CONTRIBUTIONS OF A VOLUNTARY RESPONSE TO INSTRUCTION-DEPENDENT MODULATION OF THE LONG-LATENCY STRETCH REFLEX

by

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Contributions of a voluntary response to instruction-dependent modulation of the long-latency stretch reflex

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Abstract

Successful movement control in a dynamic environment involves generating appropriate and timely motor responses to counter disturbances applied to the body. In the upper-limb, mechanical perturbations elicit responses in stretched musculature at short- (M1: 25-50 ms) and long-latency (M2: 50-100 ms). The M2 response has received a great deal of attention because it can be modified by volition; for instance, increasing when the performer is instructed to resist a perturbation and decreasing when asked to not-intervene/let-go. It remains a matter of contention whether M2 modulation results from a facilitation of the contributing neural circuitry, or from superimposition of a voluntary response. The difficulty in delineating between these alternatives is due to both responses engaging common neural circuitry and the presence of considerable overlap between the voluntary response and M2 in the muscle recordings. This dissertation investigated the contributions of a rapid voluntary response on the modulation of M2.

In theme 1, we performed behavioural manipulations that influence volition and observed the corresponding impact on M2. Theme 2 investigated contributions from a startle/StartReact mechanism. The final theme used kinesthetic motor imagery to determine whether the overt initiation of a voluntary response is a pre-requisite for M2 modulation. Taken together, the findings of this dissertation showed that even in the absence of startle, a perturbation could elicit a voluntary response at a latency (75-100 ms) that overlaps M2. Despite the early nature of these rapid voluntary actions, they could not account for all instruction-dependent M2 changes. Irrespective of voluntary latency or magnitude, a general increase to the first half of M2 (50-75 ms) was observed for all active conditions. We suggest that this generic M2 modulation is related to the intention to voluntarily respond, while more sophisticated/flexible modulation observed during the latter portion of M2 is produced in part from voluntary superimposition.
Lay Summary

Disturbances to the arm produce reflexive responses in the involved muscles. One of these responses is generated by a neural pathway that travels through the brain and has received a great deal of attention because it can change in size based on the voluntary intention of the individual. This dissertation examined the relationship between voluntary reactions and changes to this reflexive response. Through a series of eight experiments, we demonstrated that although voluntary reaction time can occur within the time period associated with the reflexive response, it does not occur early enough to account for all changes to the reflexive response. Moreover, we provided strong evidence that this reflexive response in the arm is not typically associated with a startle reaction but remains strongly linked with voluntary movement control.
Preface

This dissertation is a result of my supervisory committee, Dr. Ian Franks, Dr. Romeo Chua, and Dr. Dana Maslovat, providing invaluable guidance and support throughout all phases of the research. Conception and design of the experiments took place over many conversations with my committee. Dr. Chua and I conducted the programming and setup of the experiments. I was involved with all data collection and analysis, along with undergraduate research assistants: Laurence Chin (chapter 2), Jonathan Kim and Nicolette Gowan (chapter 4), Kimberly Bennett (chapter 5). Upon completion of data collection and analysis, my committee members assisted with interpretation of the findings. I drafted the manuscripts, figures/tables, and made modifications based on feedback from the committee and anonymous journal reviewers.

All experiments in this dissertation were conducted on human participants and ethical approval was provided by the UBC Behavioural Research Ethics Board. Chapters 2-5 were covered under ethics certificate number H09-00632 (Preparation and Control of Movement). Chapter 6 was covered under ethics certificate number H17-00145 (The Influence of Motor Imagery on Modification of the Stretch Reflex).


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To my parents and sister
Chapter 1: General Introduction

For many years, scientists have studied the rapidity with which humans can voluntarily respond to a sensory event. The original empirical work was conducted by Friedrich Bessel in the early 1800’s to understand differences among astronomers calculating star transit times (Woodworth, 1938, p. 300). Hermann von Helmholtz is credited with the first reaction time (RT) study (in the 1850’s), although the intention was to calculate the speed of human nerve conduction (Woodworth, 1938, p. 299). In a standard (choice) RT paradigm, the participant must identify a stimulus, select the appropriate motor response, and prepare the required motor commands prior to movement initiation (Donders, 1969). However in a simple RT task, where the upcoming required response is known in advance of the imperative signal, selection and preparation of the required motor commands can occur during the foreperiod (known as pre-programming), and this results in shorter RTs (Klapp, 1996). Even when minimal information processing is required, RT is still strongly influenced by external factors such as stimulus modality, stimulus intensity, and stimulus predictability. The typical findings are that more predictable (Klemmer, 1957) and more intense stimuli (Woodworth, 1938, p. 318) yield shorter RTs. In regards to modality differences, visual signals take the longest time to process and the shortest latency reactions follow auditory and somatosensory stimuli (Woodworth & Schlosberg, 1954, p. 9). Historically, it has been considered that the fastest a human can voluntarily respond to a sensory event (i.e., the physiological limit for simple RT) is around 100-120 milliseconds (ms) (Woodworth, 1938, p. 323; Woodworth & Schlosberg, 1954, p. 9).

Two phenomena have been documented where voluntary motor behaviour can be consistently expressed at latencies of less than 100 ms. The first instance involves the modulation of stretch reflexive activity by the voluntarily intention of how to respond to a
mechanical limb disturbance (Hammond, 1956). The second example implicates the interaction between a pre-programmed voluntary response and the auditory startle reflex (Valls-Solé, Rothwell, Goulart, Cossu, & Munoz, 1999). Given that these early expressions of volition are reliant on stimuli eliciting specific reflexive responses, both phenomena contribute to the debate of what distinguishes “reflexive” responses from “voluntary” responses (Forgaard, Franks, Bennett, Maslovat, & Chua, 2018; Prochazka, Clarac, Loeb, Rothwell, & Wolpaw, 2000; Pruszynski, Kurtzer, & Scott, 2008; Scott, 2016). The experiments detailed in this dissertation examined contributions of a rapid voluntary response to the modulation of stretch reflex activity that follows an upper-limb mechanical perturbation. Contributions from the neural circuitry implicated in the startle reaction were also explored.

1.1 Muscular responses to a mechanical perturbation

When the upper-limb is mechanically perturbed, short- (M1: <50 ms) and long-latency (M2/M3: ~50-100 ms) stretch responses are observed in the electromyographic (EMG) recording of surrounding musculature (Hammond, 1954; Lee & Tatton, 1975). The M1 response is produced from a monosynaptic spinal pathway (Liddell & Sherrington, 1924) and only appears in locally stretched muscles. The long-latency stretch response is most commonly studied in homonymous stretched muscles, but depending on the goal or intention of the performer, can also be flexibly routed to many different muscles throughout the body (Cole, Gracco, & Abbs, 1984; Gielen, Ramaekers, & Van Zuyl, 1988; Kurtzer, Pruszynski, & Scott, 2008; Lowrey, Nashed, & Scott, 2016; Marsden, Merton, & Morton, 1981; Mutha & Sainburg, 2009).

Often appearing as one (M2) or two (M2/M3) reflex peaks in the EMG recording, the long-latency stretch response actually reflects output from multiple neural pathways engaged by the mechanical stimulus. These include continued activation of the (group I afferent) spinal
mechanism responsible for M1 (Hagbarth, Hägglund, Wallin, & Young, 1981), activation from group II afferents travelling a spinal pathway (c.f., Kurtzer et al., 2018; Lourenço, Iglesias, Cavallari, Pierrot-Deseilligny, & Marchand-Pauvert, 2006; Matthews, 1984; c.f., Matthews, 1989), and activation of group I afferents engaging a network of supra-spinal structures such as posterior parietal cortex (Omrani, Murnaghan, Pruszynski, & Scott, 2016), primary sensory cortex (MacKinnon, Verrier, & Tatton, 2000; Omrani et al., 2016; Spieser, Meziane, & Bonnard, 2010), dorsal premotor cortex (Omrani et al., 2016), supplementary motor area (Spieser, Aubert, & Bonnard, 2013), primary motor cortex (Capaday, Forget, Fraser, & Lamarre, 1991; Cheney & Fetz, 1984; Day, Riescher, Struppler, Rothwell, & Marsden, 1991; Evarts & Tanji, 1976; Herter, Takei, Munoz, & Scott, 2015; Lewis, Polych, & Byblow, 2004; MacKinnon et al., 2000; Matthews, Farmer, & Ingram, 1990; Palmer & Ashby, 1992; Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski, Omrani, & Scott, 2014; Shemmell, An, & Perreault, 2009; Spieser et al., 2013; Spieser et al., 2010), cerebellum (Kurtzer et al., 2013; Strick, 1983), red nucleus (Herter et al., 2015), and the reticular formation (Ravichandran, Honeycutt, Shemmell, & Perreault, 2013; Shemmell et al., 2009).

The short-latency stretch response is typically only sensitive to peripheral factors such as pre-perturbation muscle activity and magnitude of the mechanical stimulus (Matthews, 1986; Pruszynski, Kurtzer, Lillicrap, & Scott, 2009; Pruszynski, Kurtzer, & Scott, 2011). In addition to being influenced by these factors, the long-latency response has been extensively studied because it can be modified by the instruction or goal given to the participant (Calancie & Bawa, 1985; Pruszynski, Kurtzer, & Scott, 2011). In the seminal work by Peter Hammond (Hammond, 1954, 1956, 1960) it was shown that verbally instructing participants to “resist” a perturbation
produced a large M2, whereas this stretch response appeared small or absent following the instruction to “let-go”.

In recent years, it has been revealed that long-latency stretch response modulation is more sophisticated than a categorical increase with “resist” conditions and decrease following the instruction to “let-go” (for a review see Pruszynski & Scott, 2012). For example, M2/M3 was shown to scale with intended movement distance (Pruszynski et al., 2008) or urgency (Crevecoeur, Kurtzer, Bourke, & Scott, 2013). This stretch response has also demonstrated evidence of an internal model of limb dynamics which can account for intersegmental limb dynamics (Gielen et al., 1988; Kurtzer et al., 2008; Pruszynski, Kurtzer, Nashed, et al., 2011; Soechting & Lacquaniti, 1988). Further exemplifying this sophistication, long-latency stretch activity accounted for the orientation of the arm (Weiler, Gribble, & Pruszynski, 2018a), modulated appropriately to aid in obstacle avoidance during a reaching task (Nashed, Crevecoeur, & Scott, 2012, 2014), and was expressed across limbs when both arms shared a goal, but not when each arm had a separate goal (Dimitriou, Franklin, & Wolpert, 2012; Mutha & Sainburg, 2009; Omrani, Diedrichsen, & Scott, 2013).

Different terms have been ascribed to the long-latency stretch response, including M2/M3 (Lee & Tatton, 1975), R2/R3 (Pruszynski et al., 2008), the functional stretch reflex (Melvill Jones & Watt, 1971), the transcortical reflex (Marsden, Merton, Morton, Adam, & Hallett, 1978), and the long-loop reflex (Tatton, Bawa, Bruce, & Lee, 1978). The distinction of two separate stretch responses (M2 and M3) during the 50-100 ms post-perturbation time period likely results from separate neural pathways contributing to activation of a given muscle over a slightly different time course. Interestingly, not all participants display a distinct M3 response (Lee & Tatton, 1975), and it has also been suggested that M3 is the early onset of a voluntary
response (Marsden et al., 1978). For the entire dissertation document, unless otherwise specified, the terms “M2” and “long-latency stretch response” were used synonymously to describe the stretch response activity from 50-100 ms following the onset of a mechanical perturbation. Due to contention on the term “reflex” (Prochazka et al., 2000; Pruszynski et al., 2008), it was used cautiously (and sparingly) throughout the document.

1.2 Long-latency stretch response modulation

The mechanisms underlying long-latency stretch response modulation have remained an unresolved matter of contention since the 1970’s. One view attributes M2 as a fast-feedback response whereby excitability (or gain) differences along one or more of the contributing neural pathways results in a modulation of the response (e.g., Calancie & Bawa, 1985; Colebatch, Gandevia, McCloskey, & Potter, 1979; Hammond, 1956; Lee & Tatton, 1975; Pruszynski et al., 2008; Spieser et al., 2010). The opposing interpretation suggests that M2 cannot be modified by the intention or goal of the performer, rather the appearance of modulation and flexibility only occurs because a voluntary or “triggered” response superimposes onto the M2 response (Crago, Houk, & Hasan, 1976; Lewis, MacKinnon, & Perreault, 2006; Manning, Tolhurst, & Bawa, 2012; Ravichandran et al., 2013; Rothwell, Traub, & Marsden, 1980; Shemwell et al., 2009). In conditions where a participant resists/compensates against the direction of a perturbation, voluntary activity appears continuous with this stretch response and the disagreement stems from the problem of clearly delineating the M2 response from the voluntary response in the EMG recordings. Moreover, disagreement exists in the latency at which the voluntary response can begin following a mechanical stimulus.

Studies that have proposed M2 modulation results from excitability differences along the contributing neural pathways (i.e., modified feedback gains) either did not provide an estimate of
voluntary RT (e.g., Spieser et al., 2010) or made estimates based on responding to a weak proprioceptive stimulus that was too small to elicit an M2 response (Hammond, 1956; Pruszynski et al., 2008; Selen, Shadlen, & Wolpert, 2012; Yang, Michaels, Pruszynski, & Scott, 2011). This method produced voluntary premotor RTs longer than 100 ms and critically, modulation of the M2 response occurred in the primary conditions (with stronger perturbations) at an earlier latency than the estimated RT. As mentioned above, voluntary RT has an inverse relationship with stimulus intensity, thus it is probable that shorter latency voluntary RTs occurred when participants responded to the stronger perturbations used to elicit the M2 response.

Researchers arguing for voluntary response (or triggered reaction) superimposition as the main contributor to the appearance of M2 modulation determined RT using the same magnitude stimulus employed to elicit M1 and M2 (Day, Rothwell, & Marsden, 1983; Evarts & Granit, 1976; Evarts & Vaughn, 1978; Manning et al., 2012; Ravichandran et al., 2013). In order to calculate RT, participants were instructed to voluntarily respond with musculature not excited by stretch response activity (i.e., antagonist or contralateral muscles). Remarkably short RTs were reported using these methods, with the earliest latency reactions beginning at ~65-70 ms. This latency is early enough to account for the appearance of most of the modulation of EMG activity occurring during the long-latency stretch response (Manning et al., 2012; Ravichandran et al., 2013). The issue of RT determination following a mechanical perturbation is detailed extensively in chapters 2 and 5 of this dissertation.

While disagreements exist on the latency of a voluntary response following a mechanical stimulus, other neurophysiological methods have also resulted in equivocal findings on whether the neural circuitry contributing to the M2 response can undergo gain modulation. For example,
some groups have shown that electroencephalography (EEG) potentials over sensorimotor cortex modulated at an appropriate time course to account for instruction-dependent M2 modulation in muscle (Abbruzzese, Berardelli, Rothwell, Day, & Marsden, 1985; Spieser et al., 2010), whereas another study reported no cortical modulation with the instruction on how to respond to a perturbation (MacKinnon et al., 2000). Single-cell recordings from primary motor cortex in non-human primates revealed that a subset of neurons modulated at an appropriate time-course to account for goal-dependent M2 modulation in the arm (Evarts & Tanji, 1976; Pruszynski et al., 2014). Neurons in the dentate nucleus of the cerebellum also displayed modulated firing rates based on the goal of how to respond to a mechanical stimulus (Strick, 1983). Using transcranial magnetic stimulation (TMS) on humans, it was shown that a silent period of supplementary motor area or primary motor cortex abolished instruction-dependent M2 modulation in the wrist flexor muscles (Spieser et al., 2013). By contrast, similar application of TMS over primary motor cortex had no influence on instruction-dependent M2 modulation in biceps brachii (Shemmell et al., 2009). Despite some studies providing a neural basis for feedback gain modulation of the long-latency stretch response, comparable to the issue of distinguishing a voluntary reaction from the M2 response in EMG recordings, the supra-spinal neural modulation could also be interpreted as resulting from the generation of a voluntary response.

It is important to mention that changes to the long-latency stretch response have been documented in some paradigms that cannot be interpreted from voluntary response superimposition. For instance, Doemges & Rack demonstrated a larger M2 response when participants engaged in a position control task compared to a force control task (Doemges & Rack, 1992a, 1992b). Shemmell and colleagues (2009) showed that the M2 response was larger when the arm was in a compliant environment compared to a stiff environment (see also Fox &
Shemmell, 2014). These examples of M2 modulation occurred when participants were instructed to “not-intervene” with the perturbation (i.e., remain passive). Interestingly, Shemmell et al. (2009) provided evidence that separate neural pathways are involved in environmental stability regulation of the arm compared to instruction-dependent M2 modulation (at least for biceps brachii). Recall that a TMS induced silent period of primary motor cortex did not influence instruction-dependent M2 modulation (previous paragraph), but interestingly, the same cortical inhibition abolished the M2 increase when participants were in a compliant environment. Similar application of TMS during the same task involving wrist flexors had minimal impact on environmental regulation of the limb during the M2 response (Fox & Shemmell, 2014). Given the current perspective that multiple neural pathways are involved in the generation of M2 (Kurtzer, 2015; Pruszynski & Scott, 2012; Shemmell, Krutky, & Perreault, 2010), the work by Shemmell and colleagues (2009) provided strong evidence that separate neural circuits may also be involved in various forms of long-latency stretch response modulation. Moreover, the neural structures involved in different forms of M2 regulation appear to display muscle specificity (Fox & Shemmell, 2014).

Many studies over the past decade have used the long-latency stretch response as a medium to test predictions of Optimal Feedback Control (OFC) theory. This theory proposes that sensory feedback is crucial in guiding volitional actions and that the M2 response displays features of a voluntary response because the two are linked as part of the same control process (Pruszynski et al., 2008; Scott, 2004). The studies testing OFC have used targets as opposed to verbal instructions to specify participant behaviour (see Pruszynski et al., 2008). While this has many advantages, especially in exploring the limits of goal-dependent M2 modulation, we do not believe a truly passive (control) condition can be specified with targets. These studies have also
operated on the assumption that the voluntary response does not occur at latencies that can overlap with the end of the M2 response, a conjecture based off RT estimates using less intense perturbations than what was used in the primary experimental conditions (Pruszynski et al., 2008; Selen et al., 2012; Yang et al., 2011). Although these OFC studies have produced some remarkable findings on the sophistication of the long-latency stretch response and the relation to volitional control (Pruszynski & Scott, 2012; Scott, 2016), issues around the potential contributions of a voluntary or triggered reaction remain unresolved.

It should be clarified that despite using “instruction-dependent” and “goal-dependent” modulation in what may appear as an interchangeable manner, these terms are not entirely synonymous. We consider goal-dependent M2 modulation to be any change in the M2 response that is brought about by changing the goal of the performer or maintaining the same goal (e.g., returning to a target) in the presence of different perturbations or environmental conditions. By contrast, instruction-dependent modulation is considered a subset of a goal-dependent modulation and is achieved by providing the performer with different verbal instructions following the same perturbation stimulus. Some examples of different verbal instructions that have been used include “resist or let-go” (Hammond, 1956; Rothwell et al., 1980), “compensate or do-not intervene” (Crago et al., 1976; Manning et al., 2012), “resist, do-not resist, or assist” (MacKinnon et al., 2000), and “oppose, do-not react, or relax” (Capaday, Forget, & Milner, 1994). However, the only true passive condition resulting in an unmodified M2 response is achieved with the “do-not intervene” instruction1 (Crago et al., 1976; Manning et al., 2012). The experiments conducted in this dissertation were primarily concerned with instruction-dependent

1 It is reasonable to assume “do-not resist” and “no-not react” are similar to “do-not intervene”. 
modulation of the M2 response in wrist flexors and the contributions from a voluntary response. Comparisons were made between passive conditions, where participants were instructed to not-intervene with a wrist extension perturbation and varying active conditions involving a voluntary intervention specified by verbal instructions and/or targets. Although these experiments were always conducted using a simple RT paradigm, and we thus encouraged the earliest possible appearance of a voluntary response (in active conditions), throughout the document, reservations were placed on interpretation of data purely from superimposition of a voluntary response. Where possible, we also interpreted our findings within the broader context of more sophisticated goal-dependent modulation. In summary, while there is clear evidence that a mechanical perturbation can elicit a voluntary/triggered response at latencies early enough to influence the M2 response, it remains unclear whether instruction-dependent M2 modulation results purely from this voluntary response superimposition, or a combination of an early voluntary response and gain modulation of the underlying neural circuitry.

1.3 The StartReact effect

A number of research groups investigating changes to the M2 response have also described the (potential) presence of a “triggered” response (Crago et al., 1976; Jaeger, Gottlieb, & Agarwal, 1982; Lewis et al., 2006; Manning et al., 2012; Ravichandran et al., 2013). Although the exact nature of a perturbation triggered response remains unclear, in the context of the long-latency stretch response in the upper-limb, triggered responses appear to have some relation with a pre-programmed voluntary response (Crago et al., 1976). Recent parallels have been drawn with the startle reaction, which like M2, is another reflexive response known to have an interesting interaction with the volitional control of movement. When participants perform in a simple RT paradigm and the upcoming motor response is fully pre-programmed, unexpected
presentation of a startling auditory stimulus (SAS; >120 decibels) elicits the voluntary response at a remarkably short (~65-70 ms) latency (see reviews by Carlsen, Maslovat, & Franks, 2012; Nonnekes, Carpenter, Inglis, Duysens, & Weerdesteyn, 2015; Rothwell, 2006; Valls-Solé, Kumru, & Kofler, 2008). Termed the StartReact effect, the triggering effects of the SAS are such that the entire pre-programmed motor response is hastened, with minimal disruption to the EMG pattern or kinematics when compared to non-SAS conditions (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004b; Valls-Solé et al., 1999). This effect has been demonstrated for many types of movements, from simple upper-limb actions (Carlsen et al., 2004b), to the vocalization of speech (Stevenson et al., 2014), and even more complex tasks such as the sit-to-stand maneuver (Queralt, Valls-Solé, & Castellote, 2008) and the anticipatory postural adjustments that precede step initiation (MacKinnon et al., 2007). The exact neural mechanism underlying the StartReact effect remains subject to debate (see chapter 4), however it is generally accepted that when a SAS activates startle circuitry, the pre-programmed voluntary response is automatically elicited.

While the startle response and StartReact effect have been most commonly studied using a SAS, startle circuitry (in the caudal pontine reticular formation) can also be activated by intense proprioceptive and vestibular inputs (Yeomans, Li, Scott, & Frankland, 2002). It was postulated that the mechanical perturbations used to elicit stretch responses in the upper-limbs may also elicit a startle response (Koshland & Hasan, 2000; Lewis et al., 2006; Shemmell et al., 2009). This hypothesis was directly tested by Ravichandran et al. (2013). These authors showed that unexpectedly delivering a perturbation in an auditory simple RT task resulted in a startle response (evidenced by early activation in sternocleidomastoid (SCM), a muscle commonly used to indicate startle), as well as the early release (73 ms) of the pre-programmed voluntary response in triceps brachii. These authors proposed that when a pre-programmed response occurs
in the same muscle as the M2 response, instruction-dependent M2 modulation results purely from the StartReact effect (i.e., the startle triggered release of a pre-programmed voluntary response). Moreover, because the StartReact effect is believed to result in the initiation of a pre-programmed voluntary response via a different mechanism than normal voluntary response initiation (on non-startle trials), this theory provides evidence that a perturbation triggered reaction is distinct from a voluntary response initiated to a non-startling stimulus.

The methodology of Ravichandran et al. (2013) differed from a majority of previous studies examining instruction- and goal-dependent M2 modulation. Critically, the perturbation was delivered unexpectedly which was potentially more likely to elicit startle. By contrast, many studies examining M2 modulation have presented a perturbation on every trial, usually as the imperative signal (Crago et al., 1976; Hammond, 1956; MacKinnon et al., 2000; Manning et al., 2012; Pruszynski et al., 2008; Rothwell et al., 1980; Spieser et al., 2013). Predictability of a startling stimulus is known to influence the probability of eliciting startle with rapid habituation following repeated stimulus presentations (Brown et al., 1991; Oude-Nijhuis, Allum, Valls-Solé, Overeem, & Bloem, 2010). Thus, it remains unclear if an expected mechanical perturbation could still elicit the startle response and whether activation of startle circuitry (and the StartReact effect) is the primary mechanism underlying the appearance of instruction- or goal-dependent facilitation of the M2 response.

1.4 Summary

This dissertation was concerned with the contributions of a volitional response and/or triggered reaction to instruction-dependent modulation of EMG activity during the long-latency stretch response period (50-100 ms post-perturbation onset). Eight experiments were designed to address the role of voluntary response superimposition, potential involvement of startle circuitry
and the StartReact effect, and whether the overt execution of a voluntary response is a prerequisite for instruction-dependent M2 modulation. The experiments are outlined below in the context of each chapter in which they appear.

- **Chapter 2 – Voluntary reaction time and long-latency reflex modulation**

  Two behavioural manipulations (accuracy and complexity) that are known to influence the latency (and shape) of a voluntary response were employed in a perturbation paradigm. These were compared with the standard conditions of compensating against a perturbation without accuracy constraints and remaining passive to the perturbation. These first experiments showed that irrespective of the latency or magnitude of a voluntary response, the intention to actively respond against a perturbation resulted in a generic increase to the first half of the M2 response (compared to the passive condition). The second half of the M2 response was sensitive to differences in the latency and magnitude of the voluntary response. Data in this chapter also provided indirect evidence that accuracy constraints may be sufficient to minimize voluntary response superimposition onto the M2 response.

- **Chapter 3 – Perturbation predictability can influence the long-latency stretch response**

  Previous perturbation studies have used a wide range of different foreperiods or inter-perturbation intervals. It is well known that RT can be influenced by the predictability of an imperative signal, but it remains unclear whether the M2 response (on passive trials) is also influenced by variations in foreperiod predictability. Potential differences in the findings of chapter 2 and some previous studies could have resulted from variations in foreperiod predictability. In experiment 3, we compared active and passive conditions in
short (2.5-3.5 seconds) and long (2.5-10.5 seconds) variable foreperiods. Similar to chapter 2, we found a general increase in EMG activity during the first half of the M2 response when participants actively responded to the perturbation. We also observed an unanticipated finding where the latter portion of the M2 response in the passive conditions was sensitive to the predictability of the foreperiod. This finding suggests that there is some form of active engagement, even in a passive task. We also found interesting contrasting aging foreperiod effects in both foreperiod conditions; for the passive conditions, the latter portion of the M2 response decreased as the foreperiod aged but in active conditions, the latter portion of the M2 response increased.

- Chapter 4 – An examination of the startle response during upper limb stretch perturbations

The fourth chapter of this dissertation examined whether the startle response and StartReact effect contribute to instruction-dependent modulation of the M2 response. In these experiments, EMG activity was recorded from sternocleidomastoid and orbicularis oculi (common muscles used to indicate startle). Experiment 4 was specifically concerned with whether a startle response was present when participants performed in active and passive conditions to an expected mechanical perturbation (i.e., similar conditions to a majority of previous stretch response studies). A startle response was not consistently observed in either the passive or active conditions, however instruction-dependent M2 modulation remained intact. Experiment 5 was designed to examine whether a mechanical perturbation was capable of eliciting a startle response. As such, perturbations were delivered unexpectedly during the performance of an auditory simple
RT task. A perturbation startle reaction was only observed on a small proportion of trials and this response looked considerably different than the auditory startle reaction.

