g. no movement, an exceptionally long movement time, or non-SAS trials with an initial peak displacement greater than 10° from required target, 4 trials). SAS trials were also omitted if no startle response was detected (defined as SCM activity within 120 ms of the stimulus; 5 trials). Finally, any trials where the auditory stimulus was delivered incorrectly (> 15 ms from desired temporal location) were omitted (30 trials). After removal of incorrect trigger trials, the mean stimulus onset for the AG1 trials was 2.6 (± 2.6) ms after AG1 onset. Mean stimulus onset for ANT-70 trials was 68.1 (± 1.8) ms before the expected ANT onset obtained from the pretest block.
3.2.6 Dependent Measures

Surface EMG burst onsets were defined as the point at which the rectified raw EMG first began a sustained rise above baseline levels (the calculated mean of activity for 100 ms preceding the IS on a trial-by-trial basis). The location of this point was determined by displaying the EMG on a computer monitor with a superimposed line indicating the time at which activity increased to more than 5 standard deviations above baseline. Onset was verified by visually locating and adjusting (if necessary) the onset marker to the point at which activity first began a sustained rise above baseline. This methodology allowed for correction of errors due to the strictness of the algorithm. A similar method was used to mark EMG offset. Premotor RT was defined as the interval from presentation of the imperative signal to onset of agonist activity. Burst durations (AG1, ANT, ANT) were defined as the interval from burst onset to offset). Inter-burst intervals (AG1-ANT, ANT-AG2, AG1-AG2) were defined as time period between a burst onset to the onset of a following burst.

3.2.7 Statistical Analysis

Similar to Experiment 1, a One-Way Repeated Measures ANOVA was conducted all dependent measures for the control conditions, to confirm that the 80.0 dB stimulus presented after the IS did not affect the muscle activation patterns. With the exception of premotor RT, which was shorter for AUD GO (than the other 3 conditions) we found no other significant differences amongst the 4 control conditions (all p values > .05). Confident that delivery of an 80.0 dB tone at AG1 onset did not disrupt EMG or kinematics, all subsequent analyses involving the “control” condition will refer to 80.0 dB presentation at the GO (AUD GO).

Premotor RT was tested using a 1 factor (Stimulus Timing: control, SAS GO, SAS AG1, SAS ANT-70) repeated-measures ANOVA. The significant main effect for Timing was
interpreted with Tukey’s Honestly Significant Difference (HSD) test. A partial eta squared ($\eta^2_p$) value was reported to convey effect size. In the main statistical analysis, planned Dunnett contrasts were used to compare the three SAS conditions (GO, AG1, and ANT-70) to the control condition. The $\alpha$ level for the entire experiment was set at .05.
3.3 Results

3.3.1 Premotor Reaction Time

Evidence of the StartReact effect was revealed by the ANOVA on premotor RTs. A main effect for stimulus timing \([F(3, 30) = 135.04, p < .01, \eta_p^2 = .93]\) was found and as expected, a Tukey HSD test confirmed that delivering a SAS with the IS elicited the response at short latency \((M = 87.8 \text{ ms})\), compared to control \((M = 122.0 \text{ ms})\). The post-hoc analysis also showed that trials with SAS delivery at AG1 onset \((M = 175.5 \text{ ms})\) and 70 ms before ANT onset \((M = 173.1 \text{ ms})\) had longer premotor RTs than control, an effect we can attribute to intersensory facilitation at the IS.
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*Table 3.1.* Experiment 2 Results for the different stimulus conditions. Values are means and standard deviations (in parentheses). AG1, first agonist burst (triceps brachii); ANT, antagonist burst (biceps brachii); AG2, second agonist burst (triceps brachii).
Figure 3.1. Boxplots of mean triphasic EMG configurations for the long (60°) movement in Experiment 2. Box length represents duration of each EMG burst. Error bars correspond to standard error of burst onsets and offsets. To account for reaction time differences between conditions, all data were time-locked to AG1 onset. Asterisks denote statistically significant differences in inter-burst timing compared to control (Dunnett’s test $p < .05$). Top panel corresponds to the control condition (80 dB presented at IS). Second panel displays SAS ANT-70. Note early onset of ANT and AG2 bursts. SAS delivery at AG1 onset is displayed in the third panel. Bottom panel represents SAS GO condition. Note that there were no significant changes in inter-burst intervals compared to control for the latter two SAS conditions.
Figure 3.2. Average displacement and EMG data (low-pass Butterworth filtered at 150 Hz) from an exemplar subject for 8 movements to the 60° target. Horizontal axis normalized to displacement onset (time 0). Vertical axis normalized to percent of maximum voluntary contraction (MVC). Startling acoustic stimulus (SAS: 124 dB) presented at 70 ms prior to the anticipated ANT onset (grey) compared to the control stimulus (80 dB) presented with the imperative stimulus (black). Startle trials represent the 8 SAS trials for each respective condition. The 8 control trials were chosen (from a total 40 trials) to most closely represent the mean intra- and inter-burst for this participant. Speaker symbol denotes SAS delivery time, approximately 70 ms before expected ANT onset. Note the ANT burst and AG2 bursts were elicited early on SAS trials.
3.3.2 Electromyography