- **Chapter 5 – Mechanical perturbations can elicit triggered reactions in the absence of a startle response**

  In chapter 4, despite not observing the startle response following an expected mechanical perturbation, we reported considerable activity in the SCM muscles near the end of the startle time criteria (~120 ms). To test whether this was a result of a postural response (associated with head/neck stabilization) or a startle response, experiment 6 employed prepulse inhibition (PPI) stimuli. Rather than attenuating the SCM responses (as was expected for startle), the PPI stimulus significantly advanced responses in SCM, suggesting the SCM activity was a result of postural control. Experiment 7 was concerned with whether a perturbation could consistently elicit voluntary RTs at a latency that overlaps the M2 response, even in the absence of startle. Participants performed voluntary wrist extension responses following a wrist extension perturbation. This allowed a determination of RT in (non-stretched) wrist extensor EMG. Two-thirds of RTs were shorter than 100 ms but these values were not as early as the auditory StartReact effect.

- **Chapter 6 – The influence of kinesthetic motor imagery on the long-latency stretch response: Overt voluntary activity is a pre-requisite for instruction-dependent modulation**

  The final experiment of this dissertation employed kinesthetic motor imagery to determine whether instruction-dependent M2 modulation is reliant on the overt execution of a voluntary response. This form of motor imagery was chosen because it is known to
activate many of the same supra-spinal regions involved in voluntary control of the same action, as well as generation of the M2 response. On ~40% of imagery trials, a small voluntary response “leaked out” into wrist flexor musculature. It was only on these leaked imagery trials that the M2 response increased compared to the passive condition. Interestingly, this increase was identical to the physically active (compensate) trials during the first half of the M2 response. The second portion of the M2 response on leaked imagery trials was larger than the passive condition but was not as large as active trials. The findings of this experiment confirmed that the overt execution of a voluntary response is required for instruction-dependent M2 modulation.
Chapter 2: Experiments 1 & 2

Voluntary reaction time and long-latency reflex modulation

2.1 Introduction

Fast perturbations applied to the upper limbs can elicit stereotypical, electromyographic (EMG) responses in the stretched muscle. The first response (M1) occurs at short-latency (~25-50 ms) and reflects input from a spinal reflex pathway (Liddell & Sherrington, 1924). This is followed by a longer latency (~50-100 ms) response (M2) which receives input from group II afferents travelling a spinal pathway (Lourenço et al., 2006; Matthews, 1984) as well as group I afferents traversing a longer transcortical route (Capaday et al., 1991; Cheney & Fetz, 1984; Evarts & Tanji, 1976; Lourenço et al., 2006; MacKinnon et al., 2000; Matthews et al., 1990; Omrani, Pruszynski, Murnaghan, & Scott, 2014; Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski et al., 2014). The magnitude of M1 is dependent on peripheral factors such as background state of the motoneuron pool and characteristics of the mechanical perturbation (Matthews, 1986; Pruszynski et al., 2009; Pruszynski, Kurtzer, & Scott, 2011). While these factors can also influence M2 (Calancie & Bawa, 1985; Pruszynski et al., 2009; Pruszynski, Kurtzer, & Scott, 2011), the size of the long-latency response can be influenced by the task requirements or instructions provided to the participant. When the participant is instructed to “resist” or “compensate” for the perturbation, M2 increases in magnitude and is followed a voluntary response; when the participant is told to “not intervene” or “let go,” M2 is smaller in comparison (e.g., Calancie & Bawa, 1985; Colebatch et al., 1979; Hammond, 1956; MacKinnon et al., 2000).

The reason for the increase in the size of M2 when instructed to act against the perturbation has been a matter of contention for some time. One view attributes the amplitude modulation to be the result of an increase in the excitability of the reflex pathways (Calancie & Bawa, 1985; Colebatch et al., 1979; Hammond, 1956; Lee & Tatton, 1978; Pruszynski et al.,
The opposing view suggests that the amplitude modulation is an artefact of the superposition of the voluntary response onto the end of the long-latency response (Crago et al., 1976; Houk, 1978; Lewis et al., 2006; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980). Part of the disagreement appears to stem from the problem of clearly delineating the M2 response from the voluntary response in EMG recordings. To distinguish between the two potential mechanisms, it is critical that the M2 and voluntary EMG responses can be clearly demarcated from one another.

Studies have previously relied on indirect techniques to determine the onset of the voluntary response (i.e., RT). For example, Hammond (1956) used a mechanical tap to the wrist (which did not evoke reflexive activity) as a proprioceptive imperative signal and found a mean voluntary change in tension of about 115 ms for elbow flexion responses. This corresponded to a mean premotor RT of ~100 ms (see Marsden et al., 1978). This type of proprioceptive RT was subsequently used as the estimate of voluntary response latency during perturbation conditions, where participants responded by flexing the elbow following an imposed extension. Hammond found that EMG activity changed in size based on instruction (“resist” vs. “let-go”) and this occurred at a latency shorter than the estimated RT. As a result, he concluded that M2 modulation likely resulted from pre-setting excitability of the contributing stretch reflex mechanism (see also Calancie & Bawa, 1985; Colebatch et al., 1979; Lee & Tatton, 1978; Pruszynski et al., 2008; Selen et al., 2012; Yang et al., 2011).

Although Hammond (1956) originally assumed that the M2 and voluntary responses did not overlap much in time, there is evidence showing that voluntary RTs to a mechanical perturbation can be considerably shorter than 100 ms (e.g., Day et al., 1983; Evarts & Granit, 1976; Evarts & Vaughn, 1978; Lewis et al., 2006; MacKinnon et al., 2000; Manning et al., 2012;
Ravichandran et al., 2013; Shemmell, 2015; Shemmell et al., 2009; Shemmell et al., 2010). As an example, to dissociate M2 from voluntary activity, Evarts & Granit (1976) instructed participants to supinate or pronate the forearm in response to a perturbation (occurring either in the supination or pronation direction). When biceps were stretched (pronation perturbation) and a supination response was required, M1, M2, and voluntary activity all occurred in the same muscle and RT could not be determined precisely. In contrast, when the same supination response was required in reacting to a supination perturbation, M1 and M2 appeared in pronators but only the voluntary response remained in biceps. Using this technique, Evarts & Granit (1976) and others (Evarts & Vaughn, 1978; MacKinnon et al., 2000; Ravichandran et al., 2013) have reported voluntary RTs to a proprioceptive stimulus to be as short as 70 ms. Assuming similar RTs when M2 and the voluntary response occurred in the same muscle, any observed M2 modulation could have resulted from overlapping voluntary activity onto the M2 response.

This overlap hypothesis was tested in an experiment by Rothwell et al. (1980). The authors manipulated predictability of the imperative (perturbation) signal, an effect known to produce voluntary reaction time differences (to visual or auditory stimuli), and compared modulation of M2 between a “resist” and “let-go” instruction. Larger changes to M2 period activity were observed in the conditions with more predictable perturbations (and presumably shorter RTs). It was concluded that rather than modulating excitability of the reflex pathways, the appearance of a facilitated M2 response resulted primarily from the overlap of a “rapid voluntary event”. However, voluntary response magnitudes were also larger for conditions expected to have shorter RTs. Therefore it is plausible RT differences were not present, instead only the magnitude of the voluntary response differed, generating the appearance of different voluntary latencies.
While substantial evidence exists suggesting that early voluntary responses may contribute to changes in M2 magnitude when participants actively compensate for a stretch perturbation, it remains unclear whether Hammond’s original mechanism of pre-setting excitability of the contributing reflex pathway(s) also plays a role. The purpose of the experiments conducted in this chapter was to further examine whether the changes in M2 magnitude result purely from a superimposed voluntary response. In order to address this question, it was imperative that we manipulated the onset of the voluntary response, in turn minimizing its overlap onto the M2 response.

One method that has been shown to increase RT (in a non-perturbation task) is to increase the movement accuracy (e.g., Fitts & Peterson, 1964; Lajoie & Franks, 1997; Sidaway, 1991) or number of movement components (e.g., Henry & Rogers, 1960; Ketelaars, Garry, & Franks, 1997; Ketelaars, Khan, & Franks, 1999; Lajoie & Franks, 1997). Previous perturbation studies, particularly those that have provided evidence of short latency voluntary RTs, have typically used simple untargeted active responses following a perturbation (e.g., Crago et al., 1976; Day et al., 1983; Evarts & Vaughn, 1978; MacKinnon et al., 2000; Manning et al., 2012; Rothwell et al., 1980). The absence of specified endpoint accuracy and/or complexity yields short RTs which increases the probability of superposition of the voluntary response into the M2 period.

In this chapter, we delayed voluntary responses during a perturbation paradigm by increasing either the accuracy (Experiment 1) or complexity (Experiment 2) requirements of the task. In Experiment 1, participants reacted to a wrist extension perturbation by flexing the wrist quickly and accurately to a narrow or a wide target. Reflexive and voluntary responses from the targeted conditions were compared to untargeted active as well as passive responses (i.e., similar
conditions used by groups arguing against modulation of the M2 response; e.g., Crago et al., 1976; Manning et al., 2012). In Experiment 2, we compared low-complexity unidirectional movements to high-complexity reversal movements. Experiment 2 had the additional goal of controlling the magnitude of the voluntary response between active conditions. Reversal movements were chosen because the beginning of the voluntary EMG response (the portion that may overlap onto M2), is similar in magnitude and shape to that of a unidirectional movement, when amplitude and size of the first target are controlled (Gottlieb, 1998). We reasoned that controlling the size of the voluntary response may permit observations of voluntary RT changes in which differences in RT are not confounded by alterations in response magnitude.

If superposition of the voluntary response is the main source of M2 period modulation (as suggested by Crago et al., 1976; Day et al., 1983; Manning et al., 2012; Rothwell et al., 1980; and others), we expected that delaying the voluntary response in both Experiment 1 and 2 would result in an M2 response approaching a similar magnitude to the passive condition. Alternatively, if excitability changes to the underlying neural circuitry is the main factor underlying M2 period modulation (e.g., Colebatch et al., 1979; Hammond, 1956; Lee & Tatton, 1978; Pruszynski et al., 2008; Spieser et al., 2010), there should be minimal influence of voluntary response timing and thus we expected the size of the M2 response to change only as a categorical function of whether or not participants actively compensated for the perturbation. Finally, if M2 period activity during active conditions is due to a combination of the two mechanisms described above, we expected M2 magnitudes to increase during all active conditions, compared to the passive condition, with a further increase observed in the latter half of the M2 period, when the voluntary overlap is greatest.
2.2 Methods

2.2.1 Participants and apparatus

Twenty right-handed volunteers (9 females, 11 males; 20-46 years), free of any neuro-muscular abnormalities and capable of correctly performing the various conditions participated in at least one of three experiments lasting approximately 90 minutes. The third experiment was a control condition, and not the primary focus of the chapter (see below). Experimental procedures were approved by the University of British Columbia ethics committee and informed written consent was collected prior to each testing session.

Participants were positioned in a height-adjustable chair facing an oscilloscope monitor (placed ~1 m in front) resting on a table. Both elbows were flexed at 100 degrees and hands were semi-pronated with the wrist joints aligned with the manipulanda rotational axes. Connected to the right manipulandum was a torque motor (Aeroflex TQ 82W-1C) and a metal handle adjoined to the motor shaft was placed near the right metacarpophalangeal joints. To prevent lateral wrist movement padded stops were adjusted on either side of the wrist. Custom molded thermoplastic enveloped the hand and allowed movement to occur about the wrist without the fingers having to grasp the metal handle. Participants were also asked to keep the hands and fingers relaxed and to only move the right wrist joint. Angular position information pertaining to the right wrist was continuously provided on the oscilloscope as feedback. The home position was 10 degrees of wrist flexion and visually defined on the oscilloscope by arrows. The left manipulandum was immovable and positioned at 20 degrees of wrist extension.

All perturbation trials began with a slight extension preload ramped slowly (over 500 ms) to 0.25 newton metres (Nm). Participants were instructed to resist by lightly activating wrist flexors against the load and to hold their right wrist at the home position. A random foreperiod
(2750-3750 ms) followed onset of the preload and was terminated by a 1.5 Nm extension perturbation lasting 150 ms.

2.2.2 Experimental paradigm

2.2.2.1 Experiment 1: movement accuracy

At the start of the experiment each participant \((n = 11)\) was provided with ~15 familiarization trials for each condition. In addition, 5 practice trials were given prior to the start of each condition to ensure correct performance of the required task. Each testing condition consisted of a single block of trials where the participant was instructed to either (1) “not intervene with the perturbation and slowly move back to the home position” (do not intervene/passive condition: DNI), (2) “flex the right wrist \textit{as fast as possible} following the perturbation” (active untargeted: ACT), or (3) “flex the right wrist into the target zone \textit{as fast and as accurately as possible} following the perturbation” (active wide target: ACT-Wi; active narrow target: ACT-Na). A target was only visible on the oscilloscope for the two targeted conditions, and was 10 degrees wide for the ACT-Wi condition and 5 degrees wide for ACT-Na. Both target positions were centered around 22.5 degrees of wrist flexion. Feedback for the ACT condition involved continuously reminding participants to “react as fast as possible”. For the ACT-Wi and ACT-Na conditions participants were reminded to respond “as fast and as accurately as possible”. Moreover, for the two targeted conditions, participants were also verbally informed by the experimenter if they either “hit the target” or “missed short or long”. This accuracy component was determined by the amplitude of the initial peak flexion displacement of the return movement in relation to the target. Collection within a block continued until at least 20 correct trials were performed (i.e., no false starts, failure to respond, or
missing the centre of the target by more than 12.5 degrees). Block order was randomized and a 5 minute rest was provided between conditions to prevent fatigue.

### 2.2.2.2 Experiment 2: movement complexity

Similar to Experiment 1, participants \((n = 10)\) were provided with \(~15\) familiarization trials for each condition and 5 practice trials were given prior to the start of each condition in the testing phase. The testing conditions consisted of 3 blocks of trials where participants were instructed to either (1) “not intervene with the perturbation and slowly move back to the home position” (DNI), (2) “flex the right wrist into the flexion target zone as fast and as accurately as possible following the perturbation” (active narrow target: ACT-Na), (3) or “flex the right wrist into the flexion target zone and immediately extend into the extension target zone as fast and as accurately as possible following the perturbation” (active reversal: ACT-R). The flexion target was identical to the ACT-Na target used in Experiment 1. The extension target position was positioned at 2.5 degrees of extension, and was 5 degrees wide. When performing in the ACT-Na and ACT-R conditions, participants were continuously reminded to respond “as fast and as accurately as possible”. After each trial, participants were also informed if they either “hit the target(s)” or “missed short or long”. Block order was randomized and collection within a block continued until at least 20 correct trials were performed. A 5 minute rest was provided between blocks to prevent fatigue.

### 2.2.2.3 Visual RT conditions

To ensure our manipulations of movement accuracy and complexity influenced RT, each participant performed an additional visual RT task after completing the perturbation conditions. The home position was 30 degrees of wrist extension, approximately the position the motor was expected to move the wrist to in the perturbation conditions (determined from pilot testing). The
warning signal was represented by a line on the oscilloscope moving up to the home position. Following a variable foreperiod (2750-3750 ms) the line jumped into the centre of the flexion target area (22.5 degrees of flexion). The participants in Experiment 1 were instructed to either “flex their wrist as fast as possible after the line jump” (visual active untargeted: V-ACT), or to “flex the right wrist into the target zone as fast and as accurately as possible following the line jump” (visual active wide target: V-ACT-Wi; visual active narrow target: V-ACT-Na). The participants in Experiment 2 were instructed to either “flex the right wrist into the flexion target zone as fast and as accurately as possible following the line jump” (V-ACT-Na), or “flex the right wrist into the flexion target zone and immediately extend into the extension target zone as fast and as accurately as possible following the line jump” (visual active reversal: V-ACT-R). The targets were placed in the same position as the perturbation conditions and feedback was provided on a trial-by-trial basis. Block order was randomized and participants performed 10 practice trials for one condition, immediately followed by at least 20 testing trials. The testing blocks were separated by a 5 minute rest period.

2.2.2.4 Startle control conditions

Recent work has shown that perturbations applied to the elbow (Ravichandran et al., 2013) or ankle (Campbell, Squair, Chua, Inglis, & Carpenter, 2013) can elicit a startle reflex, characterised by activation of sternocleidomastoid (SCM). Important for the context of the present study, startling stimuli are known to involuntarily trigger prepared voluntary movements at short latencies (<100 ms), a phenomenon known as the StartReact effect (for reviews see Carlsen et al., 2012; Rothwell, 2006; Shemmell, 2015). If the perturbations used in Experiments 1 and 2 elicited a startle reflex and the StartReact effect, potential voluntary RT differences (as observed in the Visual RT conditions) could be nullified (Maslovat, Hodges, Chua, & Franks,
Thus, to examine whether the wrist perturbations and the experimental protocols used in this study were capable of eliciting a startle reflex, we collected data from 3 participants (recruited for their known disposition to being startled), and monitored indicators of the startle reflex (EMG data were recorded bilaterally from sternocleidomastoid (SCM) and unilaterally from left orbicularis oculi (OOc)). Participants were positioned in a setup identical to Experiments 1 and 2, with the addition of a loudspeaker placed 30 cm behind the head capable of eliciting a startling acoustic stimulus (SAS) (see Forgaard, Maslovat, Carlsen, Chua, & Franks, 2013; Maslovat et al., 2011a; Maslovat, Hodges, Chua, & Franks, 2011b for a similar setup).

At the beginning of testing, participants were asked to remain relaxed and fixate on the oscilloscope monitor. After 5-10 seconds a 120 dB (1000 Hz, 50 ms, SAS) was delivered via the loudspeaker. This first trial was used to examine each participant’s baseline auditory startle reflex in the absence of any motor preparation. Following the baseline startle trial, participants performed 15 practice trials followed by 25 testing trials in both a DNI block and an ACT (untargeted) block (identical protocols and instructions to Experiment 1). To make direct comparisons with the auditory startle reflex, on 5 random testing trials, a 120 dB SAS was delivered simultaneous with perturbation onset.

### 2.2.3 Data collection and analysis

Surface EMGs were collected from the right wrist flexor (FCR) and extensor (ECR) carpi radialis muscles (as well as left OOc and bilaterally from SCM, startle control conditions only) using bipolar pre-amplified surface electrodes connected to an external amplifier (Experiment 1: Model 544, Therapeutics Unlimited Inc., Iowa City, IA; Experiments 2 and startle control condition: Model DS-80, Delsys Inc., Natick, MA). EMGs were amplified at 2-4 K and bandpass filtered from 30-1000 Hz in Experiment 1 and 20-450 Hz in Experiments 2 and startle control.
condition. Positional data were collected using a potentiometer (Bourns, Model 6637S-1-103, Riverside, CA) connected to the right wrist manipulandum. All signals were sampled at 2 kHz using a 1401Plus data acquisition system and a computer running Spike2 (CED, Cambridge, UK) software. Offline data analysis was accomplished using Spike2 and custom-written LabVIEW (National Instruments, Austin, TX) software.

At the beginning of analysis, EMG data were baseline corrected and full-wave rectified. A 700 ms window (200 ms pre- to 500 ms post-) was placed around each mechanical perturbation or visual imperative signal. While error trials were recycled online during data collection, we conducted an additional check of individual trial data from EMG recordings and the displacement profiles to ensure correct performance. Good trials were submitted to an individual condition ensemble average for each participant.

For Experiments 1 and 2, the ensemble averages for each perturbation condition (from each participant) were used to determine stretch response onset and offset times (using a similar method to MacKinnon et al., 2000; Manning et al., 2012). Mean background EMG and standard deviation (SD) were first determined from 100 to 5 ms before delivery of the perturbation. A horizontal cursor was placed at 3SD above mean activity. The first rise in activity (~20-35 ms post-perturbation), greater than 3SD above baseline was marked as M1 onset. The onset of M2 was more difficult to determine because M1 and M2 often overlapped, thus the trough in activity between these two events (occurring around 50 ms) was marked as M2 onset. Due to overlapping voluntary activity in the active conditions, M2 offset could only be determined in the DNI conditions. This point was marked as the first decrease in activity below 3SD above baseline following M2 onset.
For the main analysis, we analyzed the wrist flexor EMG data in five time periods relative to perturbation onset. The first epoch was used to determine baseline EMG, occurring 25 ms before onset of the perturbation. The second epoch contained the short latency (M1) response, occurring 25-50 ms post perturbation. While M2, our main response of interest, occurs 50-100 ms following the perturbation, we chose to divide this period further into M2a (50-75 ms) and M2b (75-100 ms). Finally, magnitude of the voluntary response was captured in an epoch (VOL) occurring between 100 and 200 ms post-perturbation. Previous studies (Lee & Tatton, 1978; Pruszynski et al., 2008; Ravichandran et al., 2013) have used a similar temporal segregation to more closely analyze the time-course of M2 as well as the voluntary response.

Integrated area of each of the predefined epochs (Background, M1, M2a, M2b, VOL) was calculated on a trial-by-trial basis using the raw rectified EMG data. The means and standard deviations from each condition were then normalized to the largest M2 value obtained from each participants’ DNI ensemble average (Experiment 1 DNI Mean Peak: 0.25 mV, SD: 0.11 mV; Experiment 2 DNI Mean Peak: 0.18 mV, SD: 0.07 mV). In Experiment 1, mean raw rectified background EMG was 0.021 mV (± 0.009 mV) and for Experiment 2 mean background EMG was 0.018 mV (± 0.006 mV).

Kinematic profiles following each perturbation were also analyzed. Spike2 software was used to identify Peak Extension Amplitude and the subsequent initial Peak Flexion Amplitude on a trial-by-trial basis. These marker positions were verified visually and exported along with the associated latency values (Peak Extension Latency and Peak Flexion Latency) relative to perturbation onset.

For the visual RT conditions, we were interested in quantifying onset of voluntary activity on a trial-by-trial basis. Onset of activity in FCR (RT) was defined as the point at which
rectified EMG began a sustained rise above baseline levels (-100 to -5 ms before the imperative signal). The location of this point was determined by displaying FCR activity on a monitor with a superimposed horizontal cursor indicating 3SD above baseline activity. A vertical cursor was placed at the first point (after the imperative signal) that activity increased above the horizontal cursor, and remained above this level for >5 ms.

Identical procedures were used to determine the onset of activity in startle indicator muscles (L-SCM, R-SCM, and L-OOc) for the startle control condition. The startle reflex elicited by either auditory or somatosensory stimuli is characterised by early activity in OOc, and symmetric bilateral bursts in the left and right SCM. Thus for a trial to be considered as showing a positive startle reflex, both left and right SCM as well as OOc had to be activated within 120 ms of the SAS or the perturbation (Álvarez-Blanco, Leon, & Valls-Solé, 2009; Brown et al., 1991; Carlsen, Dakin, Chua, & Franks, 2007; Carlsen, Maslovat, Lam, Chua, & Franks, 2011).

2.2.4 Statistical analysis

Kinematic landmarks (Peak Extension Amplitude, Peak Extension Latency, Peak Flexion Amplitude, Peak Flexion Latency) and integrated normalized EMG data from the first four epochs of interest (Background, M1, M2a, M2b) were analyzed using 4 Condition (Experiment 1: DNI, ACT, ACT-Wi, ACT-Na) and 3 Condition (Experiment 2: DNI, ACT-Na, ACT-R) repeated measures analyses of variance (ANOVA). Because a voluntary response was not present for the DNI conditions, integrated normalized EMG data for the VOL epoch was analyzed using a 3 Condition (Experiment 1: ACT, ACT-Wi, ACT-Na) repeated measures ANOVA or a paired-samples t-test (Experiment 2: ACT-Na, ACT-R). Trend analysis (Experiment 1) and a paired-sample t-test (Experiment 2) were used to analyze the visual RT data. For the ANOVA results, to adjust any violation to the assumption of sphericity,
Greenhouse-Geisser corrected $p$ values were reported, along with the uncorrected degrees of freedom. Partial eta squared ($\eta_p^2$) were used to convey effect sizes and post-hoc analyses were performed using the Dunn-Bonferonni corrected $t$-test. For all measures, the level of statistical significance was set at $p = .05$.

2.3 Results

2.3.1 Experiment 1

2.3.1.1 Behavioural differences in visual RT

In the visual RT experiment, we found a significant linear trend between the three conditions, $F(2,20) = 30.61, p < .001, \eta_p^2 = .75$. Mean RT was shortest for untargeted condition (V-ACT: 168.9 ms), increased ~10 ms when participants aimed for the 10 degree target (V-ACT-Wi: 179.2 ms), and another ~10 ms when aiming for the narrow (5 degree) target (V-ACT-Na: 190.8 ms). Please see Figure 2.1 for a distribution of visual RTs from the three different accuracy conditions.
Figure 2.1. Reaction time frequency distributions of the visual RT conditions in Experiment 1. Mean values are displayed by vertical arrows above the X-axis. Note how the distribution (and mean value) for the V-ACT (untargeted, dashed grey line/arrow) condition is earliest, followed by V-ACT-Wi (wide target, solid grey line/arrow). The longest RTs were observed in the V-ACT-Na condition (solid black line/arrow). Reprinted with permission of the American Physiological Society.

2.3.1.2 Kinematics following a rapid wrist perturbation

The rapid wrist perturbation induced large changes in the angle of the wrist; however, the intention to respond actively changed the four kinematic measures examined. See Figure 2.2A for the group ensemble displacement profiles for the various perturbation conditions (also see Table 2.1 for mean values and statistical results of all comparisons). The DNI condition resulted in greatest (34.9 degrees) Peak Extension Amplitude, \((F(3,30) = 43.11, p < .001, \eta^2_p = .81)\), at a significantly longer latency (134.2 ms), \((F(3,30) = 35.17, p < .001, \eta^2_p = .78)\). No significant differences with regards to Peak Extension were observed between the three active conditions.
(ACT: 24.3 degrees, 112.1 ms; ACT-Wi: 22.9 degrees, 112.6 ms; ACT-Na: 22.9 degrees, 112.6 ms). A different pattern of results was observed when Peak Flexion Amplitude ($F(3,30) = 30.82$, $p < .001$, $\eta_p^2 = .76$) was examined. Participants moved furthest into flexion ($p$ values < .001) when actively responding as fast as possible, but not aiming for a target (ACT: -39.8 degrees). Even though the target zones were centred about the same position for the two targeted conditions, participants consistently moved further into flexion when aiming for the wide target ($p = .045$; ACT-Wi: -27.7 degrees; ACT-Na: -25.9 degrees). Despite differences in flexion amplitude between the three active conditions, Peak Flexion Latency did not differ significantly ($p$ values $> .88$; ACT: 232.0 ms; ACT-Wi: 226.4 ms; ACT-Na: 239.5 ms). The active conditions did however differ from DNI, where peak flexion was reached significantly later ($p$ values < .002; 381.2 ms), $F(3,30) = 30.46$, $p < .001$, $\eta_p^2 = .75$. 