Similar to Experiment 1, delivery of a SAS with the IS, or at AG1 onset had minimal effect on EMG timing for a large amplitude movement. The important finding from this experiment was that presenting a SAS 70 ms prior to the expected ANT onset led to early onset of the ANT and AG2 bursts, relative to AG1 (Figure 3.1 and Figure 3.2, grey line).

Planned Dunnett contrasts were used to determine whether delivery of a SAS at the IS, AG1 onset, or 70 ms prior to the estimated ANT onset had any effect when compared with 80.0 dB delivery at the IS (control). Irrespective of delivery time, duration of the AG1 burst was not modified by the SAS (all p values > .10). Duration of the ANT burst was only shortened for the SAS AG1 (p < .05; M = 71.9 ms) condition compared to control (M = 81.9). Duration of AG2 was not different than control for any SAS condition (all p values > .10). Examination of the inter-burst intervals revealed a time-dependent effect of the startle stimulus. A SAS delivered 70 ms before the expected ANT onset shortened both the AG1-ANT (p < .05; M = 97.2 ms) and AG1-AG2 intervals (p < .01; M = 147.9 ms) compared to control (AG1-ANT M = 108.9 ms; AG1-AG2 M = 164.7 ms). Delivering a SAS with the IS or at AG1 onset had no significant effect on these intervals (all p values > .10). Interestingly, for the ANT-AG2 interval, no significant differences were found between the three startle conditions and control (all p values > .10). Collectively, these inter-burst timing results indicate that by eliciting the ANT burst early (for the SAS ANT-70 condition), the AG2 burst was also triggered early relative to AG1, but relative timing between ANT and AG2 appeared to be preserved.
3.4 Discussion

The focus of Experiment 2 was to examine the existence of a critical time window for early elicitation of the ANT related to normal ANT latency. Whereas we did not find early elicitation when a SAS was presented with the IS or time-locked to AG1 onset (replicating Experiment 1 findings), we did trigger the ANT early when the SAS was delivered prior to the expected onset of ANT. These findings suggest the time period in which the ANT burst can be perturbed with a SAS is related to ANT timing, rather than a set interval after AG1 onset.

A StartReact effect was observed when a SAS was presented with the IS, as premotor RT was reduced by ~35 ms without significantly affecting the structure of response. In contrast, when we presented a SAS 70 ms before the expected ANT, the whole response was not elicited early, rather the AG1-ANT interval was shortened by ~15 ms. Additionally, the AG2 burst was initiated ~15 ms early relative to AG1 onset, but was not changed in relation to the onset of ANT. Despite an early onset, duration of the ANT and AG2 bursts were not modified from control trials. These results confirmed that for a long(er) 60˚ movement, the ANT burst was programmed separately from AG1, and approximately 70 ms before expected onset, ANT preparation was at a sufficient level to enable early SAS-mediated release. Although our results are still supportive of a serial model of triphasic generation, we have provided strong evidence that the first 30-40 ms of AG1 was not always critical in determining onset time of the ANT; instead the critical ANT trigger window appeared to scale with movement amplitude, occurring in close temporal proximity with the nominal ANT onset.
4  Chapter: General Discussion

Previous investigations have shown that during a RT task, prepared movements are elicited early if a startling acoustic stimulus is delivered simultaneous with the imperative signal (see reviews by Carlsen et al., 2012; Rothwell, 2006; Valls-Solé et al., 2008). The present data not only verify that the SAS can trigger pre-programmed movements but also extends the early triggering effects to individual muscle bursts within a movement. Indeed, through precise temporal delivery of the SAS during performance of ballistic single-joint actions, a significant disruption of the triphasic EMG pattern responsible for movement control was observed. By manipulating movement amplitude (and thus some temporal characteristics of the triphasic pattern), we also observed a differential triggering effect of the SAS providing evidence that the time course of preparation of the bursts occurred differently for short and long movements. Our findings support the argument that the triphasic EMG pattern is not initiated as a single entity but rather the ANT burst may be programmed separately from AG1 (MacKinnon & Rothwell, 2000), with execution of each burst occurring in a serial manner (Irlbacher et al., 2006). Furthermore, the trigger signal responsible for initiating antagonist activity appears to occur not in relation to the AG1 burst, but rather in close temporal proximity to the expected onset of the ANT.

The primary finding in Experiment 1 was that for the short movement (20° elbow extension), delivery of a SAS at AG1 onset triggered the ANT burst early (Figure 2.2B). Previous work has shown motor commands could only be elicited early by startle if prepared in advance of SAS delivery (see Carlsen et al., 2004); therefore it appeared for these short movements, the ANT burst was programmed (and awaiting separate initiation) at the time of AG1 onset. We also manipulated amplitude and by applying the same perturbation, examined whether the time course of ANT programming was affected by movement distance. In contrast to
the effect observed for the short movement, SAS delivery at AG1 onset did not elicit the ANT burst early for the long movement (60° elbow extension) (Figure 2.3B). This inability to trigger the ANT independently for the long movement suggests the ANT was not completely prepared at the time of AG1 onset.

The principal reason for using two movement amplitudes was to modify ANT burst latency on control trials. For the short movement, the AG1-ANT interval was ~70 ms whereas this value increased to ~110 ms on long movements. Because of the discrepant startle effects in the first experiment, we designed the second experiment to provide further insight into the time course of ANT programming for a long movement. We hypothesized the critical window in which ANT readiness can be probed with SAS may be tied to normal onset of the ANT, rather than a specific time after AG1. To test this, each participant’s mean AG1-ANT interval was calculated, and a SAS was delivered at a set time before the ANT burst was expected. Presenting the SAS 70 ms before normal onset of the ANT triggered this burst early, confirming separate ANT generation for the long movement (Figure 3.2).

The findings obtained from the current experiments are compatible with predictions made by recent TMS investigations of triphasic generation. In one study, motor cortex excitability was probed at different time points throughout the pattern (MacKinnon & Rothwell, 2000). Cortical excitability was found to increase immediately prior to the AG1 burst, but when the same muscle functioned as the antagonist, the excitability changes were absent. These authors suggested even though the AG1 burst may be mediated by primary motor cortex, ANT appears to be generated separately and subcortically. Because careful delivery of a SAS in the present study was able to elicit the ANT early and independent from AG1, our results provide support for separate programming of the ANT. In addition, we found evidence of separate ANT generation for more
than one movement amplitude, thereby extending the predictions previously based only on short wrist flexion/extension actions.

Following up on MacKinnon and Rothwell’s (2000) study, Irlbacher et al. (2006) investigated the influence of a carefully timed cortical silent period at different points throughout the pattern. When the silent period occurred within the first 40 ms of a muscle burst, onset of the next burst was delayed, but relative timing between subsequent bursts was not affected. However applying the silent period outside of the critical first 40 ms of a burst did not disrupt inter-burst timing. These authors proposed that the triphasic pattern is executed serially, with primary motor cortex generating the trigger signal for each burst, 30-40 ms into the preceding burst. The findings from our short movement are compatible with this hypothesis. The control AG1-ANT interval (~70 ms) from our study was similar to that obtained by Irlbacher and colleagues. By presenting a SAS at AG1 onset, we reasoned we could influence this critical window determining ANT onset. Confirming this prediction, a carefully timed SAS pulse elicited the ANT burst ~20 ms early.