Figure 2.2. Group ensemble displacement and wrist flexor EMG data for the perturbation conditions in Experiment 1. A. Wrist displacement data, normalized by participant ($n = 11$). DNI condition: dashed black line. ACT: dashed grey. ACT-Wi: solid grey. ACT-Na: solid black. Positive values signify wrist extension and negative values wrist flexion. Target positions are represented by the solid black lines (wide target) and dashed black lines (narrow target). B. Group ensemble EMG data, normalized by participant. EMG amplitude value of 1 corresponds to peak reflex value from DNI condition from each participant. C. Expanded view of the epochs of interest shown in panel B. Reprinted with permission of the American Physiological Society.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Omnibus ANOVA</th>
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<td>ACT-Wi</td>
</tr>
<tr>
<td>Peak Extension,°</td>
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<td>24.3 (7.3)</td>
<td>22.9 (8.0)</td>
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<td>ACT-Na</td>
</tr>
<tr>
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<td>112.6 (8.7)</td>
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<td>ACT</td>
<td>ACT-Wi</td>
<td>ACT-Na</td>
</tr>
<tr>
<td>Peak Flexion,°</td>
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<td>39.8 (6.0)</td>
<td>27.7 (1.8)</td>
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<tr>
<td>DNI</td>
<td>ACT</td>
<td>ACT-Wi</td>
<td>ACT-Na</td>
</tr>
<tr>
<td>Peak Flexion Latency, ms</td>
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<td>320 (16.2)</td>
<td>226.4 (35.9)</td>
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<td>DNI</td>
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<td>ACT-Na</td>
</tr>
<tr>
<td>M1 onset, ms</td>
<td>20.5 (3.0)</td>
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<td>29.5 (2.2)</td>
</tr>
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<td>ACT</td>
<td>ACT-Wi</td>
<td>ACT-Na</td>
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<td>M2 onset, ms</td>
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<td>46.4 (3.0)</td>
<td>49.0 (3.7)</td>
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<td>ACT-Na</td>
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<tr>
<td>M2 offset, ms</td>
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<td>—</td>
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<td>ACT-Na</td>
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<tr>
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<td>ACT-Na</td>
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<tr>
<td>M1 activity, NU ms</td>
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<td>7.3 (3.2)</td>
<td>7.7 (3.0)</td>
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<td>ACT-Na</td>
</tr>
<tr>
<td>M2b activity, NU ms</td>
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<td>21.8 (7.7)</td>
<td>17.0 (6.6)</td>
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<td>ACT-Wi</td>
<td>ACT-Na</td>
</tr>
<tr>
<td>VOL Activity, NU ms</td>
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<td>11.8 (4.1)</td>
<td>90.0 (4.2)</td>
</tr>
<tr>
<td>DNI</td>
<td>ACT</td>
<td>ACT-Wi</td>
<td>ACT-Na</td>
</tr>
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Table 2.1. Means, standard deviations (in parentheses), and statistical results from Experiment 1. ms, milliseconds; NU, normalized unit (value of 1 corresponds to peak reflex EMG value from DNI condition); NU*ms, integrated area of normalized EMG data. Following a significant omnibus test, post-hoc testing was conducted using the Dunn-Bonferroni corrected t-test. Reprinted with permission of the American Physiological Society.
2.3.1.3  EMG Activity following a rapid wrist perturbation

Rapidly perturbing the right wrist into extension evoked at least two clear responses in the EMG recording from wrist flexors. See Figure 2.2B/C for group ensemble averages from each condition (also see mean values and a statistical summary in Table 2.1). The first response, M1, had mean onset latencies of 29.5 (± 3.0) ms, 29.5 (± 3.1) ms, 29.3 (± 2.2) ms, and 29.5 (± 2.5) ms for the DNI, ACT, ACT-Wi, and ACT-Na conditions, respectively. Corresponding onset times for the second response, M2, were 48.6 (± 3.4) ms, 48.4 (± 3.1) ms, 49.0 (± 3.7) ms, and 49.2 (± 3.2) ms. M2 offset was obtained only for the passive condition and was 94.2 (± 8.4) ms. The voluntary response was observed only for the active conditions. As the voluntary response sometimes began earlier than 100 ms, and overlapped with the end of M2, we could not determine its exact onset latency, nor could we mark the end of M2 for these conditions.

Prior to each perturbation, a slight extension preload was applied by the motor. To counter the load, and maintain the limb at the home position, participants generated a small contraction in wrist flexors. This pre-perturbation muscle activity did not differ significantly between DNI, ACT, ACT-Wi, and ACT-Na, $F(3,30) = 0.33, p = .81$, $\eta^2_p = .03$. Likewise, EMG activity during the M1 period was not statistically different across the four conditions, $F(3,30) = 0.52, p = .67$, $\eta^2_p = .05$.

While preparing to respond against the perturbation did not influence background activity or the short-latency response, marked differences were observed between the passive and active conditions for the epochs containing the long-latency response (see Figure 2.2B/C). For the first half of M2 (M2a), a significant main effect was found, $F(3,30) = 12.76, p < .001$, $\eta^2_p = .56$. Post-hoc tests revealed increased activity for the active conditions (ACT: 20.1 Normalized Units*ms;
No significant differences were found between the three active conditions ($p$ values > .99). Analysis of the latter half of M2 (M2b) also revealed EMG differences, $F(3,30) = 33.84, p < .001$, $\eta^2_p = .77$, but post-hoc tests uncovered a different pattern of results. As expected, the DNI instruction resulted in the lowest level of activity ($M = 7.6$ NU*ms; $p$ values ≤ .001). Asking the participants to flex the wrist as fast as possible (ACT) produced the highest level of activity ($M = 21.8$ NU*ms; $p$ values ≤ .04), while having the participants flex the wrist with an accuracy component produced significantly less activity (ACT-Wi: 17.0 NU*ms; ACT-Na: 17.6 NU*ms). As we will argue, the increased activity observed in the M2b period for the ACT condition resulted from a larger and likely shorter latency voluntary response, producing increased superposition onto the end of the M2 response.

While modulation of EMG activity was clearly observed during the epochs containing the M2 response, we also found significant EMG differences between active conditions in the VOL (100-200 ms) epoch, $F(2,20) = 17.75, p < .001$, $\eta^2_p = .64$. Post-hoc analysis revealed significant differences ($p$ values ≤ .035) between all three active conditions (see Figure 2.2B/C). Activity was highest in the ACT condition (118.7 NU*ms), lowest for ACT-Na (81.8 NU*ms), and intermediary for the ACT-Wi condition (93.0 NU*ms).

2.3.2 Experiment 2

2.3.2.1 Behavioural differences in visual RT

We observed significant RT differences between the unidirectional and reversal conditions, $t(9) = 3.2, p = .011$. As expected, mean RT was significantly longer for the reversal movement (V-ACT-R: 193.1 ms) compared to the unidirectional movement (V-ACT-Na: 180.7
Please see Figure 2.3 for a distribution of visual RTs for the two conditions. Also note that the mean RT for the ACT-Na condition was ~10 ms earlier than it was for Experiment 1.

Figure 2.3. Reaction time frequency distributions of the visual RT conditions in Experiment 2. Mean values are displayed by vertical arrows above the X-axis. Note how the distribution (and mean RT value) for the V-ACT-Na (unidirectional movement, black line/arrow) condition is earlier than the V-ACT-R (reversal movement, solid grey line/arrow) condition. Reprinted with permission of the American Physiological Society.
Table 2. Means, standard deviations (in parentheses), and statistical results from Experiment 2. NU, normalized unit (value of 1 corresponds to peak reflex EMG value from DNI condition); NU*ms, integrated area of normalized EMG data. Following a significant omnibus test, post-hoc testing was conducted using the Dunn-Bonferroni corrected test. Reprinted with permission of the American Physiological Society.

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<td>17.0 (8.9)</td>
<td>25.6 (19)</td>
<td>26.5 (19)</td>
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<td>&lt;0.01</td>
<td>0.03</td>
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<td>Post-Emerg. Lantry</td>
<td>17.7 (5.4)</td>
<td>30.5 (16)</td>
<td>29.4 (16)</td>
<td>2.18</td>
<td>&lt;0.01</td>
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<td>17.0 (8.9)</td>
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<td>Post-Exposure</td>
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<td>0.08</td>
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</table>
2.3.2.2 Kinematics following a rapid wrist perturbation

Similar to Experiment 1, the perturbation produced large excursions in wrist angle, and the intention to respond influenced all kinematic measures examined (see Figure 2.4A and Table 2.2). Peak Extension Amplitude was significantly larger, $F(2,18) = 50.96, p < .001, \eta^2_p = .85$, and occurred later, $F(2,18) = 31.23, p < .001, \eta^2_p = .78$, for the DNI condition ($p$ values = .001; 36.8 degrees, 137.0 ms). There were no differences between the active conditions (ACT-Na: 27.9 degrees, 117.5 ms; ACT-R: 26.5 degrees, 117.0 ms). Analysis of Peak Flexion also revealed differences in the Amplitude, $F(2,18) = 13.34, p = .002, \eta^2_p = .60$, and Latency, $F(2,18) = 85.85, p < .001, \eta^2_p = .91$. Values obtained for the DNI condition were latest (377.9 ms) and of smallest amplitude (-21.7 degrees). As expected, because participants aimed for the same flexion target in the ACT-Na and ACT-R conditions, Peak Flexion Amplitude did not differ significantly ($p = .32$). Although also not statistically significant ($p = .10$) the latency of Peak Flexion was ~6 ms later for the ACT-R condition. As will be argued in the Discussion, this small latency difference (see Figure 2.4A) in reaching peak flexion might provide indirect evidence of a RT difference between ACT-R and ACT-Na. For the ACT-R condition, we also calculated measures associated with the second extension (i.e., reversal) movement. The target was placed from 0 to 5 degrees of extension, and participants reached an average wrist angle of 4.0 degrees of extension, 394.9 ms after perturbation onset.
Figure 2.4. Group ensemble displacement and wrist flexor EMG data for the perturbation conditions in Experiment 2. A. Wrist displacement data, normalized by participant ($n = 10$). DNI condition: dashed black line. ACT-Na: solid black. ACT-R: dashed grey. Positive values signify wrist extension and negative values wrist flexion. Target positions are represented by the dashed black lines. B. Group ensemble EMG data, normalized by participant. EMG amplitude value of 1 corresponds to peak reflex value from DNI condition from each participant. C. Expanded view of the epochs of interest shown in panel B. Reprinted with permission of the American Physiological Society.
2.3.2.3   EMG Activity following a rapid wrist perturbation

See Figure 2.4B/C for group ensemble EMG averages from each condition (also see values and a statistical summary in Table 2.2). M1 had mean onset latencies of 29.7 (± 2.0) ms, 30.3 (± 1.6) ms, and 30.0 (± 2.1) ms for the DNI, ACT-Na, and ACT-R conditions, respectively. Onset times for M2 were 50.4 (± 3.9) ms, 50.9 (± 4.3) ms, and 50.6 (± 3.8) ms. M2 offset was only obtained for DNI and was 93.7 (± 5.9) ms. The third response, representing voluntary activity, was observed during the ACT-Na and ACT-R conditions. As this response was often continuous with the end of M2, we did not mark its onset latency, or the offset of M2 for these conditions. However, because the magnitude of activity during the VOL epoch did not differ, and the voluntary response appeared of similar magnitude and shape (see Figure 2.4B/C), we inferred a small voluntary response latency shift between active conditions (see below).

Analysis of EMG activity during the pre-perturbation and reflex periods replicated the findings of Experiment 1. No significant differences were found for the pre-perturbation, $F(2,18) = 1.48, p = .26, \eta_p^2 = .14$, or M1 epochs, $F(2,18) < 0.01, p = .98, \eta_p^2 < .01$; however, differences were found for the epochs containing the M2 response (M2a: $F(2,18) = 7.76, p = .004, \eta_p^2 = .46$; M2b: $F(2,18) = 15.51, p = .001, \eta_p^2 = .63$). Activity during the M2a and M2b epochs was larger for both active conditions compared to DNI; but the two active conditions (ACT-Na, ACT-R) did not differ significantly ($p$ values ≥ .71) (see Figure 2.4B/C and Table 2.2).

Similar to the M2b period, analysis of EMG activity for the VOL epoch revealed no significant difference between the two active conditions, $t(9) = 0.34, p = .74$. Furthermore, inspection of the group ensemble EMG profiles (see Figure 4B/C) revealed a comparable magnitude voluntary response between ACT-Na and ACT-R. Because voluntary response
magnitude was similar, we examined the group EMG ensemble to infer a latency shift (see Figure 2.4B/C). Indeed the voluntary response for the ACT-R (Solid Grey Line) condition appeared delayed relative to the ACT-Na (Solid Black Line) condition. Despite inferred differences in voluntary response onset, M2b activity did not differ significantly between the active conditions ($p = .71$), suggesting minimal voluntary superposition onto the end of M2.

### 2.3.3 Control conditions to examine potential activation of startle circuitry

All participants displayed positive indicators of a startle reflex on the control SAS trial. After onset of the SAS, L-OOc was activated first at a mean latency of 37 ms followed by bursts in left and right SCM at 58 and 60 ms, respectively. For the DNI perturbation-only trials, we found no incidence of activity in either SCM or OOc. However, when a SAS was delivered simultaneous with a perturbation (DNI+SAS), 93.3% of trials displayed a positive startle reflex. OOc was activated at a mean onset of 43 ms (mean intra-participant SD = 5.1 ms), followed by right SCM at 65 ($\pm 2.9$) ms and left SCM at 66 ($\pm 3.8$) ms.

Of primary interest in these control conditions was startle reflex incidence during ACT perturbation-only trials. Based on the criteria of bilateral SCM and OOc activation within 120 ms of the stimulus (in this case the wrist perturbation), none of the trials were classified as showing a startle reflex. We did note a small general increase in late SCM activity (often greater than 120 ms); however, left and right SCM were not activated simultaneously, and this activity was never accompanied by OOc activity (see Figure 2.5B/D). This is in stark contrast to the ACT+SAS trials where a SAS was delivered simultaneous with the perturbation, in which 100% of trials showed indicators of a startle reflex, with OOc activated first at a mean latency of 44 ($\pm 6.7$ ms), followed by left SCM at 56 ($\pm 3.6$) ms and right SCM at 57 ($\pm 3.4$) ms (see Figure 2.5A/C). The findings of this control experiment suggested that the perturbations and the predictable
presentation protocol we used in Experiments 1 and 2 did not evoke a startle reflex. We were therefore confident that the voluntary responses observed in Experiments 1 and 2 were not responses triggered involuntarily by the startle reflex (i.e., no StartReact effect).

**Figure 2.5.** Ensemble EMG data from an exemplar participant in a control condition conducted to determine whether the perturbations used in Experiments 1 and 2 were capable of eliciting a startle reflex. Solid black: Left sternocleidomastoid (SCM); solid grey: Right SCM; dashed black: left orbicularis oculi (OOh). Black arrows denote mean onset time. A. Average SCM data from 5 ACT trials where a startling auditory stimulus (SAS) was unexpectedly paired with the perturbation. B. Average SCM data from 25 ACT trials without a SAS (identical to ACT trials in Experiment 1). C. Average OOh data from the same trials as panel A. D. Average OOh data from same trials a panel B. Note how trials with the SAS (Panels A and C) have a burst in left OOh, followed by large bilateral bursts in SCM. This is in contrast to the control ACT trials (B and D) where no OOh activity was observed and activity in left and right SCM were small and not activated symmetrically. Reprinted with permission of the American Physiological Society.
2.4 Discussion

An extension perturbation applied to the wrist evoked short and long-latency responses in the stretched wrist flexors. When participants acted against the perturbation, the short-latency response was unaffected, while activity during the long-latency response period increased and was followed by a voluntary response. This study was primarily concerned with whether changes in activity during the M2 period result from instruction- or goal-dependent reflex gain modulation, superposition of the voluntary response, or a combination of the two mechanisms. By employing behavioural manipulations that are known to influence voluntary RT, we varied the degree to which the voluntary response overlapped with the epochs containing the long-latency response. We found that activity during the first half of the M2 epoch (M2a) was influenced by the intention to respond, but was not sensitive to the latency or magnitude of the voluntary response (see Figures 2.2B/C and 2.4B/C). Similarly, activity during the latter half of the M2 epoch (M2b) was greater when participants compensated for the perturbation. However, unlike M2a activity, under certain conditions (i.e., ACT condition, Experiment 1), M2b was also influenced by the voluntary response. Our findings support a hybrid mechanism in which both reflex gain modulation and a superpositioned voluntary response can influence EMG activity during the long-latency response period. Moreover, these findings showcase instruction-dependent wrist flexor M2 period modulation in the absence of a startle reflex or StartReact effect.

2.4.1 Reaction time, triggered reactions, and modulation of the long-latency reflex

Researchers examining the long-latency response have long understood the importance of estimating voluntary RT in perturbation paradigms. While the seminal work by Hammond (1956) calculated proprioceptive RT at ~100 ms, subsequent studies have reported RT values in
response to muscle stretch at latencies as short as ~70 ms (e.g., Evarts & Granit, 1976; Evarts & Vaughn, 1978; MacKinnon et al., 2000; Manning et al., 2012; Ravichandran et al., 2013). While we are not arguing that voluntary superposition cannot play a role in M2 period modulation, we found clear modulation of M2 activity at a latency (50-75 ms) shorter than the earliest reported voluntary response. Moreover, modulation of M2a period activity was similar across all active conditions which (as argued below) exhibited different amounts of voluntary response superposition during the M2b period. Previous studies have shown sub-70 ms M2 changes between a “let-go” and “compensate” task (Colebatch et al., 1979) or a target analogue version of the verbal “do-not intervene” vs. “compensate” (Pruszynski et al., 2008; Yang et al., 2011), or even between two “do-not intervene” conditions that differed only in the stability of the environment in which they were performed (Shemmell et al., 2009). However a majority of studies arguing against instruction/goal-dependent modulation of the long-latency response compared the verbal “do-not intervene” to a “compensate or resist” instruction (Crago et al., 1976; Manning et al., 2012; Ravichandran et al., 2013). These studies have often only found modulation during the M2b period, thus attributing the findings to voluntary response superposition. Pruszynski and colleagues (2008) suggested there may be ambiguity associated with verbal instructions and participants may interpret instructions differently. Even though we made comparisons using the verbal “do-not intervene” instruction with various active conditions, we have provided clear evidence of instruction-dependent M2a period modulation, a finding in line with the recent work using targets (as opposed to verbal instructions) to specify participant behaviour (e.g., Pruszynski et al., 2008; Yang et al., 2011).

The manipulations of accuracy requirements (Experiment 1) and response complexity (Experiment 2) were intended to influence the latencies of the voluntary response. Analysis of
EMG data from our visual RT conditions confirmed that these manipulations of response requirements resulted in significant voluntary RT differences ranging from ~10 to ~20 ms in response to a visual stimulus (see Figures 2.1 and 2.3). However, analysis of the voluntary responses to the stretch perturbations indicated that the perturbations resulted not only in shorter voluntary response onsets but also may have compressed the magnitude of RT differences between some of the active conditions. In order to infer voluntary latency differences between perturbation conditions, we examined the interaction between wrist kinematics, voluntary response magnitude, and magnitude of activity in the M2b epoch.

The perturbation we applied moved the wrist into extension; however the intention to compensate resulted in a smaller (and shorter latency) Peak Extension Amplitude compared to the passive motion in the DNI condition. This finding is in line with work showing the urgency or intention to respond to a perturbation can influence peak joint excursion (Crevecoeur et al., 2013). However we found no significant differences between active conditions (in both Experiments 1 and 2) in terms of the joint angle or latency at which peak extension was reached (see Figures 2.2A and 2.4A), suggesting a similar level of urgency when compensating for the perturbation. There were differences in Peak Flexion Amplitude, depending on the active condition. When there was no accuracy requirement (ACT, Experiment 1), participants moved further into flexion compared to when a target was present (ACT-Wi and ACT-Na, Experiment 1). Despite differences in Peak Flexion Amplitude between all three active conditions in Experiment 1 (ACT > ACT-Wi > ACT-Na), peak flexion positions were reached at similar latencies (all p values >.80). This was likely a result of RT differences and the corresponding change in the magnitude of the voluntary response between conditions (ACT > ACT-Wi > ACT-Na). Certainly both shorter RTs and larger voluntary responses can account for why participants
moved further distances in a similar period of time. In Experiment 2, when subjects had to move
to the same initial target position (ACT-Na vs. ACT-R), we found no significant differences in
flexion amplitudes nor in the magnitudes of the voluntary responses. We did however note a
small increase in Peak Flexion Latency, being \( \sim 6 \) ms later for the ACT-R condition (see Figure
2.4A). Although this difference only approached statistical significance \( (p = .10) \), 6 ms was
similar to the difference observed between onsets of the voluntary responses in the ensemble
EMG profiles (see Figure 2.4B/C, solid grey vs. solid black profiles).

If the voluntary responses to the perturbations were not sensitive to our response
accuracy/complexity manipulations, but instead were automatically “triggered” by the stretch
erpurbation (e.g., Crago et al., 1976; Evarts & Granit, 1976; Evarts & Vaughn, 1978;
Ravichandran et al., 2013; Shemmell, 2015), similar RT latencies would be expected between
the three active conditions in Experiment 1 and the two active conditions from Experiment 2.
Because the magnitude of EMG activity during the VOL epoch differed between active
conditions in Experiment 1, the triggered response hypothesis would predict these differences to
also appear in the M2b period. However, we only found increased M2b activity for the ACT
condition, whereas the values obtained for ACT-Wi versus ACT-Na did not differ. We can
interpret this finding as further evidence of response latency differences between active
conditions. Similar to the visual RT data, the ACT condition had what appeared to be the earliest
(and largest) voluntary response and thus exhibited the greatest degree of superposition. Adding
an accuracy component delayed the voluntary response, thus minimizing superposition onto the
end of M2. Furthermore because magnitude of activity during the VOL epoch differed between
targeted conditions (ACT-Wi and ACT-Na), but M2b period activity did not, we reason these
two targeting conditions may have had minimal overlap from the voluntary response in the M2b period.

While the visual RT data from Experiment 1 demonstrated differences ranging from 10 to 20 ms (V-ACT < V-ACT-Wi < V-ACT-Na; see Figure 2.1), closer analysis of the M2b period for the perturbation conditions revealed a selective increase in activity for the ACT condition occurring 20 to 25 ms earlier than ACT-Wi or ACT-Na (see Figure 2.2B/C). Typically when RT is reduced (in this situation from the visual to proprioceptive imperative signal), differences in RT between conditions may also be expected to decrease (e.g. Maslovat et al., 2011a). This raises an intriguing question as to why the temporal separation between ACT and the two targeting conditions may have increased. One possibility is the presence of a “triggered response” in the ACT condition, but not for ACT-Wi or ACT-Na. Although the definition of a triggered response varies in the literature, in the context of studies which have examined M2, the triggered response is believed to be the voluntary response elicited at shortened latency (see Lewis et al., 2006; Manning et al., 2012). The ACT condition in our study was similar to that used by studies which have proposed the concept of a triggered response (i.e., untargeted movement with participants instructed to respond as quickly as possible; e.g., Crago et al., 1976; Evarts & Granit, 1976; Evarts & Vaughn, 1978). However, exactly why we would observe a triggered response for the untargeted condition, but not the conditions with imposed accuracy constraints remains unclear. As an alternative mechanism, magnitude of the long-latency response may have scaled with the intended distance to move against the perturbation. This may be similar to the recent work showing the size of the long-latency response in elbow flexors can increase (or decrease) with the distance moved against (or with) an elbow perturbation (Pruszynski et al., 2008). In Experiment 1 of the present study, participants moved the wrist ~15
degrees further into flexion in the ACT condition, compared to ACT-Wi and ACT-Na. However, despite these large kinematic differences, M2a activity remained similar between the three active conditions suggesting the differences in the M2b period may not have been from scaling of the long-latency response. Future studies may be needed to further distinguish whether modulation of M2 period activity results from overlap of a triggered response or scaling of the long-latency response magnitude with intended movement distance.

Although an exact mechanism for a perturbation “triggered response” and how it differs from a voluntary response remains elusive in the literature (Crago et al., 1976; Evarts & Vaughn, 1978; Lewis et al., 2006; Manning et al., 2012; Pruszynski, Kurtzer, & Scott, 2011), comparisons have been made with the StartReact effect (Lewis et al., 2006; Ravichandran et al., 2013; Shemmell et al., 2009). Ravichandran and colleagues (2013) showed that unexpectedly delivering an elbow flexion or extension position perturbation when participants were planning an elbow extension movement elicited a startle reflex as well as the intended voluntary response in elbow extensors at short latency (73 ms). Because it is well known that auditory startle stimuli can elicit a startle reflex which triggers a prepared voluntary response (i.e., StartReact effect), Ravichandran et al. (2013) argued that the StartReact effect is a mechanism responsible for perturbation triggered responses. We conducted a control condition to investigate whether the perturbations in our protocol were capable of eliciting a startle reflex. While the auditory startle stimulus reliably evoked a startle reflex (see Figure 2.5), the wrist perturbations never elicited startle in either the DNI or the ACT condition. Despite the lack of a startle reflex (and therefore StartReact effect), as discussed above, the results of the ACT condition in Experiment 1 demonstrated that the voluntary response could be elicited at short latency, such that it superimposed onto the end of M2. Thus a perturbation evoked startle reflex was likely not the
mechanism responsible for triggering the voluntary response at short latency. Contributions from
the startle response and the StartReact effect were investigated in detail in chapters 4 and 5.

One of the first studies to suggest that modulation of activity during the M2 period is due
to an overlapping voluntary response also implemented a behavioural manipulation in attempt to
vary RT. Rothwell and colleagues (1980) changed the predictability of the perturbation and
found the largest increase in M2 activity between “let-go” and “resist” conditions when the onset
of the perturbation was most predictable. Although this work has been cited extensively as
evidence for voluntary response superposition contributing to increased M2 activity (e.g., Lewis
et al., 2006; Manning et al., 2012; Shemmell et al., 2009), we believe this interpretation may be
tempered by differences in the magnitude of voluntary responses across the “resist” conditions.
Comparable to our findings in Experiment 1, the magnitude of the voluntary response was
largest when RT was expected to be shortest (Rothwell et al. also could not estimate RT directly
in the perturbation conditions). It is plausible that RT differences between active conditions were
negligible (for both Rothwell et al., 1980 and Experiment 1); instead, it was the magnitude of the
voluntary response that differed, creating the appearance of latency differences between the
voluntary responses. Even though the findings from the two targeting (ACT-Wi and ACT-Na)
conditions in Experiment 1 provided evidence that this was likely not the case (i.e., different
magnitude voluntary responses but no differences in M2b activity), we can turn to the results of
our second experiment to further disentangle the interactions between voluntary response
magnitude, onset latency, and superposition into the M2 period.

In Experiment 2, activity during the M2a and M2b epochs was larger during the active
conditions (see Figure 2.4). Similar to the targeting conditions in Experiment 1, we found no
further modulation of M2b activity as a function of the active condition. In contrast to the first
experiment, the magnitude of EMG activity in the VOL epoch was similar between active conditions, and furthermore, the size and shape of the ensemble voluntary responses appeared qualitatively comparable (Gottlieb, 1998; see Figure 2.4 B/C). This allowed us to indirectly infer a RT difference between the unidirectional and reversal movements. As expected (Ketelaars et al., 1997; Ketelaars et al., 1999; Lajoie & Franks, 1997), the ensemble EMG profile of the reversal condition (ACT-R, Gray Trace) was delayed relative to unidirectional condition (ACT-Na, Solid Black Trace). Inspection of the M2b epoch revealed that the two active conditions did not significantly differ. A difference in voluntary response onset, but no significant difference in M2b period activity suggests the voluntary response was sufficiently delayed in both conditions, minimizing superposition onto the end of M2. Taken together with the findings from the targeting conditions in Experiment 1, the use of accuracy constraints following a perturbation may delay the voluntary response outside of the long-latency response epochs.

### 2.4.2 Conclusion

The present study examined sources that may contribute to stretch response modulation following expected torque perturbations applied at the wrist. Across all active conditions, we noted a general increase in the size of the long-latency reflex, beginning early in the appearance of the response (~50 ms). Because this increased activity began at a latency shorter than the earliest reported voluntary responses or triggered reactions, we can attribute the magnitude changes to a facilitation of reflex circuit excitability (or gain modulation). Activity during the latter half of M2 was also found to be larger for all active conditions (compared to DNI) but we found a further increase in activity for the condition with the earliest and largest voluntary response. However we also showed that early triggering of the voluntary response was not a result of the StartReact effect. In summary, the findings of this chapter support a hybrid
mechanism by which both changes in reflex gain and a superimposed voluntary response contribute to instruction-dependent modulation of the long-latency response period.