While our short movement findings are supportive of previous work, the long movement results provided evidence the first 30-40 ms of AG1 was not always critical in determining onset for the ANT. Because the ANT occurred later for the longer movement, and SAS delivery at AG1 onset did not trigger this burst early, it appeared the critical ANT burst window was not always related to early AG1 activity. Instead this critical period may have occurred later, closer to the expected onset of the ANT. We explored this issue further in Experiment 2. Consistent with Experiment 1, we first replicated the lack of effect of the SAS at AG1 onset; however presenting a SAS 70 ms before normal ANT burst onset elicited the ANT early. Because an early ANT burst was only observed when the SAS was delivered at a set time before each participant’s
expected ANT, our findings confirmed the critical window for a larger amplitude movement occurred later and was related to normal timing of the ANT.

Despite only using two movement distances, the findings obtained here should generalize to a wider range of single-joint movements performed with the speed-insensitive strategy. Because ANT latency is proportional to movement distance (Gottlieb et al., 1989a, 1989b; Gottlieb et al., 1992), the window in which ANT triggering can be probed (with a SAS or TMS) also likely scales with the size of movement. For very short movements, we have provided evidence that processes involved in ANT preparation occur in advance of response onset (AG1 initiation). For longer movements, it appeared that at least final preparation of the ANT burst occurred online, after AG1 initiation. Based on this pattern of results, we reason there likely exists a threshold movement distance whereby ANT preparation switches from occurring before AG1 onset to afterwards. This threshold distance likely varies slightly person to person, based on previous agonist-antagonist interactions reaching the target.

The serial execution model proposed by Irlbacher et al. (2006) also suggested that the trigger signal for AG2 was determined during the first 30-40 ms of ANT. While the present investigation was not designed to specifically test AG2 generation, on trials where the ANT burst was elicited early, agonist activity was also disrupted. For the short movement, it appeared as if either duration of the AG1 burst was prolonged, or the AG2 burst was elicited early, converging with the end of AG1 (see Figures 2.1 and 2.2B). Because AG2 initiation was proposed to be determined during early ANT, by eliciting ANT early, we reasoned the AG2 trigger signal was also initiated early. This interpretation was confirmed in Experiment 2. For the 60˚ movement, AG2 was also disrupted, but because AG1 and AG2 were further separated, the two agonist
bursts did not converge (see Figures 3.1 and 3.2). It appeared that by advancing ANT, irrespective of movement amplitude, AG2 was also advanced by a similar amount.

Although the exact mechanisms underlying triphasic EMG generation and the StartReact effect are relatively unknown, combining recent descriptions for both provides a possible explanation of the results obtained here (see Figure 4.1). As previously mentioned, considerable evidence suggests AG1 is cortically generated and delivered via the corticospinal tract (e.g. Chen et al., 1998; Lamarre et al., 1983; Lamarre et al., 1981; MacKinnon & Rothwell, 2000), but less evidence supports a direct involvement of motor cortex control over the ANT. Instead it was suggested that subcortical structures such as the cerebellum may be more directly involved in its production (Irlbacher et al., 2006; MacKinnon & Rothwell, 2000). In order to appropriately time ANT activity relative to AG1, the cerebellum has to be informed when AG1 was initiated. It has been suggested that a signal from M1 may be sent to the cerebellum presumably by way of cortico-pontine to ponto-cerebellar fibers (see Appendix A as well as Hore & Vilis, 1985 for a similar interpretation regarding antagonist activity following long-latency reflex responses). Upon reaching the cerebellum, this signal either begins the process of initiating the ANT burst or directly acts as a trigger signal. The ANT burst would then travel down a brainstem-spinal pathway to the appropriate spinal level (MacKinnon & Rothwell, 2000).

To account for the movement triggering effects following SAS delivery at an imperative signal, it has been hypothesized that ascending activation from pontomedullary reticular formation travels to thalamus and then motor cortex to involuntarily elicit pre-programmed motor commands (Alibiglou & MacKinnon, 2012; Carlsen et al., 2012). Here we propose that by precisely timing the SAS to occur 70 ms before normal ANT onset, this ascending activation interacted (at the level of motor cortex) with the trigger signal for the ANT burst. This trigger
signal was elicited early and travelled to the cerebellum, ultimately producing the ANT burst 15-20 ms early.
**Figure 4.1.** Possible mechanism to account for agonist-antagonist interactions and the triggering of the ANT burst in the present study. AG1 is believed to be generated by primary motor cortex, travelling to spinal motor neurons via the corticospinal (CS) tract. After AG1 initiation, a signal from primary motor cortex is sent to the cerebellum via cortico-pontine (CP) to ponto-cerebellar (PC) fibers. This signal either triggers the ANT burst from the cerebellum, or starts the process to program and initiate the burst. The commands responsible for the ANT are then output onto brainstem-spinal pathways. When a SAS is delivered, the intense stimulus is believed to activate the caudal pontine reticular nucleus (nCPR). In addition to activating reticulo-spinal tract (producing the startle reflex, not shown), ascending activation travels first to thalamus and then primary motor cortex. When we delivered the SAS 70 ms before normal ANT onset, this ascending activation may have influenced the ANT trigger signal at a critical time. This trigger signal was prematurely elicited and ultimately produced the ANT burst early.
The findings of the current study show that an SAS can be used to investigate the internal elements of a single movement provided there is delivery of the stimulus at a specific time relative to the pertinent variable. We have shown that for the braking force in the triphasic pattern of muscle activation, this critical variable is the ANT onset and a time window exists about 70 ms prior to its nominal onset where SAS triggering can occur. We reason that by carefully delivering a SAS at specified times, either after onset of one muscle burst, or prior to the estimated onset of another, the time course of generating motor commands responsible for the control of movement can be determined for wider range of movement amplitudes and a variety of actions.
Bibliography


Appendix A.

Efference Copy Model of Antagonist Generation

A fast perturbation to a joint in a relaxed subject evokes a monosynaptic reflex (M1) in the stretched muscle at a latency of 20-50 ms. When the instruction involves resisting the stretch, the monosynaptic reflex is unaffected, but beginning 50-80 ms post-perturbation, a second burst of activity appears in the stretched muscle. This is referred to as the long latency stretch reflex (M2), and is believed to involve a longer transcortical pathway beginning with activation of Ia afferent fibres, up the dorsal column to sensorimotor cortex, and back down the corticospinal pathway (e.g. Rothwell, 1994). Some researchers have argued that modulations in activity during the M2 period are a result of gain modification to a transcortical reflex pathway (e.g. Pruszynski, Kurtzer, & Scott, 2008; Pruszynski, Kurtzer, & Scott, 2011; Pruszynski & Scott, 2012), but others have argued that M2 is merely an involuntarily triggered prepared movement (e.g. Manning, Tolhurst, & Bawa, 2012; Rothwell, Traub, & Marsden, 1980). Regardless of its origin, correcting for a perturbation requires M2 activity to move the joint opposite to the direction of perturbation. If the perturbation is of short duration (e.g. 40 ms) an appropriately timed burst of activity in the antagonist muscle (~60 ms after M2 onset) is necessary to brake the return motion of the limb (Hore & Vilis, 1984, 1985; Vilis & Hore, 1980). The ANT burst usually precedes stretch of the antagonist muscle and thus must be centrally driven, not the result of a monosynaptic stretch reflex.

In this appendix we focus on the ANT burst following M2 and its relation to a model of triphasic EMG generation presented in the main thesis document. The goal is not to resolve whether long-latency response modulations are due to a transcortical reflex, or reflective of a triggered response. It is however interesting to note that the stereotypical coupling between M2
and ANT following a perturbation is very similar to the interaction between AG1 and ANT underlying fast voluntary movements. Thus the series of studies reviewed here may provide indirect support for the hypothesis that the long latency reflex is part of an involuntary triggered, yet otherwise normal voluntary response.