2.5 Bridging summary

The findings from the experiments in chapter 2 showed that when participants transitioned from a passive task (with the instruction to “not-intervene”) to an active task (involving a voluntary intervention against the perturbation), there was a general increase in the first half of the M2 response (M2a: 50-75 ms). The latter portion of the M2 response (M2b: 75-100 ms) was influenced by the latency and/or magnitude of the voluntary response. This work was originally motivated as a follow-up to a study which argued for instruction-dependent M2 modulation being produced purely from voluntary superimposition (Manning et al., 2012). We noted that this previous study used long variable foreperiods (3-10 seconds) to reduce predictability of a perturbation, however in chapter 2, we used short variable foreperiods (2750-3750 ms). The range of variable foreperiods used between other previous studies also differed considerably. The purpose of the experiment in the following chapter was to determine whether differences in variable foreperiod predictability could influence the M2 response. We were specifically concerned with whether differences in findings between studies may have arisen because of potential differences in the magnitude of the M2 response on passive trials (often used as the control condition).

2 This foreperiod was originally intended to be 2500-3500 ms. This range of values was used for subsequent experiments (unless otherwise specified).
Chapter 3: Experiment 3

Perturbation predictability can influence the long-latency stretch response

3.1 Introduction

Mechanical perturbations applied to joints in the upper limbs elicit stereotyped responses in the electromyographic (EMG) recording of the stretched muscle. The first event, termed the short-latency (M1) response, is produced by a monosynaptic spinal reflex pathway and appears in the EMG recording ~25-50 ms after the onset of muscle stretch (Liddell & Sherrington, 1924). Immediately following M1, occurring between ~50-100 ms, is a second event termed the long-latency (M2) stretch response (Hammond, 1956). M2 is generated (at least in part) by a transcortical pathway involving the primary sensory and motor cortices (Cheney & Fetz, 1984; Evarts & Tanji, 1976; Lewis et al., 2004; MacKinnon et al., 2000; Matthews et al., 1990; Omrani et al., 2014; Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski et al., 2014). While M1 is usually resistant to voluntary intervention, a remarkable feature of M2 is that it can be modulated based on the goal or intent of the subject. For instance, when instructed to counteract the perturbation, M2 increases in magnitude thus aiding to offset the imposed load. This modulation is believed to result from pre-setting excitability of the contributing neural circuitry (Hammond, 1956; Pruszynski et al., 2014); although alternative explanations have also been provided (Crago et al., 1976; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980).

While numerous studies have examined the influence of intentional set, other factors such as habituation (Rothwell, Day, Berardelli, & Marsden, 1986), event predictability (Kimura, Haggard, & Gomi, 2006), and temporal anticipation of the perturbation (Goodin & Aminoff, 1992; Rothwell et al., 1986) have also been shown to influence the M2 response. For example, Rothwell et al. (1986) reduced the temporal uncertainty of perturbation delivery, by either cueing perturbation onset with a weak electric stimulus, or having participants self-deliver the perturbation by pressing a button. Stretch responses from these predictable conditions were
compared with a less predictable condition, where the perturbation was delivered every 5 seconds. M1 did not change across conditions; however M2 was reduced in magnitude on trials with increased temporal certainty. Similar results were observed from an experiment where participants were asked to actively respond by either “letting go” or “opposing” the perturbation (Goodin & Aminoff, 1992). Like the study by Rothwell and colleagues (1986), predictable perturbations were self-delivered; however in the experiment by Goodin & Aminoff (1992), unpredictable perturbations were given randomly every 8-116 seconds (i.e., at very unpredictable intervals). Comparable to previous findings, M1 was not affected by either intentional set or perturbation predictability. Of particular interest was the finding that M2 was smaller following predictable perturbations, and this occurred for both the “let-go” as well as the “oppose” conditions.

The experiments discussed above highlight findings from the extremes of temporal predictability. In order to prevent anticipation of perturbation delivery, many studies examining M2 have used variable intervals between the warning signal and perturbation (i.e., foreperiod), or between subsequent perturbations (if no warning signal was provided). However, the range of intervals used differs considerably between studies. For example, a recent study by Pruszynski et al. (2014) employed short variable foreperiods of 1-1.5 seconds; in Experiments 1 and 2 of this dissertation we used foreperiods ranging from 2750-3750 seconds; while Manning et al. (2012) used long variable foreperiods of 3-10 seconds. It remains unclear whether the range of possible variable foreperiod length influences the circuitry contributing to the M2 response. We were concerned that a less predictable (i.e., long) foreperiod may result in a larger M2 response on passive trials. This could reduce the potential for any further increases in excitability when participants actively respond to the perturbation and may account for why some studies have not
observed goal-dependent modulation of the M2 response beyond what can be accounted for by voluntary response superimposition (e.g., Manning et al., 2012).

The purpose of experiment 3 was to examine whether the predictability produced by the length of variable foreperiod can influence the M2 response. We compared blocks of short (2.5-3.5 seconds; high predictability) and blocks of long (2.5-10.5 seconds; low predictability) variable foreperiods where participants were instructed to either not intervene (passive) or to compensate (active) for the perturbation as quickly as possible. It was expected that M2 would be larger on active trials (compared to passive), but of primary interest was whether M2 could also be modulated as a function of the range of foreperiod durations.

3.2 Methods

3.2.1 Participants

Fifteen right-handed healthy participants (8 male, 7 female; mean age of 22 ± 3 years) participated in an experiment lasting approximately 90 minutes. Informed written consent was collecting prior to each testing session and the procedures were approved by the University of British Columbia behavioural ethics board.

3.2.2 Experimental setup

Participants sat in a height-adjustable chair with right arm secured to a manipulandum that allowed movement to occur in the horizontal plane about the wrist joint. The elbow was flexed at 100 degrees and hand semi-supinated with the wrist joint aligned to the rotational axis of the manipulandum. A torque motor (Aeroflex TQ 82W-1C) was connected to the manipulandum and a metal handle attached to the motor shaft was positioned near the metacarpophalangeal joints. Foam stops were tightened on either side of the wrist to prevent lateral movement and custom-molded thermoplastic was tightened around the hand of each
participant allowing movement to occur without the fingers grasping the metal handle. An
oscilloscope was placed on a table ~1 m in front of the manipulandum and provided continuous
feedback of wrist position and a LED lightbox was placed on top of the oscilloscope.

3.2.3 Task and stimuli

The starting position for each perturbation trial was 10 degrees of wrist flexion, a point
defined visually on the oscilloscope by arrows. Trials began with the torque motor ramping up
(over 500 ms) to a small extension preload of 0.25 newton metres (Nm). To resist the preload
and keep the wrist at the home position, participants generated a slight contraction in wrist
flexors. A warning signal was generated by the lightbox when the preload reached its peak. If the
light turned red participants were instructed to “not intervene with the perturbation” (i.e.,
passive, do not intervene condition: DNI), while if the light was green participants were
instructed to “flex the wrist as quickly as possible following the perturbation” (i.e., active
condition: ACT). The foreperiod was terminated with a large wrist extension perturbation of 1.5
Nm lasting 150 ms. Following the perturbation, the preload level of torque remained for 450 ms.

Differences in foreperiod duration can influence reaction time (e.g., Klemmer, 1957);
however, due to preceding reflexive responses, RT cannot be determined on a trial-by-trial basis
in a perturbation paradigm (for more detail see Manning et al., 2012; and chapter 2). We
included two separate conditions to determine whether RT differences between the two
foreperiod ranges may have occurred in our perturbation conditions. Procedures were identical to
those described above and included all four types of trials (ACT and DNI, Short and Long
variable foreperiods); however the imperative stimulus was an 80 dB auditory tone (no
perturbations occurred in these blocks) and the starting position was 30 degrees of wrist
extension (approximately where the perturbation displaced the wrist; chapter 2). While this was
not meant to provide an estimate of RT in the perturbation conditions, the auditory cue conditions were meant to provide evidence that RT differences were present in the foreperiod ranges that we used and that this would presumably also be present during perturbation trials (albeit at a different response latency due to shorter RT on perturbation trials).

Participants performed 4 blocks of 50 experimental trials (200 trials total) in which the variable foreperiod between the warning signal and the perturbation (or auditory imperative signal) was short (2.5-3.5 seconds: SFP) or long (2.5-10.5 seconds: LFP). Prior to commencing the experimental blocks, participants were provided 10 ACT and 10 DNI practice trials for each foreperiod length. Within an experimental block, 25 ACT and 25 DNI trials were randomly interleaved. To control the inter-stimulus rate (between trials conducted in different blocks), a minimum interval between onset of each trial was 15 seconds. Experimental block order was randomized across participants and separated by a 5 minute rest period.

3.2.4 Data collection, reduction, and analysis

Surface EMG data were collected from the right wrist flexor (FCR) and extensor (ECR) carpi radialis muscles using bipolar preamplified silver/silver chloride surface electrodes connected to an external amplifier (Model 544, Therapeutics Unlimited Inc., Iowa City, IA). EMGs were amplified at 2-4K and bandpass filtered from 30-1000 Hz. Signals were sampled at 2 kHz using a 1401Plus data acquisition system and a computer running Spike2 (CED, Cambridge, UK). Offline data analysis was accomplished using Spike2 and custom-written LabVIEW (National Instruments, Austin, TX) software.

EMG data were baseline corrected and full-wave rectified. A 700 ms epoch (200 ms pre to 500 ms post) was defined around each imperative signal. Visual inspection was conducted on individual trial data from FCR, ECR, and the displacement profile to ensure correct performance.
Reasons for trial exclusion included responding before the imperative signal (i.e., false starts), not responding on an ACT trial, or responding on a DNI trial. Of the 3500 experimental trials collected, 2.6% were omitted due to error.

For the perturbation conditions, individual participant ensemble averages (of ~25 trials) were used to obtain stretch response onset and offset times (MacKinnon et al., 2000; Manning et al., 2012). Average baseline EMG and standard deviation (SD) were calculated from -200 to 0 ms relative to perturbation onset. M1 onset was determined as the point at which activity first increased 3 SD above baseline levels. Due to overlap between the end of M1 and onset of M2, we could not use the same criteria to mark M2 onset, therefore this point was defined as the trough in activity (occurring around 50 ms). With voluntary activity overlapping onto the end of M2 in the ACT conditions, M2 offset was only marked from DNI conditions (where no voluntary response was present). This was determined as the first decrease in activity below 3 SD above baseline following M2 onset.

For the perturbation conditions, integrated values from the wrist flexor EMG data were analyzed in four 25 ms epochs relative to perturbation onset (on a trial-by-trial basis). The first time period was used to examine baseline EMG, occurring 25 ms prior to the onset of the perturbation. The second epoch contained the M1 response, 25-50 ms post-perturbation. While the M2 response in wrist flexors typically occurs between 50 and 100 ms, a current hypothesis in the literature is that activity during this interval is not generated by a single pathway; rather multiple spinal and supra-spinal circuits make contributions (e.g., Lourenço et al., 2006; Pruszynski & Scott, 2012; Shemmell, 2015). Furthermore, on ACT trials, the voluntary response (sometimes referred to as a triggered reaction) may also superimpose onto the latter half of the M2 response (e.g., Forgaard, Franks, Maslovat, Chin, & Chua, 2015; Manning et al., 2012;
Ravichandran et al., 2013). Therefore, taking a similar approach to other authors (e.g., Forgaard et al., 2015; Pruszynski et al., 2008; Ravichandran et al., 2013), we have divided the M2 period into two parts, M2a (50-75 ms) and M2b (75-100 ms).

The mean integrated activity from the M1 epoch (across the four perturbation conditions) was used to normalize the integrated EMG data for each participant. A value of 1.0 corresponds to integrated EMG values obtained in the M1 epoch. We were also interested in whether normalized integrated activity within each epoch changed as a function of foreperiod length on a given trial. We computed a Pearson correlation coefficient from each epoch versus the time at which the perturbation occurred. In addition, we calculated the raw peak M1 (25-50 ms) and peak M2 values (50-100 ms) from each trial. These values are presented in Table 3.1. For the auditory conditions, mean baseline (from -200 ms to the imperative signal) and standard deviation (SD) of FCR activity was calculated on each ACT trial. RT was determined as the first point that activity exceeded 3 SD above baseline and remained above this level for at least 20 ms. Onset marker positions were verified visually and adjusted only on the rare occasion where the algorithm resulted in an obvious error. DNI trials from these conditions were analyzed to ensure participants successfully withheld a voluntary response on these trials.

RT values from the auditory conditions were analyzed using a paired samples t-test comparing ACT trials from the 2 Foreperiods (SFP, LFP). A paired samples t-tests was also used to compare M2 offset between the two perturbation DNI conditions. For the perturbation conditions, M1/M2 onsets and the integrated normalized EMG data from each of the predefined epochs (Background, M1, M2a, M2b) were analyzed using a 2 Foreperiod (SFP, LFP) × 2

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3 Note that we originally performed the same normalization procedure as chapter 2, however on request of a Reviewer, wrist flexor EMG were normalized to the M1 response. This change in normalization procedure had no influence on the findings of the experiment.
Intentional Set (DNI, ACT) repeated measures analysis of variance (ANOVA). We used partial eta squared $\eta^2_p$ to express effect size and significant Foreperiod × Intentional Set Interactions were interpreted using simple main effects analysis. The level of statistical significance for these tests was set at $p = .05$. For the correlation analysis between trial-by-trial foreperiod length and normalized integrated EMG activity within each epoch, Pearson $r$ values underwent a Fisher $r$ to $Z$ transformation. The transformed values were statistically analyzed using one-sample $t$-tests to determine whether any values differed significantly from zero. To correct for four comparisons (DNI SFP, DNI LFP, ACT SFP, ACT LFP) within each epoch, the level of statistical significance for these tests was set a $p = .0125$.

3.3 Results

Group wrist displacement profiles, wrist flexor EMG ensembles, and integrated EMG values from each epoch of interest are presented in Figure 3.1. A statistical summary is also provided in Table 3.1.

When participants responded to an auditory signal, RT values from the SFP block (141.1 ms) were shorter than values obtained in the LFP block (154.5 ms), a difference that approached statistical significance ($t(14) = 2.14, p = .051$).

The perturbation produced two clear responses in wrist flexors. The first response (M1) had a mean onset latency of 27.5 ms, a value which did not differ across the four conditions. The second response (M2) had a mean onset of 49.8 ms and also was not different between conditions. The offset of M2 could only be determined from the two DNI conditions; this value occurred earlier ($t(14) = 5.03, p < .001$) for the SFP block (93.4 ms) compared to the LFP block (97.7 ms).
Figure 3.1. Group ($n = 15$) displacement and wrist flexor EMG data. A. Ensemble wrist displacement in degrees. B. Normalized integrated wrist flexor EMG values for the epochs of interest. Values were normalized to each participant’s mean integrated EMG activity in the M1 epoch. C. Normalized rectified wrist flexor EMG along same time scale as A. Values were normalized to each participant’s mean peak M1. D. Same as C, but zoomed-in to focus on the time periods of interest. Reprinted under the Creative Commons Attribution license.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Short Foreperiod</th>
<th>Long Foreperiod</th>
<th>Omnibus Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNI</td>
<td>ACT</td>
<td>DNI</td>
</tr>
<tr>
<td>M1 Onset (ms)</td>
<td>27.6</td>
<td>27.3</td>
<td>27.7</td>
</tr>
<tr>
<td>M2 Onset (ms)</td>
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<td>49.7</td>
</tr>
<tr>
<td>M2 Offset (ms)</td>
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<td>-</td>
<td>97.7</td>
</tr>
<tr>
<td>M1 Peak (mV)</td>
<td>0.18</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>M2 Peak (mV)</td>
<td>0.48</td>
<td>0.71</td>
<td>0.51</td>
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<tr>
<td>Baseline Epoch (NU)</td>
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<td>0.31</td>
<td>0.29</td>
</tr>
<tr>
<td>M1 Epoch (NU)</td>
<td>1.01</td>
<td>1.08</td>
<td>0.92</td>
</tr>
<tr>
<td>M2a Epoch (NU)</td>
<td>2.90</td>
<td>3.48</td>
<td>2.81</td>
</tr>
<tr>
<td>M2b Epoch (NU)</td>
<td>1.89</td>
<td>4.50</td>
<td>2.36</td>
</tr>
</tbody>
</table>

Table 3.1. Mean values (and inter-participant standard deviations) from our dependent measures of interest and omnibus ANOVA results for Experiment 3. Onset/Offset data are in milliseconds. M1 and M2 peak values are in millivolts. Epoch data are normalized to each participant’s mean integrated M1 values (normalized units). Reprinted under the Creative Commons Attribution license.
Analyzing integrated area of the baseline and M1 epochs revealed no main effects or interactions between conditions (see Table 3.1 for mean values and a statistical summary). By contrast, differences were found for the epochs containing the long-latency stretch response (M2a/M2b). Analysis of the M2a period revealed a significant main effect of Intentional Set, \( F(1, 14) = 10.14, p = .007, \eta_p^2 = .42 \), indicating that activity on ACT trials was significantly larger than on DNI (see Figure 3.1). A main effect of Intentional Set was also observed for the M2b epoch, \( F(1, 14) = 20.38, p < .001, \eta_p^2 = .59 \); however this was superseded by a significant Intentional Set × Foreperiod Interaction \( F(1, 14) = 6.68, p = .022, \eta_p^2 = .32 \). Simple main effects analysis revealed increased M2b activity for the DNI LFP (2.36 Normalized Units: NU) condition compared to DNI SFP (1.89 NU; \( p = .029 \)) (see Figure 3.1, Solid Grey vs. Solid Black profiles). Significant foreperiod differences between the two ACT conditions \( (p = .83) \) were not observed; nevertheless both ACT conditions remained significantly larger than DNI.

The trial-by-trial correlation analysis between foreperiod length and integrated activity revealed no significant correlations for the baseline, M1, or M2a epochs (all \( r \) values -.09 to .11; all \( p \) values > .06. This suggested that activity during these time periods was not modulated as a function of foreperiod length on a given trial. By contrast, analysis of the M2b epoch revealed significant, or nearly significant (critical \( p = .0125 \)) correlation values for each condition (see Figure 3.2 for mean lines of best fit from the M2b epoch). The ACT conditions showed a small positive trend (SFP: \( r = 0.21, t(14) = 4.29, p = .001 \); LFP: \( r = 0.18, t(14) = 3.95, p = .001 \)), which was likely due to an aging foreperiod effect on RT (i.e., more voluntary superimposition because RT of the voluntary response was expected to be shorter as the foreperiod aged). A small negative trend was observed for the DNI conditions (SFP: \( r = -0.19, t(14) = -3.16, p = .007 \); LFP:
\[ r = -0.15, t(14) = -2.68, p = .018. \] This we believe was due to the participants being better able to anticipate perturbation delivery as they neared the end of the potential foreperiod range.

Importantly, within a given Intentional Set (ACT or DNI), similar aging foreperiod effects were found between the two foreperiod conditions (SFP and LFP).

![Figure 3.2](image-url)

**Figure 3.2.** M2b group mean (and standard error) lines of best fit for the normalized integrated wrist flexor EMG values as a function of trial-by-trial foreperiod length. DNI conditions: solid lines. ACT conditions: dashed lines. A. Short foreperiod (2.5-3.5s) conditions. B. Long foreperiod (2.5-10.5s) conditions. For ACT conditions, as the foreperiod aged, M2b values tended to increase. For the DNI conditions, M2b values decreased with the aging foreperiod. Reprinted under the Creative Commons Attribution license.

### 3.4 Discussion

The present study investigated whether the temporal predictability of a limb perturbation can influence the long-latency stretch response. While previous investigations have compared the extremes of temporal (un)certainty (Goodin & Aminoff, 1992; Rothwell et al., 1986), we made comparisons between two variable foreperiods of different potential duration. Replicating previous research (Hammond, 1956; MacKinnon et al., 2000; Pruszynski et al., 2008) and findings from chapter 2, M2 activity was increased on trials where participants were instructed to compensate for the perturbation compared to passive trials where participants were asked not to
intervene. The main finding from this study was that even though variable foreperiods are often used to prevent anticipation of a perturbation, the range of possible foreperiod duration still influenced the M2 response on passive trials. Specifically, the more predictable (short) variable foreperiod resulted in reduced M2b activity compared to the less predictable (long) variable foreperiod condition. Our study also revealed “aging foreperiod” effects during the M2b epoch for all perturbation conditions; these effects provide further evidence that temporal predictability of a mechanical perturbation can influence the long-latency stretch response.

Integrated area within a predefined epoch is influenced by the amplitude as well as the duration of activity. Analysis of our stretch response timing data revealed no differences between the conditions with regards to the onset of M1 or M2. We did however find an unexpected difference when examining M2 offset. Specifically, the duration of M2 was ~4 ms shorter for the SFP DNI condition compared to the LFP DNI condition. By contrast, peak M2 values did not significantly differ between DNI conditions (see Table 3.1). Therefore the reduced integrated M2b activity from the SFP block appeared to result from a shorter M2 duration, as opposed to a smaller M2 peak. The previous investigations examining perturbation predictability (Goodin & Aminoff, 1992; Rothwell et al., 1986) only reported integrated EMG records, so it is unclear whether differences between conditions resulted from M2 timing and/or amplitude differences.

A majority of studies examining the M2 response have used perturbations which rapidly stretched a muscle of interest, but muscular responses occurring over a similar time-course have also been observed following the sudden application of load in a precision grip task. These latter responses are believed to be elicited by cutaneous tactile afferents in the fingers. Kourtis, Kwok, Roach, Wing, and Praamstra (2008) compared the predictability of a sudden load perturbation on the size of the long-latency response in a precision grip task. Participants were asked to hold an
object between the right thumb and index finger and to not let it slip following application of a downward load. In one condition the load was given predictably every 2 seconds, whereas in another condition the load was randomly applied between 0.7 and 4.3 seconds. Similar to the stretch response findings of the present study, Kourtis et al. found that the long-latency response was reduced following the predictable load change. These authors reported an amplitude difference between the two conditions, but from their figures it appeared that duration of the long-latency EMG response was also shorter following the predictable perturbation.

A long-standing debate in the stretch response literature is whether goal-dependent modulation of activity during the M2 response epochs are produced from changes in excitability of the underlying circuitry (e.g., Hammond, 1956; Pruszynski et al., 2014) or is an artifact of the voluntary or triggered response superimposing onto the stretch response (e.g., Crago et al., 1976; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980). An early study which argued for the latter mechanism also made comparisons using foreperiods of varying stimulus predictability (Rothwell et al., 1980). Because trials with more predictable stimulus onsets can have shorter RTs (Klemmer, 1957), Rothwell and colleagues hypothesized these conditions would show the greatest M2 period modulation between “resist” and “let-go” instructions. Indeed, the greatest change to M2 was observed from conditions where perturbation onset was most predictable. However, a finding not mentioned by Rothwell et al. (but evident from their figures and pointed out by other authors; e.g., Goodin, Aminoff, & Shih, 1990) were differences between the “let-go” conditions. M2 activity was reduced (nearly in half) when participants performed in the most predictable stimulus condition. Because there was no overlapping voluntary response on “let-go” trials, but changes to M2 were still observed, this supports the hypothesis that changes in reflex circuit excitability can influence the M2 response. The “let-go”
condition has however been criticised because it may not represent an unmodified stretch response (e.g., Calancie & Bawa, 1985; Crago et al., 1976); the instruction implies participants actively relax upon receiving the perturbation (Calancie & Bawa, 1985). In our study we observed M2 differences using the passive “do-not intervene” instruction. Like the “let-go” instruction, the DNI condition is also not confounded by a superimposed voluntary response, therefore the M2b modulation that we observed likely reflected differences in contributions from the underlying M2 circuitry as opposed to a superimposed voluntary response.

Pathways mediating the long-latency stretch response have been extensively investigated since the 1970’s. Early work focused on whether M2 was generated by spinal circuitry (e.g., Hagbarth et al., 1981; Matthews, 1984), or a longer transcortical pathway (e.g., Cheney & Fetz, 1984; Evarts & Tanji, 1976; Lee & Tatton, 1975). A current proposition is that M2 is not produced by a single pathway; rather it receives contributions from multiple spinal and supraspinal pathways (Lourenço et al., 2006; Pruszynski & Scott, 2012; Shemmell, 2015). Primary motor cortex (Omrani et al., 2014; Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski et al., 2014) and the cerebellum (Kurtzer et al., 2013; Strick, 1983) have been highlighted as important structures involved in modulation of the M2 response. Interestingly, both structures have also been implicated in temporal anticipation and predictability of sensory stimuli (e.g., Davranche et al., 2007; Goodin & Aminoff, 1992; Ivry, 2000; Tesche & Karhu, 2000). Lee & Tatton (1975) reported the presence of two reflex peaks in the M2 period; the first was referred to as M2 and the second was called M3. These authors proposed that M2 was produced via primary sensory and motor cortex and M3 traversed the cerebellum prior to engaging motor cortex. Although we did not observe two distinct reflex peaks during the M2 epochs, the M2b epoch may correspond closely to the M3 response reported by Lee and Tatton. Because both cerebellar and motor cortex
circuitry potentially make contributions to the M3 response (Lee & Tatton, 1975), and both the cerebellum (Ivry, 2000; Tesche & Karhu, 2000) and motor cortex (Davranche et al., 2007; Goodin & Aminoff, 1992) have been shown to be involved in temporal anticipation, it is plausible that both structures may contribute to the M2b differences observed between our DNI conditions.

When participants actively respond to a perturbation, voluntary RTs have been observed at latencies as short as ~70 ms (Crago et al., 1976; MacKinnon et al., 2000; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980) making it difficult to interpret changes to the latter portion of M2 for ACT conditions (Forgaard et al., 2015; Manning et al., 2012). Even though we did not observe significant foreperiod differences between the two ACT conditions, this does not necessarily preclude the possibility that differences in M2 and/or voluntary response overlap may have occurred. For example, RTs were expected to be shorter for trials from the SFP block (Klemmer, 1957) and indeed, the voluntary response appeared earlier and larger for this condition (Figure 3.1 C/D: dashed black profile) compared to the LFP block (dashed grey profile). This would lead one to expect increased M2b activity for the SFP block, a result of greater voluntary response superimposition. By contrast, if differences in the M2 response itself occurred (e.g., duration, similar to the DNI conditions), we would predict trials from the LFP block to have a longer M2 response compared to trials from the SFP block. In other words, poor perturbation anticipation was expected to increase duration of the M2 response and result in a later voluntary response onset (and hence less voluntary response superimposition onto M2). By contrast, enhanced anticipation (SFP condition) may result in reduced M2 activity, but also shorter voluntary response latency (and more overlap onto M2). The net result of these
two factors would be an M2b period of similar integrated area between ACT conditions differing in predictability of the perturbation.

Although our study was not specifically designed to examine the effects of foreperiod aging on modulation of perturbation elicited stretch responses, a correlation analysis of trial-by-trial foreperiod length and activity in each predefined epoch revealed interesting findings. The duration of the foreperiod on a given trial had no influence over activity during the baseline, M1, or M2a epochs. By contrast, all perturbation conditions showed an effect of trial-by-trial foreperiod length on activity during the M2b epoch (see Figure 3.2). The active conditions showed increased M2b activity as the duration of the foreperiod increased. This was likely the result of a typical aging foreperiod effect on RT of the voluntary response, with RT decreasing as the foreperiod aged and imperative signal delivery became imminent (Drazin, 1961). The opposite trend was observed for DNI conditions with M2b activity decreasing as the foreperiod progressed. The reduced M2b activity may have resulted from a similar temporal anticipation mechanism responsible for our main DNI findings (i.e., more predictable perturbations resulting in reduced M2b activity).

In summary, while previous investigations have shown self-delivered (Goodin & Aminoff, 1992; Rothwell et al., 1986) and temporally cued (Rothwell et al., 1980) perturbations can modulate the M2 response, the novel finding from our study was that the M2 response was also sensitive to small differences in the range of potential foreperiod durations. In our study, and the previous studies that have shown temporal perturbation predictability can reduce M2 activity, the direction of the perturbation was always known in advance (Goodin & Aminoff, 1992; Rothwell et al., 1986). A recent study (Nikaido et al., 2016) suggested that temporal predictability may actually have the opposite effect on the M2 response when perturbation
direction is not precued. Therefore, while we do not advocate researchers use only short or long variable foreperiods, we do believe that it is important that considerations be given to the effect that foreperiod variation can have on the long-latency stretch response.

3.5 Bridging summary

The experiments conducted in chapters 2 and 3 of this dissertation employed various behavioural manipulations that are known to influence a voluntary response in order to examine voluntary response contributions to instruction-dependent M2 modulation. The results of these experiments demonstrated that regardless of the latency or magnitude of a voluntary response, there was a general increase in the first half of M2 when participants performed an ACT task (compared to DNI). The behavioural manipulations of the voluntary response only influenced the latter portion of the M2 response. It has been suggested that the reason a pre-programmed voluntary response can appear at latencies that overlap the M2 response (specifically the latter portion) is because a mechanical perturbation elicits a startle reaction and therefore exploits the StartReact effect (Ravichandran et al., 2013). The startle control conditions in chapter 2 provided preliminary evidence that an expected mechanical perturbation stimulus does not elicit startle. Despite this, we still observed considerable activity in SCM near the end of the startle time criteria. The objectives of the next two experiments (4 and 5) reported in chapter 4 was to determine the conditions under which a mechanical perturbation may be capable of eliciting startle, whether instruction-dependent M2 modulation is produced purely from the StartReact effect, and to examine the interaction between a mechanical perturbation stimulus and the auditory startle response.
Chapter 4: Experiments 4 & 5

An examination of the startle response during upper limb stretch perturbations


This manuscript was used in Christopher Forgaard’s successful application for the (2017) Franklin Henry Young Scientist Award at the Canadian Society for Psychomotor Learning and Sport Psychology (SCAPPS).
4.1 Introduction

Mechanical perturbations applied to the upper limbs can elicit multi-peaked electromyographic (EMG) responses in the stretched musculature. The earliest response (M1) occurs at short-latency (~25-50 ms) and is dominated by spinal contributions (Liddell & Sherrington, 1924). Immediately following M1 is a longer latency (~50-100 ms) response (M2) which receives input from both spinal (Hagbarth et al., 1981; Lourenço et al., 2006; Matthews, 1984) as well as supra-spinal sources (Cheney & Fetz, 1984; Evarts & Tanji, 1976; Lourenço et al., 2006; MacKinnon et al., 2000; Omrani et al., 2014; Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski et al., 2014). While M1 is relatively immutable to intentional modulation, M2 can exhibit several characteristics of voluntary motor behavior. Referred to as instruction- or goal-dependent modulation, M2 increases in magnitude when the participant is instructed to resist or move against the direction of the perturbation, and decreases when instructed to not-intervene or let-go (Calancie & Bawa, 1985; Forgaard et al., 2015; Hammond, 1956; MacKinnon et al., 2000; Pruszynski et al., 2008; for a comprehensive review see Pruszynski and Scott, 2012). When the participant compensates against the perturbation, M2 is followed by and often continuous with a voluntary response. Numerous groups have postulated that the majority of goal-dependent EMG modulation during the M2 period results from an early triggering of the voluntary response superimposing onto M2 (Crago et al., 1976; Houk, 1978; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980; Shemmell et al., 2009). Indeed, onset latencies (for the voluntary response) have been reported as early as 70 ms, well within the interval (50-100 ms) containing M2 (Evarts & Granit, 1976; Evarts & Vaughn, 1978; MacKinnon et al., 2000; Manning et al., 2012; Ravichandran et al., 2013).
Reaction times (RT) of similar short latency have been reported by groups examining the interaction between the auditory startle response and the preparation of voluntary movement (e.g., Carlsen et al., 2004b; Nonnekes et al., 2014; Valls-Solé et al., 1999). While it can take anywhere from 100 to 400 ms to initiate movement following non-startling auditory or visual stimuli, unexpected delivery of a startling auditory stimulus (SAS; >120 decibels: dB) with the imperative signal, results in the hastened initiation (<100 ms) of the preplanned voluntary response (termed the StartReact effect). Despite the temporal advancement, kinematic and EMG characteristics of the intended response remain relatively unchanged from control trials (for reviews see Carlsen et al., 2012; Nonnekes et al., 2015; Rothwell, 2006; Valls-Solé et al., 2008).

While circuitry involved in the startle response has been well documented in animal models (Yeomans & Frankland, 1995; Yeomans et al., 2002), the exact neural mechanisms underlying the StartReact effect remains a matter of debate (Carlsen et al., 2012; Nonnekes et al., 2015; Valls-Solé et al., 2008). It is however agreed that neural activation associated with the startle response acts as a fast initiation trigger of a preplanned voluntary response.

Given the comparably short reaction times following limb perturbations and a SAS, it was recently suggested that the StartReact effect (usually associated with SAS delivery) may also be responsible for initiating the voluntary response following a limb perturbation (Ravichandran et al., 2013; Shemmell, 2015; Shemmell et al., 2009; Shemmell et al., 2010). Ravichandran et al. (2013) showed that when participants performed a simple reaction time task to a non-startling auditory stimulus, the unexpected presentation of an elbow perturbation resulted in a startle response (which the authors characterised as activation of the left sternocleidomastoid (SCM) muscle in <120 ms) that consequently triggered the voluntary response at shortened latency (~73 ms). These authors proposed that when the intended
voluntary response occurred in the same muscle stretched by the perturbation, the increased EMG activity observed during the M2 epoch resulted entirely from superimposition of the startle-triggered voluntary response. However, an important difference exists between the experimental design of Ravichandran et al. and a majority of studies that have examined goal-dependent modulation of the M2 response. Ravichandran et al. delivered limb perturbations on a small proportion of unexpected trials simultaneous with the imperative (auditory) signal, whereas most studies examining M2 modulation delivered the perturbation on every trial as the imperative signal (e.g., Calancie & Bawa, 1985; Crago et al., 1976; Forgaard et al., 2015; Manning et al., 2012; Pruszynski et al., 2008; Rothwell et al., 1980). It is known that predictability of a SAS affects the startle response, with rapid habituation following repeated stimulus presentations (Brown et al., 1991; Oude-Nijhuis et al., 2010). Thus in the case of many studies examining M2, the expected, repeated limb perturbations may be less likely to evoke a startle response and consequent StartReact effect.

The purpose of the experiments conducted in the present chapter was to examine the relationship between perturbation predictability and elicitation of the startle response. Furthermore, we tested whether the StartReact effect is responsible for instruction-dependent modulation of EMG activity during the time period containing the M2 response. In Experiment 4, participants were instructed to either “flex the right wrist as fast as possible” (ACT), or “do not intervene” (DNI), following a large wrist extension perturbation (1.5 Nm). This design was similar to previous studies examining M2 (e.g., Calancie & Bawa, 1985; Hammond, 1956; Manning et al., 2012; Pruszynski et al., 2008; Rothwell et al., 1980); the large mechanical perturbation was used as the imperative signal and was thus expected. On random trials a SAS Probe (120 dB) was presented simultaneous with the imperative signal. This allowed a
comparison between perturbation elicited EMG activity and auditory startle elicited EMG activity in startle indicator muscles (SCM and orbicularis oculi: OOc). In Experiment 5, participants performed ACT and DNI conditions in response to a small wrist extension perturbation (0.5 Nm) or a non-startling auditory stimulus (80 dB). Similar to a previous study showing that limb perturbations could elicit a startle response (Ravichandran et al., 2013), on random trials we delivered a large wrist extension perturbation (Pert Probe; 1.5 Nm) or a SAS Probe at the imperative signal. It was expected that across both experiments, the SAS Probe would reliably evoke early bursts (<120 ms) of EMG activity in startle indicator muscles (OOc and SCM) as well as initiate the preplanned response (wrist flexion) on ACT trials at reduced latency (<100 ms). Of primary interest was whether the large wrist perturbation could evoke similar responses in startle indicators and wrist flexors. We reasoned that if limb perturbations are indeed startling, a startle response and StartReact effect should most likely be observed on trials where the large perturbation was delivered unexpectedly (Experiment 5). By contrast, if limb perturbations evoke a startle response even when the perturbation was expected (Experiment 4), the startle response and StartReact effect should be consistently observed across all perturbation conditions. Finally, if the StartReact effect underlies instruction-dependent M2 period modulation, increased M2 activity should only be observed on ACT trials displaying a positive startle response.

4.2 Methods

4.2.1 Participants

Eighteen (11 female, 7 male, mean age 22 years) healthy right-handed volunteers participated in two experiments conducted in a single testing session lasting 90 minutes. However the primary analysis focused on data from 14 participants in Experiment 4 and 12
participants in Experiment 5 who consistently displayed a startle response following the SAS Probes (e.g., Carlsen et al., 2011). The excluded participants had a lower than 50% startle response incidence following SAS Probes on ACT trials. Data from non-startling participants are presented separately in the Results section. Protocols were approved by the University of British Columbia ethics committee and written consent was obtained from each participant prior to testing.

4.2.2 Apparatus and setup

The experiments took place in an acoustically dampened testing room. Participants sat in a height-adjustable chair and were positioned with the right arm in a manipulandum that allowed flexion/extension movement of the wrist. The right elbow was flexed at 100 degrees and the hand was semi-pronated with the wrist joint aligned to the rotational axis of the manipulandum. The manipulandum was connected to a torque motor (Aeroflex TQ 82W-1C) and a metal handle adjoined to the motor shaft was placed near the right metacarpophalangeal joints. Lateral wrist movements were prevented by padded stops on either side of the wrist and a thermoplastic cast enveloped the hand allowing wrist movement to occur without the fingers grasping the metal handle. Continuous position feedback of the right wrist was provided on an oscilloscope placed 0.5 m in front of the participant. The home position was 0 degrees of wrist flexion and defined on the oscilloscope. Auditory stimuli were delivered via a loudspeaker placed 30 cm behind the participants’ ears.

4.2.3 Task and stimuli

The main portion of testing took place in two experiments, the order of which was counterbalanced across participants. All trials began with an auditory warning signal (80 decibels, 50 ms, 500 Hz) followed by an extension preload ramped slowly (over 500 ms) to 0.25
newton metres (Nm). Participants were instructed to resist by lightly activating wrist flexors against the load and to hold their wrist at the home position. A random foreperiod (2,500-3,500 ms) followed the warning signal and was terminated by an imperative signal (which varied depending on the condition, see below). Following the imperative signal, the preload level of extension torque (0.25 Nm) was maintained for 1000 ms.

4.2.4 Experiment 4: expected large perturbations

Experiment 4 involved conditions differing by intentional set and probe stimulus. Participants were instructed to either (1) “flex the right wrist as fast as possible following a large perturbation (1.5 Nm, 150 ms) imperative signal” (active condition: ACT), or (2) “not intervene with the large perturbation imperative signal” (do not intervene/passive condition: DNI). Each testing condition consisted of a single block of 30 trials (two blocks and 60 trials total). Within each testing block, 6 trials consisted of a SAS Probe (120 dB, 50 ms, 1000 Hz) presented simultaneously with the imperative signal. SAS Probe trials were interleaved pseudo-randomly such that they did not occur on the first 3 trials of a block or on consecutive trials. Ten practice trials (without probe stimuli) were given prior to the start of each condition to ensure familiarization with the task and a brief rest period was provided between the two blocks.

4.2.5 Experiment 5: unexpected large perturbations

Participants performed four testing conditions for Experiment 5; each condition differed by instructional set and/or modality of the imperative signal. The perturbation stimulus that was used as the imperative signal in Experiment 4 was used as a probe stimulus in Experiment 5. Participants were instructed to either (1) “flex the wrist as quickly as possible following an auditory imperative signal” (ACT 80 dB IS; 80 decibels, 50 ms, 1000 Hz), (2) “flex the wrist as quickly as possible following a perturbation imperative signal” (ACT Pert IS; 0.5 Nm, 150 ms),
(3) “not intervene with the auditory imperative signal” (DNI 80dB IS) or (4) “not intervene with the perturbation imperative signal” (DNI Pert IS). Each condition involved two blocks of 30 trials (8 blocks and 240 trials total). Within each block, 6 trials consisted of either a SAS or a Pert Probe (1.5 Nm, 150 ms) presented at the imperative signal. In the 80 dB IS blocks, the Pert Probe was presented with the 80 dB tone. On SAS Probe trials, the SAS was presented in lieu of the 80 dB tone. For the Pert IS blocks, the SAS Probe was presented with the small perturbation. On Pert Probe trials, the small perturbation was replaced by the large perturbation. Each block of 30 trials included 24 No probe trials (IS only), 3 Pert Probe trials, and 3 SAS Probe trials. Probe trials were interleaved pseudo-randomly and 10 practice trials were given prior to the start of each condition. The testing order of the conditions was randomized and counterbalanced across participants and brief rest periods were allotted between blocks.

4.2.6 Analysis and classification of startle

Surface EMG data were collected from the muscle bellies of right flexor carpi radialis (FCR), right extensor carpi radialis (ECR), left orbicularis oculi (OOr) and left and right sternocleidomastoid (SCM) using pre-amplified surface electrodes connected to an external amplifier (Model DS-80, Delsys Inc., Natick, MA). EMG data were amplified at 3K and bandpass filtered from 20-450 Hz. Signals were digitized at 2 kHz using a 1401plus data acquisition system and Spike2 (CED, Cambridge, UK) computer software. A custom-written LabVIEW (National Instruments, Austin, TX) program was used for offline data analysis.

At the beginning of analysis, EMG data were baseline corrected and full-wave rectified. Burst onsets were marked as the first point at which rectified EMG traces began a sustained rise above background levels (mean of activity for 200 ms preceding the imperative signal on a trial-by-trial and an individual muscle basis). EMG traces were displayed on a computer monitor with
a superimposed line indicating the first point at which activity increased to more than 3SD above background and remained above this level for at least 10 ms. Onset markers were verified visually and adjusted if necessary to the onset of the appropriate muscle burst. At this stage of analysis, we also removed any error trials. This resulted in the omission of 1.3% of trials. Reasons for omission included artefacts in EMG recordings, participants not responding in an ACT condition, or beginning their response prior to the imperative signal (i.e., false starts).

The startle response to intense auditory stimuli has been well documented; it begins with closure of the eyes and contraction of facial musculature, followed by flexion of the neck and arms (e.g., Brown et al., 1991; Carlsen et al., 2011; Landis & Hunt, 1939). By contrast, the startle response following somatosensory stimuli has received less attention. Recent work using upper-limb electrical stimulation (Álvarez-Blanco et al., 2009) and mechanical perturbations to the upper- (Ravichandran et al., 2013) or lower-limbs (Campbell et al., 2013; Oude-Nijhuis et al., 2010) have suggested that while the somatosensory startle response does involve activation of the same muscles used as indicators of auditory startle, the response is delayed by 20-30 ms and of smaller amplitude.

While the startle response following auditory or somatosensory stimuli appears to involve activation of OoC and SCM, these muscles can also be activated for non-startle related reasons. For instance, loud auditory stimuli also elicit a blink reflex, a response that is physiologically distinct and more resistant to habituation than startle-related SCM activation (Álvarez-Blanco et al., 2009; Brown et al., 1991; Kofler, Müller, Reggiani, & Valls-Solé, 2001). Responses in SCM are sometimes also present on non-SAS Probe trials (e.g., Carlsen et al., 2004b). This has been attributed to a postural response for stabilizing the head while performing ballistic upper limb movements. Postural SCM activity typically occurs just after onset of the voluntary response and
is therefore usually later than the 120 ms startle criteria (on non-SAS Probe trials). However, in the present study, we were very concerned that because the voluntary response to a mechanical perturbation can begin at sub-100 ms latency, postural SCM may also occur earlier, within the 120 ms startle criteria. If we only recorded from SCM as a startle indicator, this early postural activity may confound observations of a startle response. Further compounding this issue, the delayed onset of a startle response to a somatosensory stimulus could result in considerable overlap between postural- and startle-related SCM activation. Whereas most StartReact studies now only examine EMG activity from SCM (e.g., Alibiglou & MacKinnon, 2012; Forgaard et al., 2013; Ravichandran et al., 2013; Stevenson et al., 2014), we recorded from OOc as well as bilaterally from SCM. We did this to distinguish between startle- and postural-related SCM activity. For a trial to be classified as showing a positive startle response, EMG burst onsets had to be observed in left OOc, and bilaterally in left and right SCM within 120 ms of onset of the probe stimulus (Brown et al., 1991; Carlsen et al., 2007; Carlsen et al., 2011). Incidence data were converted to a proportion of total trials within a given condition and then subjected to an arcsine transform prior to statistical analysis. The non-transformed mean values are reported in the Results section (and Tables 4.1 and 4.2), along with statistical results conducted on transformed data.

In Experiment 4, the imperative signal (large perturbation) always elicited stretch responses (M1/M2) in wrist flexors; therefore, modulation of the evoked activity was also analyzed. Similar to previous studies (e.g., Forgaard et al., 2015; Pruszynski et al., 2008; Ravichandran et al., 2013) pre-defined 25 ms epochs were used. The first epoch examined baseline EMG, occurring 25 ms prior to the imperative signal. The second examined the short-latency response, 25-50 ms following the imperative signal. M2 was divided into two periods,
M2a from 50-75 ms, and M2b from 75-100 ms. Superimposed voluntary responses have been shown to influence M2b activity (Forgaard et al., 2015; Ravichandran et al., 2013), but not the M2a epoch (Forgaard et al., 2015). Integrated values from each epoch were taken from rectified EMG traces on a trial-by-trial basis. The mean integrated baseline values obtained from each condition (for each participant) were used to normalize the respective data from the M1, M2a, and M2b epochs. A value of 1 corresponds to integrated area equivalent to the baseline epoch.

In Experiment 4, startle incidence and stretch response data were statistically analyzed using a 2 (Intentional Set: ACT vs. DNI) × 2 (Probe Stimulus: SAS Probe vs. No Probe) repeated measures analysis of variance (ANOVA). To analyze startle incidence data in Experiment 5, we used a 2 (Intentional Set: ACT vs. DNI) × 2 (Imperative Signal: 80dB vs. Small Perturbation) × 3 (Probe Stimulus: SAS Probe vs. Pert Probe vs. No Probe) repeated measures ANOVA. Greenhouse-Geisser corrected $p$ values were reported and partial eta squared ($\eta_p^2$) was used as a measure of effect size. Significant interaction effects were interpreted using Simple Main Effects analysis and main effects (for conditions with more than 2 factors) were examined using Dunn-Bonferroni corrected $t$-tests. In Experiment 5, we also examined the StartReact effect; Premotor RT was analyzed using a $t$-test to compare the two conditions where onset of the voluntary response could be marked on a trial-by-trial basis (80 dB IS: ACT No Probe and SAS Probe). The level of statistical significance for all tests was set at $p = .05$.

4.3 Results

4.3.1 Experiment 4: expected large perturbations

See Table 4.1 for mean values and standard deviations from all conditions. While our Results focused on startle response incidence, EMG activity was also present in startle indicators on non-probe trials (particularly SCM), although typically at a later time frame which may relate
more to postural responses (Carlsen et al., 2004b; for more detail see below). Thus in Table 4.1
we also reported incidence and onset latency of activity in the SCM and OOC muscles, not
constrained by the 120 ms startle response time window.

<table>
<thead>
<tr>
<th>Measure</th>
<th>ACT</th>
<th>DNI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAS Probe</td>
<td>No Probe</td>
</tr>
<tr>
<td>Startle Reflex Incidence (%)</td>
<td>87.9</td>
<td>(11.8)</td>
</tr>
<tr>
<td>L-OOC Incidence (%)</td>
<td>96.4</td>
<td>(9.3)</td>
</tr>
<tr>
<td>L-OOC Latency (ms)</td>
<td>44.1</td>
<td>(15.3)</td>
</tr>
<tr>
<td>L-SCM Incidence (%)</td>
<td>95.0</td>
<td>(7.9)</td>
</tr>
<tr>
<td>L-SCM Latency (ms)</td>
<td>68.9</td>
<td>(8.0)</td>
</tr>
<tr>
<td>R-SCM Incidence (%)</td>
<td>97.6</td>
<td>(5.8)</td>
</tr>
<tr>
<td>R-SCM Latency (ms)</td>
<td>73.7</td>
<td>(12.0)</td>
</tr>
<tr>
<td>M1 (NU)</td>
<td>3.5</td>
<td>(1.4)</td>
</tr>
<tr>
<td>M2a (NU)</td>
<td>11.7</td>
<td>(3.9)</td>
</tr>
<tr>
<td>M2b (NU)</td>
<td>16.9</td>
<td>(6.8)</td>
</tr>
</tbody>
</table>

Table 4.1. Experiment 4 mean values and inter-participant standard deviations (in parentheses). ACT: active conditions; DNI: passive conditions; SAS Probe: 124 dB; No Probe: imperative signal only. Incidence data reported as a percentage (%) of trials from within a given condition. Latency values reported in milliseconds (ms). M1 (25-50 ms), M2a (50-75 ms), M2b (75-100 ms) are in normalized units (NU: value of 1 corresponds to integrated EMG activity from the baseline epoch). Reprinted with permission of Elsevier BV.

4.3.1.1 Expected perturbations do not elicit startle

The primary objective of this study was to establish whether mechanical perturbations could elicit a startle response similar to a SAS Probe. When participants performed in blocks of trials to an expected large perturbation, we observed significant main effects of Probe Stimulus, $F(1,13) = 82.96, p < .001, \eta_p^2 = .87$, and Intentional Set, $F(1,13) = 12.19, p = .004, \eta_p^2 = .48$;
however, these were superseded by a significant Intentional Set × Probe Stimulus interaction, $F(1,13) = 10.94, p = .006, \eta^2_p = .46$. Simple main effects analysis showed that the SAS Probe was more likely ($p = .005$) to elicit startle on ACT trials (87.9%) compared to DNI (58.3%), but no differences ($p = .17$) of Intentional Set were observed on No probe (perturbation only) trials (ACT: 0.6% vs. DNI 0%).

![Figure 4.1. Experiment 4 averaged startle indicator data from an exemplar participant. A. ACT Conditions. B. DNI Conditions. No probe: black lines. SAS Probe: grey lines. On SAS Probe trials, OOc was activated first, followed by large bilateral bursts in SCM. On ACT No probe trials, no activity was observed in OOc, but small bursts were observed in SCM, beginning around 100 ms. No activity was observed in OOc or SCM muscles for the DNI No probe condition. Reprinted with permission of Elsevier BV.](image)
See ensemble startle indicator data from an exemplar participant in figure 4.1. Also see figure 4.2 for group mean integrated EMG from startle indicator muscles, separated into 10 ms bins. The SAS Probe elicited a clear startle response in both ACT and DNI conditions, as evidenced by the early O Oc and bilateral SCM data (grey traces). By contrast, for ACT No Probe trials, activity was absent in O Oc, and smaller, postural-related bursts were observed in left and right SCM (black traces). No O Oc or SCM activity was observed for DNI No Probe trials.

**Figure 4.2.** Experiment 4 group mean integrated EMG from left O Oc, left SCM, and right SCM with standard error bars, divided into 10 ms epochs. A. ACT Conditions. B. DNI Conditions. No probe: black lines. SAS Probe: grey lines. Reprinted with permission of Elsevier BV.
We also examined SCM and OOc data, not constrained by the 120 ms startle criteria. SCM incidence analysis was performed using a 2 (Intentional Set: ACT vs. DNI) × 2 (Probe Stimulus: SAS Probe vs. No Probe) × 2 (Side: Left SCM vs. Right SCM) ANOVA. This test found a significant main effect of Intentional Set, \( F(1,13) = 35.86, p < .001, \eta_p^2 = .73 \), as well as Probe Stimulus, \( F(1,13) = 35.86, p < .001, \eta_p^2 = .80 \), suggesting SCM bursts occurred more frequently on ACT trials as well as on trials with a SAS Probe. The main effect for Side was nonsignificant (\( p = .72 \)), indicating that irrespective of condition, the left and right SCM muscles were activated with similar incidence. Analysis of SCM onset latency was conducted only on ACT trials (most participants had no SCM activity on DNI No Probe trials). Furthermore, three participants did not have any SCM activity on ACT No Probe trials; therefore, analysis of SCM onsets were conducted on the remaining 11 participants using a 2 (Probe Stimulus: SAS Probe vs. No Probe) × 2 (Side: Left SCM vs. Right SCM) ANOVA. This analysis revealed a significant main effect for Probe Stimulus, \( F(1,10) = 351.76, p < .001, \eta_p^2 = .84 \), demonstrating that the SAS Probe produced significantly earlier SCM onsets (71.0 ms) compared to No Probe trials (132.1 ms). The main effect for Side was nonsignificant (\( p = .51 \)) suggesting that the left and right SCM muscles were activated at a similar latency within their respective probe conditions.

OOc incidence was examined using a 2 (Intentional Set: ACT vs. DNI) × 2 (Probe Stimulus: SAS Probe vs. No Probe) ANOVA. This test revealed a significant main effect for Probe Stimulus, \( F(1,13) = 174.73, p < .001, \eta_p^2 = .93 \), suggesting that SAS Probe trials had a higher incidence of OOc (96.4%) than No Probe trials (16.1%). All fourteen participants that met our startle inclusion criteria displayed consistent responses following the SAS Probe; however, only four of these participants had trials with responses in OOc on No Probe trials. Statistical
analysis of OOc onset data was therefore conducted only on SAS Probe trials using paired samples $t$-test. This test of Intentional Set (ACT vs. DNI) was nonsignificant, $t(13) = .20, p = .84$, indicating that regardless of whether participants prepared to compensate for the perturbation (ACT; 46.1 ms) or not intervene (DNI; 45.6 ms), presentation of a SAS Probe elicited a response in OOc at similar latency.

### 4.3.1.2 Startle response is not required for instruction-dependent M2 modulation

With participants compensating for or not intervening with a large mechanical perturbation for Experiment 4, we also examined modulation of the stretch responses in wrist flexors. See the group ensemble displacement and wrist flexor EMG data in figure 4.3. As expected, no differences of Intentional Set, $F(1,13) = 0.85, p = .37, \eta^2_p = .06$, or Probe Stimulus, $F(1,13) = 2.28, p = .16, \eta^2_p = .15$, were observed during the M1 epoch. This was in contrast to activity during the M2a epoch where we found significant main effects for both Probe Stimulus, $F(1,13) = 18.01, p = .001, \eta^2_p = .58$, and Intentional Set, $F(1,13) = 9.00, p = .010, \eta^2_p = .41$. These findings indicated that both the intention to respond as well as presentation of an unexpected SAS Probe resulted in increased activity during the M2a period (DNI No Probe = 6.2 Normalized Units (NU: value of 1 corresponds to integrated activity during the baseline epoch); ACT No Probe = 8.1 NU; DNI SAS Probe = 8.6 NU; ACT SAS Probe = 11.7 NU). Examining the M2b epoch also revealed main effects of Intentional Set, $F(1,13) = 43.04, p < .001, \eta^2_p = .77$, and Probe Stimulus, $F(1,13) = 28.18, p < .001, \eta^2_p = .68$, but these were superseded by an Intentional Set $\times$ Probe Stimulus interaction, $F(1,13) = 7.68, p = .016, \eta^2_p = .37$. Post hoc analysis showed that although both ACT conditions were significantly larger than the DNI
conditions ($p$ values < .001 for both comparisons), the addition of the SAS Probe produced an even larger difference between the two Intentional Sets (DNI No probe = 3.6 NU; ACT No probe = 10.6 NU; DNI SAS Probe = 6.1 NU; ACT SAS Probe = 16.9 NU). In summary, despite the No probe (perturbation only) conditions having zero incidence of startle, the wrist flexor stretch response data displayed clear M2 modulation between ACT and DNI conditions, suggesting the startle response was not necessary for instruction-dependent modulation of EMG activity during the epochs containing the long-latency stretch response.
Figure 4.3. Experiment 4 group wrist position and wrist flexor EMG data. ACT conditions: solid lines/bars. DNI conditions: dashed lines/bars. No probe: black lines/bars. SAS Probe: grey lines/bars. A. Wrist angle (in degrees). Positive values denote extension and negative values flexion. B. Group integrated EMG data (with inter-participant standard error bars) from the 3 stretch response epochs examined. M1 (25 to 50 ms); M2a (50-75 ms); M2b (75-100 ms). Asterisks denote statistically significant differences ($p < .05$). C. Rectified wrist flexor EMG data, along the same time course as the displacement data presented in panel A. D. Wrist flexor EMG data focused on the time periods of interest (0-200 ms). Reprinted with permission of Elsevier BV.
4.3.1.3 Non-startling participants

Our main analysis was conducted on the fourteen participants that consistently displayed a startle response following a SAS Probe. Typically, we have excluded non-startling participants from our previous StartReact studies (e.g., Forgaard et al., 2013; Stevenson et al., 2014). However, for the present study, it was important to present their data as most studies examining the long-latency stretch response have not recorded EMG data from startle indicators (c.f. Ravichandran et al., 2013), and therefore likely also included data from non-startlers. These participants had a 16.7% incidence of startle on ACT SAS Probe trials, but a 0% incidence on DNI SAS Probe, ACT No Probe, and DNI No Probe. Despite the low incidence of startle across all conditions, the non-startling participants showed evidence of instruction-dependent M2a/M2b period modulation (see figure 4.4). While the M1 epoch values were similar across the conditions (see figure 4.4), both ACT conditions appeared to result in increased activity in the M2a and M2b epochs compared to the DNI conditions. We did not statistically analyze the data (due to the low number of participants), but qualitatively it would appear that non-startling participants also displayed instruction-dependent modulation of the M2 response.
Figure 4.4. Experiment 4 wrist flexor data from 4 non-startling (to a SAS Probe) participants. ACT conditions: solid lines/bars. DNI conditions: dashed lines/bars. No probe: black lines/bars. SAS Probe: grey lines/bars. A. Integrated EMG data (with inter-participant standard error bars) from the 3 stretch response epochs examined. M1 (25 to 50 ms); M2a (50-75 ms); M2b (75-100 ms). B. Rectified wrist flexor EMG data. Reprinted with permission of Elsevier BV.
### 4.3.2 Experiment 5: unexpected perturbations

See Table 2 for mean values and standard deviations from the Experiment 5 conditions.