John Hore and colleagues in the 1980’s investigated the nature of the “triggered” antagonist following an elbow perturbation. In these studies, monkeys were trained for many months to return a manipulandum to a home position following either a torque pulse (20, 40, 60, 80, 100, 120, 140 ms) or step (2000 ms) perturbation. The first experiment (Vilis & Hore, 1980) investigated the relationship between cerebellar (dys)function and cortical responses associated with task performance. Cryogenic lesions were induced around the ipsilateral (to movement side) dentate nucleus with the purpose of blocking as much of the lateral cerebellar hemisphere as possible. When the lesion was activated, a short duration (40 ms) pulse perturbation resulted in a normal M2 response, but a delayed ANT which occurred at a latency following antagonist stretch. Single cell recordings were also obtained from agonist and antagonist motor cortex neurons. The neurons corresponding to agonist were not affected by cerebellar lesion and cortical responses began before the onset of M2. Motor cortical responses before ANT were delayed but still led EMG activity in the antagonist muscle. These authors reasoned that an intact cerebellum was necessary to signal primary motor cortex neurons to trigger the ANT burst. In the case of a cerebellar lesion, this pathway was blocked and the antagonist cells in motor cortex were instead responding to stretch of the antagonist. Vilis and Hore concluded that phase advancing of the ANT on non-lesion trials had a predictive component based on learning. The cerebellum appeared to be involved with prediction and after sufficient practice could learn an appropriate response to terminate the corrective M2 with an active ANT burst.
In a follow up study, Hore and Vilis (1984) investigated the relative afferent and central drive responsible for the triggered antagonist activity. A range of conditions were used, but the optimal condition for evoking ANT was when the antagonist muscle was preloaded, and the perturbation was 40 ms long. The 40 ms pulse perturbation was compared with a longer step perturbation (2000 ms) which normally resulted in M1, M2, and M3 (“voluntary” response), but no antagonist activity. When participants were awaiting a pulse but received a step, they had smaller M2 and M3, and only some ANT. However, when they were prepared for a step, but received a pulse the return move overshot the target due to a delayed ANT, a small increase in M2, and the appearance of M3. The function of set in this paradigm appeared to direct activity after 60 ms to agonist (for an expected step perturbation) or to antagonist (for an expected pulse perturbation). In another condition, cryogenic probes were placed around the dentate. When the lesion was activated, the commands ascribed to set appeared abolished. Instead of a feed-forward mechanism responsible for ANT or M3, these delayed responses appeared to be driven by afferent feedback. These authors concluded that set might be mediated (at least in part) by dentate nucleus. When a perturbation was appropriately predicted, the cerebellum switched activity from agonist to antagonist cortical motoneurons (or vice versa).