<table>
<thead>
<tr>
<th>Measure</th>
<th>ACT: Auditory</th>
<th>Small Pert</th>
<th>DNI: Auditory</th>
<th>Small Pert</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAS Probe</td>
<td>Pert Probe</td>
<td>No Probe</td>
<td>SAS Probe</td>
</tr>
<tr>
<td>Startle Incidence (%)</td>
<td>93.1</td>
<td>(14.4)</td>
<td>27.8</td>
<td>(9.8)</td>
</tr>
<tr>
<td>Premotor RT (ms)</td>
<td>136.5</td>
<td>(23.0)</td>
<td>-</td>
<td>88.5</td>
</tr>
<tr>
<td>L-Q0c Incidence (%)</td>
<td>100</td>
<td>(36.7)</td>
<td>62.5</td>
<td>(21.1)</td>
</tr>
<tr>
<td>L-Q0c Latency (ms)</td>
<td>40.0</td>
<td>(5.7)</td>
<td>93.0</td>
<td>(47.0)</td>
</tr>
<tr>
<td>L-SCM Incidence (%)</td>
<td>95.5</td>
<td>(14.4)</td>
<td>80.6</td>
<td>(32.5)</td>
</tr>
<tr>
<td>L-SCM Latency (ms)</td>
<td>86.6</td>
<td>(12.9)</td>
<td>118.5</td>
<td>(32.4)</td>
</tr>
<tr>
<td>R-SCM Incidence (%)</td>
<td>100</td>
<td>(0)</td>
<td>95.8</td>
<td>(13.8)</td>
</tr>
<tr>
<td>R-SCM Latency (ms)</td>
<td>77.6</td>
<td>(17.6)</td>
<td>108.0</td>
<td>(22.1)</td>
</tr>
</tbody>
</table>

**Table 4.2.** Experiment 5 mean values and inter-participant standard deviations (in parentheses). ACT: active conditions; DNI: passive conditions; Auditory: 80 dB imperative signal; Small Pert: 0.5 Nm imperative signal; SAS Probe: 124 dB; Pert Probe: 1.5 Nm perturbation; No probe: imperative signal only. Incidence data reported as a percentage (%) of trials from within a given condition. Premotor RT and latency data are reported in milliseconds (ms). Reprinted with permission of Elsevier BV.
4.3.2.1 Unexpected large perturbations inconsistently elicit startle

Analysis of startle incidence data revealed significant main effects of Intentional Set, $F(1,11) = 48.12, p < 0.001, \eta^2_p = .82$, and Probe Stimulus, $F(2,22) = 103.84, p < .001, \eta^2_p = .90$; however, these main effects were superseded by three separate 2-way interactions (Intentional Set × Imperative Signal, $F(1,11) = 5.28, p = .042, \eta^2_p = .32$; Intentional Set × Probe Stimulus, $F(2,22) = 31.30, p < .001, \eta^2_p = .74$; and Imperative Signal × Probe Stimulus, $F(2,22) = 3.66, p = .043, \eta^2_p = .25$). Simple main effects analysis of the Intentional Set × Probe Stimulus interaction showed that for the SAS Probe ($p < .001$) and Pert Probe ($p = .010$) trials, the ACT conditions had a higher incidence of startle than the DNI conditions. By contrast no differences of Intentional Set were observed on No Probe trials ($p = .34$). Analysis of the Imperative Signal × Probe Stimulus interaction showed that for ACT conditions, all three probe stimuli differed in terms of startle incidence (SAS Probe: 93.8%; Pert Probe: 17.4%; No Probe: 2.8%). By contrast, for DNI conditions, only the SAS Probe (37.5%) resulted in an increased incidence of startle (Pert Probe: 0%; No Probe: 0%). Simple main effects analysis of the Intentional Set × Imperative Signal interaction showed that for the ACT conditions, no significant differences ($p = .13$) were present between the 80 dB (41.3%) or Small Perturbation (34.7%) imperative signals. However, for the DNI conditions, there was a nonsignificant ($p = .069$) trend for trials from the Small Perturbation condition to have an increased startle incidence (14.8% vs. 10.2%). In summary, when participants performed in ACT conditions to an 80 dB tone or a Small Perturbation, unexpected presentation of a Pert Probe (large perturbation) elicited a startle response on a small proportion (17.4%) of trials. Therefore, while an unexpected large perturbation can be startling, the incidence was significantly lower than trials where a SAS Probe (93.8%) was presented.
Figure 4.5. Averaged startle indicator data from probe trials for a participant in the ACT 80 dB Imperative Signal condition of Experiment 5. SAS Probe: grey lines; Pert Probe: black lines. Note how the SAS Probe elicited early and large bursts in startle indicator muscles. By contrast, the Pert Probe elicited responses that were delayed (by ~25 ms) and were of smaller amplitude. Reprinted with permission of Elsevier BV.

While the SAS Probes consistently elicited clear responses in OOc and SCM, the activity in startle indicator muscles was not as apparent on the ACT Pert Probe trials classified as displaying a startle response. The startle-like activity was only observed from six participants and even amongst those participants, startle responses were not consistently observed across all trials. Figure 4.5 shows sample ensemble data for the 80 dB IS condition, comparing the Pert Probe trials (black traces) to the SAS Probe trials (grey traces). This sample participant showed the highest incidence of startle following the Pert Probe (83.3%) and while not representative of the majority of trials from the Pert Probe condition, it does display an example of a positive startle response following a perturbation. Compare this to the ensemble SAS Probe data from the
same participant (figure 4.5; grey traces). Note how activity in OOC and left/right SCM was smaller and delayed on Pert Probe trials, as compared to the large bursts elicited by the SAS Probe.

4.3.2.2 Probe stimuli elicit a preplanned response early

Exemplar ensemble wrist flexor EMG data from ACT 80 dB IS conditions are shown in figure 4.6. Presentation of the SAS Probe reduced premotor RT from 136.5 (on No Probe trials) to 88.5 ms, $t(11) = 9.90, p < .001$, confirming an auditory StartReact effect. Due to M2 preceding the voluntary response on Pert Probe trials, we could not directly determine RT; however inspection of the ensemble EMG profiles for many of the participants showed no clear demarcation between M2 and the voluntary response, suggesting RTs below 100 ms (e.g., see figure 4.6, dashed black profile).

Figure 4.6. Experiment 5 ensemble wrist flexor EMG data from an exemplar participant. No probe: solid black line. SAS Probe: solid grey line. Pert Probe: dashed black line. Onset of wrist flexor activity was ~125 ms on No probe trials. Presentation of a SAS Probe hastened response onset by ~50 ms. On Pert Probe trials, the perturbation elicited short (M1) and long (M2) stretch responses. The voluntary response appeared continuous with M2, suggesting RTs of less than 100 ms following the Pert Probe. Reprinted with permission of Elsevier BV.
4.3.3 Interaction between a SAS and a mechanical perturbation

An unexpected finding from Experiment 4 was that pairing a SAS Probe with a large wrist perturbation produced increased activity in the wrist flexor EMG data (specifically during the M2a and M2b epochs; see figure 4.3). This occurred for both the ACT and DNI conditions, suggesting it resulted from activation of startle circuitry, but not necessarily the StartReact effect (no voluntary response was preplanned during the DNI conditions). We were interested whether this increased activity stemmed from the SAS Probe and the perturbation independently influencing wrist flexor motoneurons, or whether the two stimuli engaged common circuitry. If circuitry involved in generation of M2 was independent of circuitry activated by a SAS, presentation of the two stimuli together should yield a response that was similar to the linear sum of the two stimuli presented in isolation (i.e., SAS Probe = No Probe + SAS Probe alone). However, if the two stimuli activated overlapping circuitry, we may observe a supralinear response (SAS Probe > No Probe + SAS Probe alone). This logic is similar to studies showing that a perturbation and (appropriately timed) transcranial magnetic stimuli (TMS) activate common circuitry in primary motor cortex (Day et al., 1991; Lewis et al., 2004; Palmer & Ashby, 1992; Pruszynski, Kurtzer, Nashed, et al., 2011). In order to make the necessary comparisons we used data from Experiments 4 and 5. The No probe (DNI No Probe; ACT No Probe) and SAS Probe (DNI SAS Probe; ACT SAS Probe) conditions were taken from Experiment 4 while the SAS Probe alone condition was from Experiment 5 and represented the following subtraction (DNI 80 dB SAS Probe - DNI 80 dB No Probe). This isolated any startle related activation of wrist flexors, in the absence of a preplanned voluntary response. Integrated EMG data from the M1, M2a, and M2b epochs were normalized to the baseline epoch for each condition (similar to our main analysis above). SAS Probe data from each epoch were then
normalized again to the respective No Probe + SAS Probe alone conditions (Normalized Value = \(\frac{\text{SAS Probe}}{\text{(No Probe + SAS Probe alone)}}\)). A one-sample \(t\)-test was used to determine whether the normalized data significantly differed from a value of 1. Any supra-linearity was represented by a value larger than 1.

Presentation of a SAS Probe simultaneous with a large mechanical perturbation did not significantly influence activity during the M1 epoch, above the predicted linear sum of the two stimuli presented in isolation (see statistical results in figure 4.7). However, for both ACT and DNI conditions, presenting a SAS Probe with the perturbation produced a supralinear response during the M2a and M2b epochs suggesting the two stimuli engaged overlapping circuitry contributing to the long-latency stretch response. A potential location for this interaction is discussed below.
Figure 4.7. SAS Probe and a perturbation engage common M2 circuitry. Linear prediction: (Experiment 4 No probe condition) + (SAS Probe alone). SAS Probe alone = (Experiment 5 DNI 80 dB SAS Probe) – (Experiment 5 DNI 80 dB No Probe). Observed Supralinear Interaction: (Experiment 4 SAS Probe condition). A. ACT conditions wrist flexor EMG. B. DNI conditions wrist flexor EMG. Panel C: Supralinear interaction data normalized to linear prediction (with standard error bars) for the M1, M2a, and M2b epochs. One sample t-test used to determine whether activity was significantly greater than the linear prediction. Reprinted with permission of Elsevier BV.
4.4 Discussion

It is well known that the presentation of a loud (>120 dB) auditory stimulus can elicit a startle response (e.g., Brown et al., 1991). Used as a valuable tool in the study of motor preparation, startle-related activation can also initiate a preplanned voluntary response at shortened latency (for reviews see Carlsen et al., 2012; Nonnekes et al., 2015; Rothwell, 2006; Valls-Solé et al., 2008). A separate body of literature has shown that mechanical perturbations applied to the upper limbs produce short (M1; 25-50 ms) and long-latency (M2; 50-100 ms) responses in the stretched musculature (see Pruszynski & Scott, 2012). When participants prepare a motor response to compensate for the perturbation, the M1 response is usually unaffected, whereas activity during the M2 epoch increases. Recently, it was suggested that the mechanical perturbations used to produce M1 and M2, also elicit a startle response (Ravichandran et al., 2013). The implication is that the StartReact effect (i.e., startle triggered hastening of the preplanned voluntary response) may be responsible for instruction-dependent modulation of EMG activity during the M2 epoch. The present study tested whether large mechanical perturbations applied to the wrist are capable of eliciting a startle response and whether the StartReact effect underlies M2 modulation.

4.4.1 Long-latency stretch response

The design of experiment 4 was similar to a majority of studies that have examined the long-latency stretch response (e.g., Calancie & Bawa, 1985; Hammond, 1956; Manning et al., 2012; Pruszynski et al., 2008; Rothwell et al., 1980). Participants were instructed to either not intervene (DNI) or compensate (ACT) for a large perturbation that was delivered as the imperative signal. The main finding was that on the control (No Probe) trials, for both the ACT and DNI conditions, we observed no incidence of the startle response. Despite the lack of startle,
EMG activity during the M2a and M2b epochs was increased on ACT trials (see figure 4.3). In other words, because the expected perturbation was not startling, the instruction-dependent M2 modulation observed in the wrist flexor data could not have resulted from the startle triggered release of a pre-planned voluntary response.

A long-standing debate in the stretch response literature is whether goal-dependent modulation during the M2 epochs is caused by excitability changes to the underlying neural circuitry or from superimposition of the preplanned voluntary response onto M2. The reason the issue remains unresolved is that RT, or onset latency of the voluntary response cannot be accurately determined when M2 and the voluntary response occur in the same muscle (for more detail see Forgaard et al., 2015; Manning et al., 2012). Studies have instead relied on indirect estimates of RT, obtaining values that range from ~70 ms (e.g., Crago et al., 1976; Evarts & Granit, 1976; Evarts & Vaughn, 1978; Manning et al., 2012; Ravichandran et al., 2013) to over 100 ms (e.g., Hammond 1956; Pruszynski et al., 2008). In experiment 4, we found modulation of wrist flexor EMG data beginning around 50 ms (see figure 4.3), a latency that was 20 ms shorter than the earliest reported voluntary RTs. Similar to studies that have observed M2 modulation at sub-RT latency (e.g., MacKinnon et al., 2000; Pruszynski et al., 2008), we can attribute the M2a increase to a facilitation of the underlying neural circuitry. M2b activity was also larger on ACT trials, however because voluntary response onset could not be distinguished from the end of M2, we could not determine whether this was due to superimposition of the voluntary response, instruction-dependent modulation of M2 circuitry, or a combination of the two mechanisms (Forgaard et al., 2015).

Recent investigations have demonstrated that goal-dependent M2 modulation is more sophisticated than a simple on-off switch between active and passive conditions. For instance,
EMG activity during the M2 epochs was shown to scale with intended movement distance (Pruszynski et al., 2008) or urgency (Crevecour et al., 2013), and accounted for the mechanical interaction between the shoulder and elbow (Kurtzer et al., 2008; Pruszynski et al., 2011) or shoulder, elbow and wrist (Weiler et al., 2015). Further exemplifying this capacity, M2 activity was also found to modulate appropriately to assist in obstacle avoidance during a reaching task (Nashed et al., 2014) and was flexibly routed across limbs in a bimanual coordination paradigm when both arms had a shared goal (Dimitriou et al., 2012; Mutha & Sainburg, 2009; Omrani et al., 2013). Although our study was not designed to examine the sophistication of goal-dependent M2 modulation, it is important to mention that the modulation observed by these studies was likely not produced by superimposition of a preplanned voluntary response. Critically, this flexibility and modulation occurred without advance knowledge of perturbation direction and therefore a voluntary response could not have been preplanned prior to perturbation delivery. Work on the auditory StartReact effect has shown that a SAS Probe does not hasten voluntary response onset in choice RT paradigms when a response is not prepared in advance of the imperative signal (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004a). Therefore, even if the perturbations used by the studies cited above were startling, it is unlikely that a startle hastened voluntary response was responsible for the observed M2 period modulation.

As mentioned in the Introduction, the long-latency stretch response is not a discrete process produced by a single reflex pathway, rather it reflects the output of multiple spinal (Hagbarth et al., 1981; Matthews, 1984; Lourenço et al., 2006) and supra-spinal mechanisms (e.g., Cheney & Fetz, 1984; Evarts & Tanji, 1976; Lourenço et al., 2006; MacKinnon et al., 2000; Omrani et al., 2014; Pruszynski et al., 2011; Pruszynski et al., 2014; Ravichandran et al., 2013). Early work suggested a stronger cortical influence for distal compared to proximal arm
muscles (Ghez & Shinoda, 1978; Tatton, North, Bruce, & Bedingham, 1983), but recent studies have also implicated robust cortical contributions for M2 in elbow and shoulder musculature (e.g., Omrani et al., 2014; Pruszynski et al., 2011; Pruszynski et al., 2014). Primary motor cortex has been highlighted as an important location for goal-dependent modulation. For instance, many motor cortex neurons showed stronger perturbation related response when the animal had to move against the direction of a perturbation versus letting their hand be moved into a target and this occurred at an appropriate latency to account for the M2 modulation observed in EMG recordings (Pruszynski et al., 2014; also see Evarts & Tanji, 1976). Goal-dependent modulation has also been observed in the cerebellum, specifically neurons of the dentate nucleus (Strick, 1983). In paradigms where the voluntary response was preplanned, the M2b epoch was also influenced by triggering of the preplanned voluntary response (e.g., Forgaard et al., 2015; Manning et al., 2012; Ravichandran et al., 2013) which may have involved cortical (Alibiglou & MacKinnon, 2012; Stevenson et al., 2014) and/or brainstem (Ravichandran et al., 2013; Shemmell, 2015) contributions (for more detail see next section). In summary, comparable to the generation of the long-latency stretch response, evidence suggests that goal-dependent modulation may involve the interaction of multiple neural structures; the relative contributions of each may differ between tasks and joints studied.

### 4.4.2 StartReact effect

Three alternative mechanisms have been proposed to underlie the auditory StartReact effect. The first is that it is an extreme example of a stimulus intensity effect. However, this hypothesis has been largely rejected in the literature, beginning with the work by Valls-Solé and colleagues in the 1990’s showing that presentation of a SAS Probe could elicit a preplanned response at latencies shorter than the minimal amount of time needed for cortical processing of
the auditory stimulus (Valls-Solé et al., 1999). Moreover, it was shown that irrespective of the intensity of the stimulus, the presence of a startle response (SCM and OOc activation) resulted in a RT that was consistently short (~80 ms; Carlsen et al., 2007). By contrast, trials where the startle response was absent (no indicators present or OOc only) showed stimulus intensity effects, whereby RT was progressively reduced as intensity of the stimulus increased (Carlsen et al., 2007). To account for the reliably short-latency RTs on trials with a startle response, it was suggested that in the preparation of movement, sufficient details of the required motor commands were stored subcortically (likely in reticular formation). Presentation of the SAS Probe would activate startle circuitry and initiate the subcortically stored motor commands early, via the reticulo-spinal tract.

While it is imperative to successfully elicit a startle response when examining the auditory StartReact effect, recent work has shown that the circuitry involved in StartReact may be separate from the startle response. For instance, a prepulse stimulus has been shown to attenuate the startle response but not influence the short-latency RTs produced by the SAS Probe (Maslovat, Kennedy, Forgaard, Chua, & Franks, 2012; Valls-Solé, Kofler, Kumru, Castellote, & Sanegre, 2005). By contrast, an appropriately timed TMS induced silent period, over primary motor cortex, delayed the StartReact effect, but did not influence the startle response (Alibiglou & MacKinnon, 2012; Stevenson et al., 2014). This recent evidence has led to the hypothesis that preplanned motor commands may be stored cortically, with ascending activation from startle circuitry in the reticular formation traveling an ascending reticulo-thalamo-cortical route (Alibiglou & MacKinnon, 2012; Carlsen et al., 2012; Stevenson et al., 2014). In this cortical StartReact model, the preplanned response is triggered at the level of motor cortex and travels to the appropriate spinal level via the cortico-spinal tract.
Despite the StartReact effect being most reliably observed on trials displaying a positive startle response, a recent study also reported StartReact-like RTs (<100 ms) on a small proportion of trials where a startle response was not present (Maslovat, Franks, Leguerrier, & Carlsen, 2015). These authors showed that on SAS Probe trials with a startle response, triggering of the prepared response was likely to have occurred, but on SAS Probe trials without a startle response, the prepared response might have been triggered early (by the SAS) but on most trials it was voluntarily initiated (at longer latency) to the imperative signal. This finding has important implications for initiation of the voluntary response following a perturbation. Although our experiment did not allow for a determination of voluntary response latency, previous studies with similar designs have shown that when a voluntary response is preplanned, presentation of a perturbation imperative signal can elicit sub-100 ms RTs (i.e., StartReact-like latencies; e.g., Evarts & Granit, 1976; Evarts & Vaughn, 1978; MacKinnon et al., 2000; Manning et al., 2012). It is therefore plausible that a mechanical perturbation may have access to circuitry underlying the StartReact effect, such that it can trigger the preplanned voluntary response at short-latency, even in the absence of a startle response. This issue is explored further in chapter 5.

4.4.3 Perturbation and a SAS engage common circuitry

As hypothesized for Experiment 4, the SAS Probe consistently elicited a clear startle response in both the ACT and DNI conditions and an expected perturbation was not startling (figure 4.1). An unanticipated finding was that the SAS Probe also influenced the stretch response data. Like the effect of intentional set, activity during the M1 epoch was not altered by the SAS, and activity during the M2a and M2b epochs increased (see figure 4.2 and figure 4.7). Moreover, the observed increase in the M2 epochs was greater than the linear sum of the SAS Probe and the perturbation presented separately. This finding was comparable to the increased
M2 activity observed by studies that have applied TMS over primary motor cortex while the afferent signal from the perturbation traversed motor cortex (Day et al., 1991; Lewis et al., 2004; Palmer & Ashby, 1992; Pruszynski, Kurtzer, Nashed, et al., 2011) and suggests that the SAS Probe and the perturbation engaged overlapping circuitry. Activation of startle circuitry in the reticular formation has been shown to influence the excitability of primary motor cortex 30-60 ms after onset of a SAS (Furubayashi et al., 2000; Ilic et al., 2011; Kühn, Sharott, Trottenberg, Kupsch, & Brown, 2004). Along a similar time-course, the somatosensory potential from the stretch of wrist flexors reaches motor cortex ~30 ms after onset of a perturbation (MacKinnon et al., 2000). Thus the timing is appropriate when both a wrist perturbation and SAS are delivered simultaneously; both stimuli reach primary motor cortex within approximately 30 ms. With the conduction time from motor cortex to forearm muscles estimated at ~20 ms (Pascual-Leone, Valls-Solé, Wassermann, & Hallett, 1994), the interaction of startle-related and transcortical M2 activity, in motor cortex, may account for the larger M2 observed on SAS Probe trials.

4.4.4 Perturbation startle response

Whereas the large perturbation used as an imperative signal in Experiment 4 was not startling, Experiment 5 was designed to investigate whether unexpected upper limb perturbations can elicit startle (similar to Ravichandran et al., 2013). Participants performed ACT and DNI conditions in response to a non-startling auditory stimulus or a small wrist perturbation. Comparable to a SAS Probe in an auditory StartReact paradigm (e.g., Alibiglou and MacKinnon, 2012; Forgaard et al., 2013; Ravichandran et al., 2013; Stevenson et al., 2014), the large perturbation was unexpectedly delivered as a probe stimulus. While the SAS Probe reliably elicited startle, we found the Pert Probe to elicit startle-like activity on only 17.4% of ACT trials and 0% of DNI trials. However, even on the small number of ACT trials where the Pert Probe
was startling, EMG activity in startle indicators was of later onset and smaller amplitude (compared to a SAS Probe; see figure 4.5). Ravichandran et al. (2013) also reported delayed SCM onsets following a Pert Probe and inspection of their ensemble EMG figures showed a smaller left SCM burst compared to a SAS Probe. Although direct comparisons were not made to a SAS Probe, the somatosensory startle response (OOc, Masseter, and SCM) reported by Álvarez-Blanco et al. (2009) was also delayed compared to previous reports. Similarly, Campbell et al. (2013) and Oude-Nijuis et al. (2010) observed later SCM onsets following an ankle perturbation. It was proposed that while a SAS Probe results in highly synchronized input onto common startle circuitry in the reticular formation (caudal pontine reticular nucleus; nRPC), a mechanical perturbation or somatosensory stimulus produces a delayed and more dispersed input onto the startle circuitry (Oude-Nijuis et al., 2010). Because the giant neurons of the nRPC are not modality specific but rather are sensitive to the magnitude of input, even when a mechanical perturbation is startling, the input may be more comparable to a less intense auditory stimulus. Therefore, we confirm the report by Ravichandran et al. (2013) that an unexpected perturbation applied to the upper limb can elicit a startle response. However, while Ravichandran and colleagues reported a high incidence of startle (~70%) following an elbow perturbation, we found a lower incidence when the perturbation was delivered to the wrist.

In addition to perturbations being applied to different joints, other possibilities may account for the higher startle incidence reported by Ravichandran et al. (2013. One potential may be the different types of perturbations used. Ravichandran and colleagues used a servo perturbation, moving the elbow through a predefined range in a given period of time. This produced a very quick acceleration profile and elicited a much larger M1 response compared to the torque perturbations used by ourselves and other authors (e.g., MacKinnon et al., 2000;
Manning et al., 2012; Pruszynski et al., 2008). Therefore the servo perturbations employed by Ravichandran may have been more intense and thus more likely to elicit startle.

Another important difference was the criteria used to classify the startle response. Ravichandran et al. (2013) recorded from left SCM and defined startle as activity <120 ms after stimulus onset. We were concerned that because SCM is also involved in postural control of the head during rapid upper limb movements (Carlsen et al., 2004b; for more detail see Methods), trials with postural SCM under 120 ms could be classified as displaying startle. To distinguish between postural and startle-related SCM, we also recorded from OOc, a muscle that is involved in the startle response, but not activated as part of a postural response (Carlsen et al., 2004b). We reasoned that if a perturbation was startling, OOc would be activated first, followed by bilateral bursts in the left and right SCM. In Experiment 4, OOc activity was observed only on ~20% of ACT No Probe trials, but nearly always at a latency >120 ms. SCM activity was observed on a higher proportion of trials (58%), and the mean onset latency was ~130 ms. If we had not recorded from OOc, nearly half of the ACT No Probe trials from Experiment 4 would have been classified as displaying a startle response. In Experiment 5, the ACT No Probe trials also showed a ~50% incidence of activity in the SCM muscles but the latency was later and typically well past the 120 ms startle cut-off time (see Table 4.2). By contrast, the Pert Probe trials displayed SCM activity on >60% of trials, with a mean onset of ~113 ms for Auditory IS and ~135 ms for Small Pert IS. As mentioned above, only 17.4% of these trials displayed a true startle response, so had we only recorded from SCM, a large proportion of trials with postural SCM would have been classified as startle. It does appear therefore that while large perturbations applied to the wrist may elicit a startle response on some trials, the majority of activity we observed in SCM was the result of a postural response. Since a somatosensory startle response involves smaller
and delayed muscle activation compared to a SAS Probe, and because postural SCM activity can occur early following a mechanical perturbation, we believe it is important to record from more than just SCM when examining the startle response to a perturbation.