The studies reviewed above show that an appropriate response to an expected limb perturbation requires an intact cerebellum. Hore and Vilis (1985) developed a model which can describe their perturbation results and is similar to the model of voluntary triphasic EMG generation originally proposed by Irlbacher et al. (2006) and expanded on in this thesis. A limb perturbation (either a 40 ms pulse or a 2000 ms step) produces a stretch of the agonist muscle. It is generally well accepted that stretching of muscle spindles elicits M1 as a spinal reflex, and M2 as a transcortical response. Hore and Vilis proposed that an efference copy of the cortically
triggered M2 commands travels to the cerebellum. If the animal was expecting a pulse perturbation and received a pulse; based on past experiences of appropriately responding with a given M2 command, the cerebellum would signal antagonist related motor cortex neurons to generate the ANT burst (see Figure 5.1). On the other hand, if the animal expected and received a step perturbation, the efference copy of M2 was still delivered to cerebellum, but in this case the desired response did not require the ANT burst. Instead the cerebellum signaled agonist related cortical motor neurons to produce an M3 response in the agonist muscle (see Figure 5.2). If the monkey was expecting to receive a step, but instead received a pulse, the cerebellum was not prepared for early ANT. Instead antagonist activity must await stretch of the antagonist ultimately resulting in a delayed ANT (see Figure 5.3). A similar result was obtained when the cerebellum was lesioned; the efference copy of M2 could not reach cerebellum, thus antagonist stretch was necessary to produce an ultimately delayed ANT burst (see Figure 5.4). Concluding the perturbation findings by Hore and Vilis; a single afferent trigger has the ability to elicit a train of pre-programmed commands, and each component command initiated by motor cortex is transformed by cerebellum into the next sequence of motor signals.
Figure 5.1. Diagram of efference copy pathway involved in producing EMG response to expected pulse perturbation. Adapted from Hore & Vilis, 1985.
Figure 5.2. Diagram of efference copy pathway involved in producing EMG response to expected step perturbation. Adapted from Hore & Vilis, 1985.
Figure 5.3. Diagram of pathways involved in producing EMG response to unexpected pulse perturbation. Adapted from Hore & Vilis, 1985.
Figure 5.4. Diagram of pathways involved in producing EMG response to expected pulse perturbation when the cerebellum has been lesioned. Adapted from Hore & Vilis, 1985.
The ANT Trigger Signal for Voluntary Movements

Before contrasting the Hore and Vilis (1985) model with Irlbacher et al. (2006) and the present thesis, it was necessary to describe two studies extending the perturbation findings to voluntary actions. Single-joint movements displaying bi- and tri-phasic patterns of EMG activity were studied in cerebellar lesioned monkeys (Flament & Hore, 1986) and human patients (Hore, Wild, & Diener, 1991). Small and large amplitude movements, performed quickly with elbow, wrist, and index finger were carefully examined. Similar to the movements investigated here, the speed-insensitive strategy was used (Gottlieb et al., 1989a, 1989b); larger amplitude movements (for both control and cerebellar lesions) were characterized by a longer AG1 duration and a later ANT onset latency\(^2\). Cerebellar disrupted actions were stereotyped by a target overshoot (hypermetria), and often followed by terminal oscillations about the desired endpoint. This hypermetria was seen for all movements tested, but the relative magnitude of overshoot was greatest when movement amplitude was small. When compared with control movements of the same peak velocity, lesioned movements had a decrease in peak acceleration and an increase in peak deceleration. The most obvious EMG abnormality was a delay of about 50-100 ms for ANT onset. Agonist changes were not as pronounced but duration of AG1 activity appeared prolonged and its initial rise was shallower.

On the basis of their findings, both Flament and Hore (1986) and Hore, Wild, and Diener (1991) concluded that for voluntary movements, the cerebellum contributes directly to braking the moving limb. Extending the model from perturbation responses, these authors reasoned that an efference copy of AG1 may be sent to the cerebellum. Taking into account both the required motor set and past experiences of successfully moving to the target with a given AG1 command,

\(^2\) ANT onset latency was not reported, but an examination of a control figure showed the ANT burst occurred later for larger amplitude movements (see Hore, Wild, & Diener, 1991, Figure 1).
the cerebellum generates commands responsible for an appropriately timed ANT burst. Under cases of cerebellar dysfunction, this efference copy cannot reach the cerebellum. Thus the ANT burst is delayed and may instead result instead from an abnormal transcortical stretch reflex driving cortical motoneurons.

As exhaustively described throughout this thesis, Irlbacher et al. (2006) showed that the triphasic pattern is executed serially, and primary motor cortex initiates an ANT trigger signal (which possibly travels to the cerebellum) ~40 ms after AG1 initiation. This signal either directly triggers the ANT burst or starts the process for its initiation, likely as output onto brainstem spinal pathways (MacKinnon & Rothwell, 2000). Hore and colleagues developed a similar interpretation, suggesting ANT onset is determined in part by an efference copy of AG1 (or M2) delivered to the cerebellum (Flament & Hore, 1986; Hore & Vilis, 1984, 1985; Hore et al., 1991; Vilis & Hore, 1980). Both models were originally based on movements of short amplitude, but the critical difference was that Hore and colleagues suggested the efferent signal left motor cortex simultaneous with AG1 initiation, whereas Irlbacher and colleagues showed the signal was sent out ~40 ms after AG1 initiation. John Hore’s group did extend their model to explain voluntary movement over a range of amplitudes (Flament & Hore, 1986; Hore et al., 1991), but irrespective of amplitude, it was concluded the efference copy of AG1 left cortex simultaneous with the veridical AG1. It is important to mention the critical timing of efference copy initiation was not examined or tested in particular by both Flament and Hore (1986) and Hore et al., (1991); they simply applied the model previously based on their perturbation elicited responses.