4.4.5 Conclusion

The main finding from the present chapter is that while unexpected wrist perturbations did elicit startle-like activity on a small proportion of ACT trials (Experiment 5), expected perturbations (Experiment 4) never produced a startle response. Despite the absence of startle following an expected large perturbation, we observed clear modulation of EMG activity during the M2a and M2b epochs, suggesting that startle hastening of a pre-planned voluntary response was not the primary source of instruction-dependent modulation. An unexpected finding was that for both passive and active conditions, simultaneous presentation of a SAS Probe with a perturbation produced increased activity in the M2 epochs. This interaction provides evidence that a SAS and a perturbation may engage overlapping neural circuitry, possibly in primary motor cortex. Finally, these two experiments highlighted an important distinction between postural activation in startle indicators as compared to reflexive activation as part of the startle response.

4.5 Bridging summary

The experiments conducted in chapter 4 provided clear evidence that instruction-dependent M2 modulation can occur in the absence of the StartReact effect. Despite the low incidence of startle following a wrist perturbation, considerable activity remained in SCM muscles on ACT trials, the onset of which was near the end of the startle time criteria. We reasoned that this was a result of a postural response but because SCM is used as the most reliable indicator of startle (in the context of the StartReact effect); further work was needed to
confirm that this perturbation-evoked SCM activity was not the result of a startle response. In Experiment 6, a prepulse inhibition stimulus, which is known to attenuate the startle response (but was not expected to influence postural activity), was employed in a perturbation paradigm to confirm whether SCM activity was part of a postural response or the startle response.

The first five experiments conducted in this dissertation provided evidence that instruction-dependent M2 modulation is not produced entirely through a superimposed voluntary response. However, given that all active conditions involved the voluntary response appearing in the same muscle as M2, we were unable to dissociate the two responses and determine an exact onset latency of the voluntary response. It remains uncertain whether RT of the voluntary response following a non-startling mechanical perturbation stimulus is similar to a voluntary response elicited by the auditory StartReact effect. In Experiment 7, participants performed wrist extension responses to a wrist extension perturbation. M1 and M2 occurred in wrist flexors and the voluntary response appeared in wrist extensors (allowing onset latency to be determined on a trial-by-trial basis). To make direct comparisons with the auditory StartReact effect, on some trials a SAS Probe was presented simultaneous with (or in lieu of) the perturbation.

For chapter 5, the nomenclature for different conditions was changed slightly. In the previous chapters, the active conditions involving a compensatory wrist flexion response were referred to as ACT. In chapter 5, we included perturbation assist responses involving wrist extension responses. To avoid confusion, ACT was replaced with FLEX. The assist responses were named EXTEND.
Chapter 5: Experiments 6 & 7

Mechanical perturbations can elicit triggered reactions in the absence of a startle response

5.1 Introduction

Human motor control spans a continuum between reflexive and voluntary actions, but a clear demarcation between what constitutes a “reflex” versus a “voluntary” response has been debated for many years (see Prochazka et al., 2000, for a review). Adding to the complexity, a bidirectional relationship exists between some reflexive and voluntary responses. This interaction between reflexive and volitional contribution is exemplified in the muscular activity elicited when the upper-limb is perturbed. When sufficient stretch of muscle occurs, short- (M1: <50 ms) and long-latency (M2: ~50-100 ms) responses are observed in the electromyographic (EMG) recording of lengthened musculature (Hammond, 1956). Both responses are sensitive to peripheral factors such as background state of the motoneuron pool and magnitude of the perturbation (Pruszynski et al., 2009), but the M2 response shows the remarkable feature of also modulating based on the voluntary intention of the participant (e.g., Hammond, 1956; Lee & Tatton, 1975; MacKinnon et al., 2000; Pruszynski et al., 2008). For example, when participants are instructed to “resist” or “compensate” against the perturbation, the M2 response appears large and continuous with a third (voluntary) response. By contrast, the instruction to “let-go” or “not-intervene” with the perturbation results in a reduced M2 and no voluntary response. It has been proposed that the M2 response displays instruction/goal-dependent modulation because the perturbation engages overlapping neural circuitry with the generation of voluntary activity (Scott, 2004; Pruszynski & Scott, 2012).

The elicitation of stretch responses can also influence the onset latency of the voluntary response. Voluntary RT to auditory or visual signals is typically >120 ms (Forgaard et al., 2015; Jaeger et al., 1982); however the mechanical perturbations used to elicit stretch responses can initiate the voluntary response at a reduced latency (<100 ms) such that it overlaps the M2
response (Crago et al., 1976; Evarts & Granit, 1976; Evarts & Vaughn, 1978). The early voluntary response following a perturbation causing muscle stretch has been called a “triggered reaction” (e.g., Crago et al., 1976; Houk, 1978; Manning et al., 2012). It remains a matter of contention whether the appearance of goal-dependent M2 modulation arises from excitability changes of the underlying circuitry (Hammond, 1956; Lee & Tatton, 1975; Pruszynski et al., 2008) or from superimposition of the voluntary response onto the M2 response (Crago et al., 1976; Rothwell et al., 1980; Manning et al., 2012; Ravichandran et al., 2013). Indeed, distinguishing between these alternatives has proven problematic because most studies have used tasks where the voluntary response occurred in the same muscle as M2, and thus resolving when the voluntary response begins to make contributions to the appearance of M2 modulation has been difficult to determine (see Forgaard et al., 2015).

Another example of how reflexive and voluntary actions interact can be seen when an individual is startled. The generalized startle response is a diffuse primarily upper body reaction following presentation of sufficiently intense stimuli. It begins with closure of the eyes followed by contraction of the neck, trunk, and arm muscles (Brown et al., 1991; Carlsen et al., 2011; Landis & Hunt, 1939). A number of studies over the past 20 years have investigated the effects of a startling auditory stimulus (SAS) on a preplanned action. When a voluntary response is pre-programmed and the SAS elicits a startle response, the intended action is initiated at a latency usually less than 100 ms. This phenomenon has been termed the StartReact effect (for reviews see Rothwell, 2006; Valls-Solé et al., 2008; Carlsen et al., 2012; Nonnekes et al., 2015). Despite the early elicitation of the response, EMG and kinematic characteristics of the intended response remain relatively unchanged when compared to non-SAS trials (Valls-Solé et al., 1999; Carlsen et al., 2004b). Although the exact mechanism underlying the StartReact effect remains a matter
of debate (see Discussion), it is generally accepted that neural activation from circuitry involved in the startle response acts to hasten the voluntary response (Valls-Solé et al., 2008; Carlsen et al., 2012; Nonnekes et al., 2015).

Investigations of the StartReact effect have shown that activation in sternocleidomastoid (SCM; within 120 ms of the SAS) is the best indicator that a startle response has occurred (Carlsen et al., 2007; Maslovat et al., 2015). For example, trials with a startle response in SCM show consistently short RTs (~80 ms) irrespective of intensity of the SAS, whereas trials with no activity in any startle indicators or activity in only orbicularis oculi (OOc) display a stimulus intensity effect, in which RT decreases as sound intensity increases (Carlsen et al., 2007). OOc is also activated as part of an auditory blink reflex (Brown et al., 1991) which is produced from separate circuitry from the startle response and is not implicated in the StartReact effect (Carlsen et al., 2007). It is important to note that almost all trials with true startle-related SCM activity are also accompanied by a response in OOc (Carlsen et al., 2007; Forgaard et al., 2015; Forgaard, Franks, Maslovat, Gowan, et al., 2016).

While the StartReact effect has been consistently found when using a SAS, it has been suggested that mechanical perturbations can also act as a stimulus that evokes a startle response and hence a possible initiator of the StartReact effect (Forgaard, Franks, Maslovat, Gowan, et al., 2016; Ravichandran et al., 2013). However, a majority of these studies that have examined triggered reactions and instruction-dependent M2 modulation in the upper-limb have used expected perturbations (e.g., Lee & Tatton, 1975; Crago et al., 1976; Rothwell et al., 1980; Pruszynski et al., 2008; Manning et al., 2012; Forgaard et al., 2015). We have recently shown that while expected mechanical perturbations do elicit activity in SCM within the startle time criteria (<120 ms), this was not accompanied by an early response in OOc and therefore likely
not a true startle response (Forgaard, Franks, Maslovat, Gowan, et al., 2016). Instead we suggested that the SCM activity was a result of postural responses associated with head/neck stabilization during the performance of a ballistic upper-limb movement (see also Dean & Baker, 2017). This calls into question using SCM as the sole indicator of startle when delivering upper-limb mechanical perturbations.

The purpose of the present chapter was twofold: (1) we sought to determine whether the SCM activity evoked by a mechanical perturbation is produced from the reflexive startle response or postural control: and (2) whether a startle response is a prerequisite for the early (<100 ms) elicitation of a voluntary response following a perturbation imperative signal. One way in which postural- and startle-related SCM activity can be dissociated is through the use of a prepulse inhibition (PPI) stimulus. PPI is the inhibitory influence of a non-startling sensory stimulus on the reflexive startle response, the effects of which are typically maximal when presented ~100 ms prior to a startling stimulus (Maslovat, Kennedy, et al., 2012; Valls-Solé et al., 2005; Yeomans, Lee, Yeomans, Steidl, & Li, 2006). In Experiment 6, we employed a PPI stimulus (80 dB auditory signal) in a perturbation paradigm to examine whether perturbation-evoked SCM activity results from the elicitation of a startle response, or is instead a result of postural stabilization. Participants were instructed to perform a ballistic wrist flexion response against a wrist extension perturbation (Lee & Tatton, 1975; MacKinnon et al., 2000; Manning et al., 2012; Forgaard et al., 2015). On random trials, a PPI stimulus was delivered 100 ms prior to the perturbation. If the perturbation-evoked SCM activity is the result of startle, we hypothesized that PPI would attenuate SCM responses (similar to auditory startle findings). By contrast, the PPI stimulus was not expected to have an inhibitory influence on SCM if it is activated as part of its postural role in stabilizing the head/neck.
Experiment 7 investigated whether a startle response is required for a perturbation to elicit the voluntary response at <100 ms. One confound when participants perform a movement in opposition to a perturbation (i.e., compensate/resist task) is that RT cannot be determined because the voluntary response occurs in the same muscle and over a similar time-course as the M2 response (Hammond, 1956; Manning et al., 2012; Forgaard et al., 2015). In order to overcome this, in Experiment 7 participants performed wrist extension movements following a wrist extension perturbation (i.e., assist task; Jaeger et al., 1982; MacKinnon et al., 2000; Miscio et al., 2001). Similar to Experiment 6, the perturbation elicited stretch responses (M1 and M2) in wrist flexors. Moving the voluntary response to wrist extensors allowed RT to be determined on a trial-by-trial basis because the voluntary response was not preceded by stretch responses in the same muscle. To permit startle incidence and RT comparisons with the StartReact effect, on random trials the perturbation was either replaced with a SAS Probe, or was accompanied by a SAS Probe. In order to obtain a high percentage of startle responses, intensity level of the startle probe is usually >120 dB (e.g., Valls-Solé et al., 1999; Carlsen et al., 2011). However in Experiment 7 we specifically used a less intense, 115 dB SAS, to obtain a distribution of trials both with and without a startle response (Carlsen et al., 2007; Carlsen et al., 2009). This procedure also allowed us to obtain two RT distributions on SAS trials; one which was consistently short (<100 ms) and was associated with startle, and another which was longer (>100 ms) and not accompanied by a startle response (Maslovat et al., 2015). Of primary interest was the distribution of RTs obtained on the control (perturbation only) trials. If a voluntary response can be elicited at latencies <100 ms only in the presence of a startle response, we hypothesized that these early RTs would only be observed on the trials where participants were startled. By contrast, if a mechanical perturbation can elicit the voluntary response at <100 ms
even in the absence of startle, we reasoned that the pre-programmed voluntary response would consistently be elicited early.

5.2 Methods

5.2.1 Participants

A total of twenty (11 female, 9 male, aged 20-27 years) healthy right-handed volunteers participated in these experiments. Fourteen participants were tested in Experiment 6 and thirteen were tested in Experiment 7. Six participants completed both Experiments (testing sessions were separated by a minimum of 2 weeks). All protocols were approved by the University of British Columbia Behavioural Research Ethics Board and conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from each participant prior to testing.

5.2.2 General setup and procedures

The experiments took place in an acoustically-dampened chamber. Participants sat on a height-adjustable seat and were positioned with the right arm in a manipulandum that constrained movement to flexion/extension of the wrist. The right elbow was flexed at 100 degrees and the hand was semi-pronated with the wrist joint aligned to the rotational axis of the manipulandum. Connected to the manipulandum was an Aeroflex torque motor (TQ 82W-1C). A metal handle adjoined to the motor shaft was placed near the right metacarpophalangeal joints and lateral wrist movements were prevented by padded stops. Continuous position feedback of the right wrist was provided on an oscilloscope placed 0.5 m in front of the participant. An LED lightbox was placed on top of the oscilloscope and a loudspeaker positioned 30 cm behind the participants’ ears. Each trial started with an auditory warning signal (80 decibels, 50 ms, 500 Hz) and an extension preload increased over 500 ms to 0.25 newton metres (Nm). Participants were instructed to “resist by lightly activating wrist flexors against the load and to hold their wrist at
the home position”. An evenly distributed random foreperiod (2,500-3,500 ms) followed the warning signal and was terminated by an imperative signal (which differed based on the experiment, see below). Following the imperative signal, the preload level of extension torque (0.25 Nm) was maintained for 1000 ms.

5.2.3 Experiment 6

For Experiment 6, thermoplastic casting surrounded each participants’ right hand such that wrist movement could occur without having to grasp onto the handle. We have previously used this setup for stretch response studies as it allows the wrist to move with minimal activation of hand musculature (Forgaard et al., 2015; Forgaard, Franks, Maslovat, Gowan, et al., 2016). The home position was 0 degrees of wrist flexion and visually marked on the oscilloscope. Participants were instructed to “flex their wrist as fast as possible following onset of the imperative signal”. On some random trials a PPI stimulus (80 dB, 50 ms, 1000 Hz) was presented prior to the imperative signal but participants were instructed that “this auditory stimulus was irrelevant to the task”. This experiment consisted of 2 protocols, one (Experiment 6A) to replicate the effects of PPI on the auditory startle response and StartReact effect (Maslovat, Kennedy, et al., 2012; Valls-Solé et al., 2005) and a second (Experiment 6B) to examine the effects of the same PPI stimulus on perturbation-evoked SCM activity. Protocol order was counterbalanced between the 14 participants.

In Experiment 6A, the imperative signal was a green LED light appearing for 150 ms. On 10% of trials, a SAS (120 dB, 50 ms, 1000 Hz) was presented simultaneous with the LED (condition name: SAS). On another 10% of trials, a PPI stimulus was presented 100 ms before the SAS (PPI-SAS). An additional 10% of trials had a PPI stimulus 100 ms before the imperative signal (PPI-Control) and a final 10% of trials had a PPI stimulus but the imperative signal was
withheld (PPI-Catch). Prior to testing, participants were given 20 practice trials for Control, PPI-Control, and PPI-Catch. Within the testing block (1 block of 50 trials), trial order was randomized with the stipulation that no trials with a SAS occurred within the first 3 trials, nor were any presented on consecutive trials.

For Experiment 6B, the imperative signal was a wrist extension perturbation (1.5 Nm, 150 ms). On 40% of trials, only the imperative signal was presented (Flex). On another 40% of trials, a PPI was presented 100 ms before the imperative signal (PPI-Flex). The remaining 20% of trials consisted of a PPI stimulus but no imperative signal (PPI-Catch). Trial order was fully randomized. Practice involved 20 trials and the testing phase consisted of 2 blocks of 40 trials. To prevent fatigue, 2 minutes of rest was provided between blocks.

5.2.4 Experiment 7

For the second experiment, participants were instructed to grasp onto the handle connected to the motor shaft. The home position for each trial was 10 degrees of wrist flexion and defined on the oscilloscope. The imperative signal was a wrist extension perturbation (1.5 Nm, 50 ms). Pilot testing determined that it was easier to respond with an extension response to a shorter perturbation (50 ms vs 150 ms). The longer perturbation used in Experiment 6B resulted in the wrist moving too far in extension to comfortably perform a voluntary extension response. Participants were instructed to “extend their wrist as fast as possible following onset of the perturbation”. On 10% of trials, a SAS Probe (115 dB, 50 ms, 1000 Hz) was presented simultaneous with the perturbation (SAS-Extend). On a separate 10% of trials, a SAS Probe was presented but no perturbation was delivered (SAS-Catch). On an additional 10% of trials, no perturbation and no SAS Probes were presented (Catch).
Experiment 7 began with 30 trials of practice in which only Control (Extend; 27 trials) and Catch (3 trials) trials were presented. The testing phase included 5 blocks of 40 trials (200 total trials). Each block contained 28 Extend, 4 SAS-Extend, 4 SAS-Catch, and 4 Catch trials. Trial order was pseudorandomized such that no trials with a SAS Probe occurred within the first 3 trials of a block and no 2 trials in a row contained a SAS Probe. Two minutes of rest was provided after each block.

5.2.5 Analysis and classification of startle

Surface EMG recordings were taken from the muscle bellies of right extensor carpi radialis (ECR), right flexor carpi radialis (FCR), left and right orbicularis oculi (OOc) and left and right sternocleidomastoid (SCM) using pre-amplified surface electrodes connected to an external amplifier (Model DS-80, Delsys Inc., Natick, MA). EMG signals were amplified at 3K and bandpass filtered from 20-450 Hz. Signals were digitized at 2 kHz using a 1401plus data acquisition system and Spike2 (CED, Cambridge, UK) computer software and a custom-written LabVIEW (National Instruments, Austin, TX) program was used for offline data analysis. At the beginning of analysis, EMG data were DC offset corrected (mean baseline value was determined and subtracted from each data point) and full-wave rectified. Burst onsets [in left/right OOc and SCM (both experiments), right FCR (Experiment 6A), right ECR (Experiment 7)] were marked as the first point at which rectified EMG traces began a sustained rise above baseline levels (mean of activity for 200 ms preceding the imperative signal on a trial-by-trial and an individual muscle basis). EMG traces were displayed on a computer monitor with a superimposed marker indicating the initial point where activity increased to more than 3SD above baseline and remained above this level for at least 10 ms. Onset markers were verified visually and adjusted if necessary (due to the strictness of the algorithm) to the onset of the
appropriate muscle burst. For Experiment 6B, integrated wrist flexor EMG activity was calculated (on a trial-by-trial basis) from the pre-defined stretch response epochs (M1:25-50 ms; M2a:50-75 ms; M2b 75-100 ms). Due to the elicitation of these stretch responses, RT could not be determined (Manning et al., 2012; Forgaard et al., 2015), but differences between conditions could be inferred from the M2b time period (Ravichandran et al., 2013; Forgaard et al., 2015).

In StartReact studies, short-latency activation (<120 ms) in SCM is used as the gold-standard measure of startle detection (Carlsen et al., 2011). Trials with only an auditory blink reflex (observed in OOC), or no responses in any startle indicator muscle do not show consistently early RTs (Carlsen et al., 2007). However, trials with true startle-related SCM (following either a perturbation or a SAS) have consistently early RTs and are accompanied by a response in OOC (Carlsen et al., 2007; Forgaard, Franks, Maslovat, Gowan, et al., 2016). By contrast, trials with just postural SCM activity do not typically have early OOC activity (Carlsen et al., 2004b; Forgaard, Franks, Maslovat, Gowan, et al., 2016). Therefore, to aid in separating startle and postural-SCM activity, we defined a positive startle response as bilateral activity in OOC and SCM within 120 ms of a given stimulus (Forgaard et al., 2015; Forgaard, Franks, Maslovat, Gowan, et al., 2016).

To quantify the effects of the PPI stimulus on SCM activity (Experiment 6A/B), rectified SCM data were collapsed across the left and right sides and integrated from 40-90 ms. Previous work has shown that an auditory PPI stimulus presented 100 ms before a SAS, significantly attenuates SCM activity during this interval (Maslovat, Kennedy, et al., 2012). The PPI-SAS data were expressed relative to integrated SAS data (Experiment 6A) and the PPI-Flex data were expressed relative to the integrated Flex data. A normalized value of less than 1.0 reflects attenuation and a value greater than 1.0 indicates facilitation.
5.2.6 Data reduction

Analysis for the Experiment 6A focused on the 8 participants who consistently (>50%) displayed a startle response on (non-PPI) SAS trials. 6.0% of trials were omitted due to false starts [either responding before the IS (18 trials), or responding on a PPI-Catch trial (6 trials)]. Experiment 6B focused on data from 10 participants who displayed SCM activity on more than 20% of Flex trials. This included the same 8 participants from Experiment 6A and an additional 2 participants who did not startle on SAS trials. Experiment 6B had more trials (32 trials per condition; see above) compared to the SAS trials in Experiment 6A (5 trials); thus we felt confident including participants with a lower incidence of SCM compared to startle incidence in Experiment 6A. For Experiment 6B, 2.1% of trials from the 10 participants were omitted due to false starts [responding before imperative signal (3 trials) or responding on a PPI-Catch trial (14 trials)] and 0.9% were excluded because no voluntary response appeared following the imperative signal (7 trials).

For Experiment 7, data were omitted from 1 participant due to an abnormal response profile on SAS-Catch trials. Although this participant consistently startled on these trials, the response that was elicited in the forearm was one of flexion followed by extension (the task required extension). Because this participant appeared to have been pre-programming a different motor response than what was specified, we excluded their data. No other participants displayed this behaviour on SAS-Catch trials. From the remaining 12 participants, only 1.9% of trials were removed due to any errors. Reasons for omission included trials where participants began their response prior to the imperative signal (i.e., false starts; 22 trials) or did not respond (4 trials). In addition, we excluded any trials that fell more than 3 standard deviations from a participant’s mean RT within a given condition (19 trials).
5.2.7 Dependent measures and statistics

All incidence data were converted to a proportion of trials from a given condition and arcsine transformed prior to statistical analysis. Non-transformed mean values are reported alongside statistical results conducted on the transformed data. Onset Latency values were only taken on trials from a given muscle if a response was detected in that muscle.

For Experiment 6A, Startle Incidence and RT data were analyzed using a SAS (SAS, No SAS) × PPI (PPI, No PPI) repeated measures analysis of variance (ANOVA). OOC/SCM Onset Latency values were compared on SAS trials with a Condition (SAS, PPI-SAS) × Side (Left, Right) ANOVA. For Experiment 6B, Startle Incidence and wrist flexor stretch response epoch (M1, M2a, M2b) data were evaluated using paired samples t-tests comparing Flex and PPI-Flex. Startle indicator measures (OOC/SCM Incidence, Onset Latency) were examined with a Condition (Flex, PPI-Flex) × Side (Left, Right) repeated measures analysis of variance (ANOVA). For both Experiment 6A and 6B, the normalized integrated SCM data were analyzed with a one-sample t-test against a value of 1.0.

For Experiment 7, repeated measures ANOVA tests were used to compare the effect of Condition (Extend, SAS-Extend, SAS-Catch) on Startle Incidence and RT. We also report incidence and onset latency of responses in startle indicator muscles (Left/Right OOC and Left/Right SCM) on Extend trials.

In the event of any violations to the assumption of sphericity for an ANOVA with more than two factors (determined by Mauchly’s test), Greenhouse-Geisser corrected degrees of freedom were reported, alongside the corrected $p$ values. Partial eta squared ($\eta^2_p$) was used as a measure of effect size. Dunn-Bonferroni corrected t-tests were employed to interpret significant
main effects and significant interaction effects were qualified using simple main effects analysis. Standard error of the mean is reported as an indicator of variability and the level of statistical significance for each test was set at $p = .05$.

5.3 Results

5.3.1 Experiment 6A: Replicating the effects of PPI on the startle response and

StartReact effect

Analyzing startle incidence data revealed a significant main effect for SAS, $F(1,7) = 382.42, p < .001, \eta^2_p = .98$, that was superseded by a SAS × PPI interaction $F(1,7) = 8.41, p = .023, \eta^2_p = .55$. Simple main effects analysis indicated that PPI-SAS had a lower startle incidence [$p = .032; 61.3\% (\pm 9.8\%)$] than SAS [$91.9\% (\pm 3.7\%)$]. On non-SAS trials by contrast, presentation of a PPI stimulus did not influence startle incidence [$p = .305; PPI$-Control: $5.0\% (\pm 3.1\%)$ vs. Control: $0.4\% (\pm 0.4\%)$].

Overall, on SAS trials, the PPI stimulus appeared to attenuate activity in startle indicator muscles (see figure 5.1). An analysis of OOc Onset Latency uncovered main effects for PPI, $F(1,7) = 7.06, p = .033, \eta^2_p = .50$, indicating that OOc onset was delayed [$52.9$ ms ($\pm 4.4$) vs. $40.9$ ms ($\pm 1.3$)] following the PPI stimulus. There were no significant main effects for Side ($p = .699$) or PPI × Side interactions ($p = .488$). For SCM, there was also a PPI main effect for Onset Latency, $F(1,7) = 5.90, p = .046, \eta^2_p = .46$. SCM onset was also significantly later on PPI-SAS trials [$100.0$ ms ($\pm 8.3$) vs. SAS: $77.6$ ms ($\pm 6.0$)]. The Side main effect ($p = .139$) and PPI × Side interaction ($p = .825$) were non-significant.

The analyses above relied on an EMG burst marking algorithm to determine muscle onsets. It is possible that on some PPI-SAS trials, responses in startle indicators were too small to
be detected. Thus we quantified the integrated EMG activity regardless of the presence of a muscle burst. For PPI-SAS trials, the integrated SCM activity from 40-90 ms was significantly reduced, $t(7) = -5.13, p = .004$. The mean normalized value was 0.47 (0.12) which corresponds to a 53% reduction in integrated SCM activity, compared to the SAS condition (see figure 5.1).

![Graph of EMG activity](image)

**Figure 5.1.** Group ensemble startle indicator data for Experiment 6A. SAS condition solid black lines; PPI-SAS solid grey lines. Reprinted with permission of Springer-Verlag.

An examination of RT revealed a main effect for SAS, $F(1,7) = 103.27, p < .001$, $\eta^2_p = .94$, but the main effect for PPI ($p = .281$) and the SAS × PPI interaction ($p = .127$) were non-significant. This indicates that the presentation of a SAS significantly reduced RT [SAS: 106.0 ms (± 7.2) and PPI-SAS: 108.9 ms (± 5.8)] compared to Control conditions [Control: 211.7 ms (± 10.7) and PPI-Control: 179.1 ms (± 15.4)]. In summary, the findings of Experiment 6A confirm previous reports (Castellote, Kofler, Mayr, & Saltuari, 2017; Maslovat, Kennedy, et al., 2017).
2012; Valls-Solé et al., 2005) that a PPI stimulus given 100ms prior to SAS can attenuate the startle response without a significant impact on the StartReact effect.