The present thesis was directly concerned with the time course of generating the ANT trigger signal for movements of different amplitudes. As mentioned previously, ANT latency
occurs later for longer movements, and we therefore reasoned the ANT trigger signal would display a similar relationship with amplitude.

In our first experiment, we confirmed the observations by Irlbacher et al. (2006). For a short movement where the AG1-ANT interval was normally about 70 ms, the ANT trigger signal appeared to be initiated early (within first 40 ms) in the AG1 burst. If the timing from the Hore and Vilis (1985) model had been correct, our perturbation was not expected to alter ANT latency. Because these authors predicted the signal left motor cortex simultaneous with AG1 onset, by the time neural activation from our perturbation (delivered at AG1 onset) reached motor cortex (12-20 ms after SAS delivery; Carlsen et al., 2012), the efferent signal presumably would have already been sent to the cerebellum.

Even though our short movement findings were consistent and supportive of the work by Irlbacher et al. (2006), the results from our long movement indicated this model may not generalize to all movement amplitudes. For the long movement, onset latency of the ANT burst normally occurred about 40 ms later than the short movement. Applying the same perturbation that modulated EMG inter-burst timing for the short movement had no effect on the long. In Experiment 2, we delivered a perturbation later, at a set interval (70 ms) before the ANT burst was expected for a long movement. This manipulation altered onset latency of the ANT burst, thus providing evidence that the ANT trigger signal was generated later for a longer movement.

Even though we only tested two movement distances, we believe our findings should generalize across a wider range of ballistic movement amplitudes. Unperturbed voluntary movements performed with the speed-insensitive strategy to targets placed at different distances are characterized by an altered ANT onset latency; a measure which scales linearly with distance (Gottlieb et al., 1989a, 1989b; Gottlieb et al., 1992). The cumulative results from our
experiments suggest the ANT trigger signal also scales with distance. Even though our findings show the trigger signal occurs in relation to ANT onset, this has to be timed some way relative to AG1. Because each subject has a slightly different ANT latency, the trigger signal occurs at a different point after AG1 onset for each subject, a time which happens to correspond to \(~70\) ms before ANT.

Flament and Hore (1986) and Hore, Wild, and Diener (1991) never stated (or tested) whether in voluntary unperturbed movements, the cerebellum delivers the ANT via a brainstem spinal pathway or whether it signals primary motor cortex to produce the ANT. Based on their interpretation of perturbation responses, we can assume they reasoned primary motor cortex produced the ANT. But unfortunately, they never tested this issue directly. According to MacKinnon and Rothwell (2000) and Irlbacher et al. (2006) review of single-cell recordings in primary motor cortex, even though strong corticospinal connections have been observed prior to the AG1 burst, little evidence exists for a direct role of motor cortex in producing the ANT. In fact both studies cite the work by Flament and Hore (1988) as providing evidence against a direct role of motor cortex in ANT generation. Recall that Irlbacher et al. showed primary motor cortex activity during the first 30-40 ms of ANT burst is critical to determining AG2 onset. If the cerebellum produced the ANT, but motor cortex was responsible for AG2, one could reason the motor cortex activity observed around the time of the ANT burst by Vilis and Hore (1980) was the generation of the AG2 trigger signal. Unfortunately the present work was not designed to address this issue directly. We can however safely conclude that for speed-insensitive movements, the trigger signal for the ANT burst is released from motor cortex at different times depending on the desired movement amplitude.