5.3.2 Experiment 6B: A PPI stimulus does not attenuate perturbation-evoked SCM activity

The purpose of Experiment 6B was to examine whether a PPI stimulus could influence SCM activity following a perturbation. Rather than attenuating responses in startle indicators, as would be expected for the startle response, the PPI stimulus significantly advanced the SCM responses and had no influence on OOc activity (see figure 5.2). A low proportion of trials in this Experiment were classified as displaying startle (Flex: 4.8%; PPI-Flex 8.8%; p = .501). On average, SCM activity was observed on 92.1% (± 10.7) of trials but no incidence differences were observed between left and right SCM (p = .567) or between trials with or without a PPI stimulus (p = .734). By contrast, analysis of SCM onset data showed main effects for PPI, F(1,9) = 45.43, p < .001, \( \eta_p^2 = .84 \), and Side, F(1,9) = 5.99, p = .037, \( \eta_p^2 = .40 \), without a significant interaction (p = .529). SCM activity occurred 17.4 ms earlier on PPI-Flex trials and 7.7 ms earlier in the right SCM compared to left [L-PPI-Flex: 123.0 ms (±6.0); L-Flex: 139.8 ms (±6.8); R-PPI-Flex: 114.7 ms (±6.8); R-Flex 132.7 ms (±6.7)]. OOc responses by contrast, appeared on 57.2% of trials at a mean onset of 164.9 ms. No OOc measures were significantly different between conditions [Incidence (Side: p = .827; PPI: p = .203; Side × PPI: p = .871); Onset Latency (Side: p = .258; PPI: p = .946; Side × PPI: p = .945)].

A one-sample t-test comparing the normalized integrated SCM activity from 40-90 ms on PPI-Flex to a value of 1.0, did not reveal any significant attenuating effects of the PPI stimulus, \( t(9) = 1.71, p = .122 \). The mean normalized value was 1.42 (±0.23) indicating that activity was in the direction of facilitation on PPI-Flex trials. Given that the startle response to a perturbation or
a somatosensory stimulus is ~20 ms later than the auditory startle response (Álvarez-Blanco et al., 2009; Forgaard, Franks, Maslovat, Gowan, et al., 2016; Ravichandran et al., 2013), we moved the integration window 20 ms later (60-110 ms) and we re-ran the same analysis. However, rather than finding attenuation of SCM, we now observed significant facilitation on PPI-Flex trials, \( t(9) = 2.50, p = .034 \). Integrated SCM activity was 1.61 (±0.23) times larger on PPI-Flex trials compared to Flex trials (see figure 5.2).

**Figure 5.2.** Group ensemble displacement and EMG data for Experiment 6B. Flex condition solid black lines; PPI-Flex solid grey lines. The perturbation moved the wrist into extension and participants executed a wrist flexion movement. Stretch responses and the voluntary response appeared in FCR. Panel A: All EMG data on same Y-scale. Note the postural activity in SCM. Also note the increased M2b activity and earlier voluntary response on PPI-Flex trials. In panel B, SCM data have been magnified. Note how the responses were advanced on PPI-Flex trials. Reprinted with permission of Springer-Verlag.
Analysis of the wrist flexor stretch response data showed no significant differences during the M1 ($p = .672$) or M2a epochs ($p = .453$). M2b activity however, was significantly increased, $t(9) = 3.12, p = .012$, on PPI-Flex trials [4.2 mV*ms (±0.7) compared to Flex 3.4 mV*ms (±0.6)], suggesting that the voluntary response may have occurred earlier on PPI-Flex trials which resulted in increased superimposition onto the end of the M2 response (figure 5.2).

### 5.3.3 Experiment 7: A mechanical perturbation elicits an early voluntary response in the absence of startle

An analysis of Experiment 7 startle incidence data revealed a significant main effect of Condition, $F(2,22) = 47.47, p < .001, \eta^2_p = .81$. The highest incidence of startle occurred for SAS-Extend [$p$ values < .001; 68.2% (±8.8%)] compared to SAS-Catch [18.1% (±7.6%)] and Extend [2.8% (±1.6%)]. The latter two conditions also differed significantly ($p = .029$). With respect to our initial objective, the 115 dB SAS produced a distribution of trials with and without a startle response$^4$ (see below) and the near zero incidence of startle for the Extend condition was similar to Experiment 6B as well as previous findings that a mechanical perturbation imperative signal does not consistently elicit a startle response (Forgaard et al., 2015; Forgaard, Franks, Maslovat, Gowan, et al., 2016). An analysis of RT showed a main effect of Condition,

$$F(1.02, 11.24) = 6.51, p = .026, \eta^2_p = .37.$$ RT for the SAS-Extend condition was significantly

$^4$ It is possible that our conservative startle classification criteria (bilateral OOc and bilateral SCM within 120 ms of a stimulus) contributed to the low incidence of startle (18.1%) on SAS-Catch trials. We further examined SAS-Catch condition, particularly the trials that showed bilateral SCM activity but no responses in OOc. It was rare to observe SCM activity in the absence of OOc (7 out of 237 SAS-Catch trials). Of these trials, the response only appeared in ECR on 4 trials (mean onset of 111 ms; i.e., not StartReact-like latency). Three other trials were classified as having bilateral SCM and unilateral OOc activity. The pre-programmed ECR response appeared on all 3 trials with a mean onset of 118 ms. The majority of SAS-Catch trials had bilateral OOc activity but no activity in SCM at <120 ms. Thus, the conservative classification of what constitutes a startle response used in this study [which was implemented to protect against a high proportion of false-positive startle responses (due to postural SCM) on mechanical perturbation trials; see Methods], was unlikely to produce a high proportion of false-negative startle responses.
shorter [80.8 (±2.2) ms] than SAS-Catch [$p = .035$; 118.6 (±12.9) ms] and Extend [$p < .001$; 96.8 (±1.9) ms]; however the values for SAS-Catch and Extend conditions did not differ significantly ($p = .379$).

Based on our startle response criteria (see Methods), every trial was categorized as either displaying a startle response ($\text{Startle}^+$) or not displaying a startle response ($\text{Startle}^-$). Given that the objective of Experiment 7 was to determine whether a mechanical perturbation could elicit StartReact-like RTs in the absence of a startle response, we re-analyzed RT values from the trials for $\text{Extend}_{\text{Startle}^+}$ with the trials for SAS-Extend$_{\text{Startle}^+}$ and SAS-Catch$_{\text{Startle}^+}$. Despite reducing the number of participants to 9 (3 participants had no SAS-Catch$_{\text{Startle}^+}$ trials), we observed a significant main effect of Condition, $F(2,16) = 29.68$, $p < .001$, and a stronger effect size ($\eta^2_p = .79$), compared to the preliminary test of RT. The post-hoc test also showed a different pattern of results. RT for the SAS-Extend$_{\text{Startle}^+}$ [76.4 (±2.2) ms] and SAS-Catch$_{\text{Startle}^+}$ [77.7 (±3.0) ms] conditions were not significantly different ($p > .999$), but both SAS conditions were earlier than Extend$_{\text{Startle}^-}$ [95.6 (±1.8) ms; SAS-Extend$_{\text{Startle}^+}$ $p < .001$; SAS-Catch$_{\text{Startle}^+}$ $p = .002$]. This finding provides evidence that a mechanical perturbation does not consistently elicit RTs as short as the StartReact effect. See figure 5.3 for ensemble average displacement and kinematic data from the SAS-Extend$_{\text{Startle}^+}$, SAS-Catch$_{\text{Startle}^+}$, and Extend$_{\text{Startle}^-}$ conditions. A distribution of RTs (in 10 ms bins) from these conditions is also presented in figure 5.4A. Note how the SAS-Extend$_{\text{Startle}^+}$ and SAS-Catch$_{\text{Startle}^+}$ RT distributions were consistently early and centered around 70-80 ms (figure 5.4A) and the Extend$_{\text{Startle}^-}$ RT distribution was centered around 90-100 ms. Although this Extend$_{\text{Startle}^-}$ distribution was significantly later than SAS-Extend$_{\text{Startle}^+}$ and SAS-Catch$_{\text{Startle}^+}$, 67.9% of trials still had RT values of 100 ms or less.
Figure 5.3. Group ensemble displacement and EMG data for Experiment 7. Panel A: Extend_{Startle}. Panel B: SAS-Extend_{Startle}. Panel C: SAS-Catch_{Startle}. The perturbation moved the wrist into extension and participants executed a wrist extension movement. Stretch responses appeared in FCR (c.f. panel C) and voluntary response appeared in ECR. No stretch responses were observed in Panel C because the perturbation was not delivered. In panel A, note how the voluntary response began at <100 ms in the absence of a startle response. Postural activity was observed in SCM muscles. In panels B and C, note how a startle response was observed (early activation of OOc and SCM) and the voluntary response began at ~70 ms. Reprinted with permission of Springer-Verlag.
Figure 5.4. Experiment 7 RT data distributed into 10 ms bins. Panel A: Extend\textsubscript{Startle} (solid grey), SAS-Extend\textsubscript{Startle} (solid black), and SAS-Catch\textsubscript{Startle} (dashed grey) conditions. Y-axis normalized by total number of trials classified as displaying the startle response (SAS-Extend\textsubscript{Startle} and SAS-Catch\textsubscript{Startle}) or not displaying startle (Extend\textsubscript{Startle}). Panel B: SAS-Catch\textsubscript{Startle} (dashed grey) and SAS-Catch\textsubscript{Startle} conditions normalized in the same manner as A. The number of trials in SAS-Catch\textsubscript{Startle} appears smaller because a response in ECR was only elicited on 37% of these trials. By contrast a response in ECR was elicited on 97.8% of SAS-Catch\textsubscript{Startle} trials. Reprinted with permission of Springer-Verlag.
Further analysis of the Catch and SAS-Catch conditions provides interesting insight into the StartReact effect. Recall that the imperative signal was not presented on these trials; therefore correct performance required that participants withhold the pre-programmed extension response. On the Catch trials with no SAS Probe, a response was only initiated on 1.7% of trials suggesting that participants successfully withheld the voluntary response when no perturbation or SAS was presented. By contrast, on SAS-Catch trials, the pre-programmed response was elicited on 46.4% (±8.9%) of trials but the probability and latency of response triggering differed considerably in the presence versus the absence of the startle response. On SAS-Catch_{Startle+} trials, the pre-programmed response was elicited on 97.8% (±2.1%) of trials with a mean RT of 77.7 ms (±3.0 ms). For the SAS-Catch_{Startle-} trials, the pre-programmed response only appeared on 37.0% (±8.8%) of trials and the mean onset latency was 131.4 (±13.4) ms. See figure 5.4B for a distribution of RT values for SAS-Catch_{Startle+} and SAS-Catch_{Startle-}. The SAS-Catch_{Startle+} RT values were consistently early and at a typical StartReact latency suggesting that the voluntary response was triggered by the SAS. By contrast, the SAS-Catch_{Startle-} trials fell into two distributions; one which was early (i.e. StartReact-like) and another which was later and more variable.

In a previous study (Forgaard, Franks, Maslovat, Gowan, et al., 2016), and Experiment 6B of the current project, we found that when participants compensated against a wrist extension perturbation, a considerable number of trials (>50%) showed postural SCM activity. We observed similar SCM responses in Experiment 7 where participants performed Extend responses to the perturbation. SCM activity was observed on 68.3% (±10.9%) of trials at a mean onset latency of 130.2 (±7.2) ms, which followed onset of the ECR response (i.e., RT) by 37.3 (±7.8) ms. In Experiment 6B, we found R-SCM to occur ~7 ms earlier than L-SCM; however in
Experiment 7, L-SCM (127.3 ms) now preceded R-SCM (133.1 ms) by ~6 ms, but this was not significantly different ($p = .283$). OOC activity was observed on 50.4% ($\pm 10.3$) of trials at a mean onset of 145.5 ($\pm 13.2$) ms.

5.3.4 Control conditions: Investigating the presence of a shortening reaction in ECR

A protocol in which the voluntary response occurs in shortened muscle has been used by a number of groups to determine perturbation RT (Evarts & Granit, 1976; Evarts & Vaughn, 1978; MacKinnon et al., 2000; Miscio et al., 2001; Ravichandran et al., 2013). When using this method, it is important to consider whether any muscular activity in the shortened muscle occurs as a natural consequence of the mechanical perturbation stimulus. It has been reported that passively shortened muscles can display a shortening reaction (Katz & Rondot, 1978) which begins >100 ms after perturbation onset (Miscio et al., 2001). Although the origin of this response remains unknown, it was proposed to be a deliberate response to counteract the effects of the stretch response (Miscio et al., 2001). Due to the close proximity of forearm flexors and extensors, some participants may also display “volume conduction” or “EMG cross talk” in the shortened muscle around the onset of the M2 response in the stretched antagonist (Miscio et al., 2001). The presence of either of these responses in the ECR data of our Experiment 7 protocol could confound our trial-by-trial determination of RT. Therefore, to determine the presence of volume conduction or a shortening reaction in ECR, we re-tested 3 participants. Testing involved 28 Extend trials and 28 passive trials where they were instructed to “not intervene following the perturbation” (DNI). Surface EMG was recorded from ECR and FCR and responses were marked in the same manner as Experiment 7. The same marking algorithm was used on the DNI trials to determine the presence of either a shortening reaction or volume conduction in the EMG recording of ECR.
Ensemble displacement and EMG data for each participant is presented in figure 5.5. Participant 1 had a mean RT on Extend trials of 87.2 ms. On 21.4% of DNI trials, the EMG marking algorithm revealed a small response at a mean of 135.4 ms. The Q30 (integrated rectified EMG over first 30 ms of the burst) was 21.0 times smaller than the Q30 of the voluntary response on Extend trials. The mean RT for participant 2 was 94.2 ms; however, on 64.3% of DNI trials, the EMG marking algorithm found activity at a mean of 53 ms. This was likely the volume conduction observed by Miscio et al. (2001) as it occurred near the onset of M2 response in FCR. When it appeared, the Q30 was 7.1 times smaller than the Q30 of the voluntary response on Extend trials. For participant 3, no volume conduction was observed, but 14.3% of DNI trials had a response at a mean of 114 ms. This response was 18.3 times smaller than the Q30 of the voluntary response on Extend trials. In summary, the data from Experiment 7 were unlikely to be influenced by a shortening reaction in ECR. Volume conduction around 50 ms could have been observed in some participants but we have two lines of evidence to suggest it did not confound the marking of RT. (1) No Extend trials had RTs of ~50 ms and (2) the SAS-Catch_{Startle+} (which would not have a volume conduction issue in ECR around 50 ms because no perturbation was delivered) had a similar RT distribution to SAS-Extend_{Startle+} (see figure 5.4A).
Figure 5.5. Ensemble kinematic and EMG data from the 3 participants in the control experiment to determine the level of quiescent EMG activity in ECR while participants were exposed to an extension perturbation. Extend data: solid black lines. DNI (passive) data: solid grey lines. Reprinted with permission of Springer-Verlag.
5.4 Discussion

The long-latency stretch response, perturbation-triggered reactions, and startle-triggered responses are all examples of rapid motor responses that obscure the boundary between “reflexive” and “voluntary” motor control. While auditory startle evoked responses have traditionally been studied separately from mechanical perturbation responses, recent work has posited that mechanical perturbations may also elicit a startle response and therefore the StartReact effect could underlie perturbation-triggered reactions and instruction-dependent M2 modulation (Lewis et al., 2006; Ravichandran et al., 2013; Shemmell et al., 2009). The present study examined whether activity in startle indicator muscles following a perturbation imperative signal results from the startle response or postural control (Experiment 6B) and whether an overt startle response is a prerequisite for perturbation RTs of <100ms (Experiment 7). Our findings provide clear evidence that a mechanical perturbation does not elicit startle when presented as the imperative signal in a simple RT paradigm. Despite the absence of startle, a majority of trials had RTs of <100 ms. It would therefore appear that a startle/StartReact mechanism is not necessary for the initiation of a perturbation-triggered reaction.

5.4.1 A prepulse inhibition stimulus reveals postural SCM activation

In Experiment 6A, we replicated previous findings showing that activation of SCM can be strongly attenuated when a PPI stimulus is presented immediately before a startling stimulus (Castellote et al., 2017; Maslovat, Kennedy, et al., 2012; Valls-Solé et al., 2005). In stark contrast to what was expected if a mechanical perturbation elicited startle, the SCM responses observed in Experiment 6B were significantly advanced (17.4 ms) on trials with a PPI stimulus (see figure 5.2). Therefore, when participants execute ballistic upper-limb movements following a perturbation, rather than the SCM activity being a result of a startle response, we believe it was
part of a postural response associated with head and neck stabilization (Forgaard, Franks, Maslovat, Gowan, et al., 2016; Dean & Baker, 2017). This interpretation is further supported by the asymmetric activation of SCM muscles. A hallmark feature of the startle response is bilateral activation of homologous startle indicator muscles (Carlsen et al., 2011); however on Flex trials we found that activation in the right SCM preceded the left by 7.1 ms. Despite the advancement on PPI-Flex trials, the asymmetric activation (R-SCM 8.3 ms earlier than L-SCM) was preserved, suggesting that the postural activation in homologous neck muscles was likely pre-programmed and executed together.

In the previous studies examining the effects of PPI on the startle response and StartReact effect (Maslovat, Kennedy, et al., 2012; Valls-Solé et al., 2005), and Experiment 6A of the current work, RT on SAS trials was insensitive to the presence of a PPI stimulus (i.e., the StartReact effect was not influenced by PPI. Control (non-SAS) trials by contrast were shown to have reduced RTs on PPI trials, a result of inter-sensory facilitation and/or direct cueing of imperative signal delivery (Maslovat, Kennedy, et al., 2012; Valls-Solé et al., 2005). Although our Experiment 6B protocol did not allow for a direct determination of RT, differences between conditions could be inferred from the stretch response epoch data. The M1 and M2a epochs were not affected, however activity during the M2b period increased on PPI-Flex trials (figure 5.2). The latter portion of the M2 response (M2b) can be influenced by superimposition of a voluntary response (Rothwell et al., 1980; Ravichandran et al., 2013; Forgaard et al., 2015). We therefore propose that the increased M2b activity on PPI-Flex trials resulted from a reduced RT and increased voluntary response superimposition. This finding is in accordance with non-SAS PPI RT data (Maslovat, Kennedy, et al., 2012; Valls-Solé et al., 2005) and taken together with the
advanced asymmetric SCM responses on PPI trials, provides strong evidence that a mechanical perturbation imperative signal does not elicit startle.

5.4.2 Potential mechanisms for a perturbation-triggered reaction

The StartReact effect is the hastening of a preplanned voluntary response by activation of startle circuitry. The original explanation was put forth by Valls-Solé and colleagues in the 1990s in order to account for RTs on SAS trials that were considered too short for the minimal amount of time needed for cortical processing of sound and conduction time from cortex to muscle. These authors proposed that a motor program, or pre-programmed voluntary response, may be stored subcortically (e.g., in reticular formation). Presentation of a SAS elicits the startle response and activation of startle circuitry also triggers the pre-programmed response early via the reticulo-spinal tract (Valls-Solé et al., 1999). Evidence supporting this hypothesis has come from studies showing that the StartReact effect is most reliably observed in muscles with strong reticulo-spinal connections (e.g., Carlsen, Chua, Inglis, Sanderson, & Franks, 2009) and patients with hereditary spastic paraplegia (axonal degeneration of the cortico-spinal tract) have an intact StartReact effect, despite delayed RTs on control trials (Nonnekes et al., 2014). An alternative to the subcortical storage/triggering hypothesis proposes that motor commands for a voluntary response are pre-programmed and stored cortically prior to initiation (Carlsen et al., 2012). On SAS trials, activation of startle circuitry results in ascending neural activation which travels a reticulo-thalamo-cortical route and initiates the pre-programmed response early via the cortico-spinal tract (Alibiglou & MacKinnon, 2012; Carlsen et al., 2012; Stevenson et al., 2014). This hypothesis is based on findings that the StartReact effect and startle response can be dissociated. For example (and as replicated in Experiment 6A), presentation of a PPI stimulus ~100 ms before a SAS reduces the magnitude of activity in startle response indicators, without impacting
the triggering effects of the startling stimulus on the pre-programmed voluntary response (Castellote et al., 2017; Maslovat, Kennedy, et al., 2012; Valls-Solé et al., 2005). Along a similar line of evidence, a transcranial magnetic stimulus induced silent period of primary motor cortex delays the StartReact effect, without influencing the startle response (Alibiglou & MacKinnon, 2012; Stevenson et al., 2014). Further evidence of a dissociation between the StartReact effect and the startle response are “StartReact-like” RTs on a minority of trials where no startle response was detected (Delval et al., 2012; Maslovat et al., 2015).

Recent work has drawn comparisons between the StartReact effect and perturbation-triggered reactions (Koshland & Hasan, 2000; Lewis et al., 2006; Ravichandran et al., 2013; Shemmell, 2015; Shemmell et al., 2009; Shemmell et al., 2010). It is well documented that activation of startle circuitry during situations of motor preparation results in a hastening of the pre-programmed voluntary response and this effect has been most commonly demonstrated using intense auditory stimuli (Carlsen et al., 2012; Nonnekes et al., 2015; Valls-Solé et al., 2008). However, the neurons responsible for the startle response (in the caudal pontine reticular nucleus NRPC) are not modality specific; they can also be activated by intense proprioceptive and vestibular inputs (Yeomans et al., 2002). For instance, NRPC neurons respond at short-latency (along with neurons in other pontine reticular formation nuclei) following unexpected platform perturbations in cats (Stapley & Drew, 2009). In humans, lower-limb perturbations have been shown to be startling, from platform perturbations that elicit strong vestibular and proprioceptive signals (Campbell et al., 2013; Oude-Nijhuis et al., 2010) as well as single-joint knee perturbations (Castellote et al., 2017). In the context of triggered reactions in the upper-limb, it has been proposed that the mechanical perturbations used to elicit stretch responses also evokes a startle response and therefore, the StartReact effect may underlie early initiation of the pre-
programmed voluntary response (Lewis et al., 2006; Ravichandran et al., 2013; Shemmell, 2015; Shemmell et al., 2009; Shemmell et al., 2010).

The StartReact effect as a mechanism underlying triggered reactions is appealing and was likely responsible for the early RTs observed by Ravichandran et al. (2013), and potentially other groups that have also used unexpected perturbations (e.g., Koshland & Hasan, 2000). However, a majority of studies examining triggered reactions and instruction-dependent M2 modulation have presented the mechanical perturbation in an expected manner, usually as the imperative signal (e.g., Crago et al., 1976; Evarts & Vaughn, 1978; MacKinnon et al., 2000; Manning et al., 2012; Pruszynski et al., 2008; Rothwell et al., 1980). The present findings complement previous work showing that expected perturbations do not elicit startle (Forgaard, Franks, Maslovat, Gowan, et al., 2016). Here we provide clear evidence that even in the absence of startle, a mechanical perturbation imperative signal can consistently elicit RTs of <100 ms in shortened muscle. Assuming the same RT distribution when the voluntary response occurs in stretched muscle, a majority of trials will have some superimposition of the voluntary response into the M2b time period.

Despite a lack of an overt startle response on Flex trials (Experiment 6B) and Extend trials (Experiment 7), we should consider the possibility that the perturbation may have still acted as a “sub-threshold” startling stimulus. In Experiment 7, we observed the highest incidence of startle on SAS-Extend trials (~68%). Because this was considerably greater than the startle incidence of the SAS-Catch condition (~18%), where only the 115 dB auditory stimulus was delivered, it is possible that neural activation from the SAS Probe and the mechanical perturbation summated to activate the NRPc startle neurons above threshold. This is in line with animal work showing that cross-modal stimuli (e.g., tactile and auditory) are more effective at
eliciting startle than intra-modal stimuli (see Yeomans et al., 2002). It also raises the possibility that on Extend trials, the mechanical perturbation may have produced subthreshold activation (below the level needed for a startle response) of startle circuitry and is similar to our previous report showing that this sort of wrist perturbation is startling on up to 30% of trials when delivered unexpectedly (Forgaard, Franks, Maslovat, Gowan, et al., 2016). Because a somatosensory or proprioceptive startle response is ~20 ms later than the auditory startle response (Álvarez-Blanco et al., 2009; Forgaard, Franks, Maslovat, Gowan, et al., 2016; Oude-Nijhuis et al., 2010; Ravichandran et al., 2013), the time-course is in accordance with our observation of ~20 ms longer RTs on control Extend trials (compared to SAS-ExtendStartle+ and SAS-CatchStartle+ trials). While these results cannot distinguish between the subcortical storage and triggering hypothesis versus cortical involvement in the StartReact effect, they are in accordance with other work suggesting that the startle response and early elicitation of a pre-programmed voluntary response are sometimes dissociable (Alibiglou & MacKinnon, 2012; Dean & Baker, 2017; Maslovat, Kennedy, et al., 2012). Although this is one potential explanation of sub-100 ms RTs following a perturbation, as no overt startle response was observed on Extend trials, it cannot be considered a result of the StartReact effect. This mechanism of response triggering also operates on the assumption that StartReact circuitry has a lower threshold of activation than startle response circuitry, something that has not been confirmed in the literature.

We believe it is more likely that the sub-100 ms RTs following the perturbation resulted from a different mechanism than what underlies the StartReact effect. A comparison of behavioural evidence shows definitive differences between the StartReact effect and elicitation of a voluntary response by a perturbation. For example, one hallmark feature of the StartReact
effect is that it only occurs in situations where a voluntary response has been pre-programmed (Valls-Solé et al., 1999; Carlsen et al., 2004a). In choice RT conditions, where no advanced programming can occur, no hastening of the appropriate voluntary response is observed on SAS trials (Carlsen et al., 2004a). By contrast, studies examining perturbation RTs have shown that early voluntary responses occur even in choice conditions where the required motor response is unknown in advance of perturbation delivery (Crago et al., 1976; Evarts & Vaughn, 1978; Glencross & Koreman, 1979; Manning et al., 2012). Moreover, the onset latency increase over a simple RT condition was shown to be minimal (10-50 ms, likely a result of the high compatibility between the stimulus and the required response (the limb with the required response was directly stimulated by the perturbation). Larger increases (~70 ms) were seen in a non-compatible choice condition where the required response involved only responding with the opposite, unperturbed limb (Glencross & Koreman, 1979). It is plausible that the sub-100 ms RTs following a perturbation are the result of the stimulus directly activating circuitry involved in producing the required voluntary response.

Upper-limb perturbations produce short-latency neural responses in many circuits throughout the central nervous system including areas involved in voluntary movement control such as the cerebellum, red nucleus, posterior parietal cortex, primary sensory cortex, primary motor cortex and dorsal premotor cortex (Evarts & Tanji, 1976; Herter et al., 2015; MacKinnon et al., 2000; Omrani et al., 2016; Pruszynski et al., 2014; Strick, 1983). These supra-spinal areas also make contributions to the long-latency stretch response and it has been proposed this stretch response displays characteristics of voluntary movement control because of this shared neural circuitry (Pruszynski et al., 2008; Scott, 2004). Extending this, we believe the voluntary response can be initiated at a remarkable (<100 ms) latency because the perturbation directly facilitates
activation of circuitry involved in producing the voluntary response. This explanation could account for early voluntary responses in both simple and compatible choice tasks, whereas the StartReact effect only accounts for voluntary response triggering in a simple RT paradigm.

5.4.3 The triggered reaction

Reports of perturbation-triggered reactions have appeared in the literature since the 1970’s; however there have been disagreements on the precise nature of these responses. Part of the contention appears to stem from different tasks, effectors studied, and various different types of stimuli delivered. The original reports described the triggered reaction in upper-limb musculature as a voluntary response that appears at an earlier latency to a proprioceptive stimulus than the voluntary response following stimuli of other modalities (Crago et al., 1976; Evarts & Vaughn, 1978; Houk, 1978). Similar to this, the StartReact effect is the mechanism underlying startle-triggered reactions, which are also believed to be the same voluntary response as non-startle trials, just initiated earlier and via a separate pathway (Valls-Solé et al., 1999; Carlsen et al., 2004b). Other authors have described triggered reactions as rapid postural adjustments following a mechanical perturbation that compromises whole body stability during standing balance (Nashner & Cordo, 1981). However, these rapid postural adjustments are highly stereotyped and shown to be separate from a voluntary response (Nashner & Cordo, 1981). By contrast, perturbations during a precision finger grip task (Johansson & Westling, 1988) and lip perturbations during speech (Abbs, Gracco, & Cole, 1984) revealed flexible fast-feedback responses which are coordinated across multiple effectors in order to achieve a given goal. Interestingly, the M2 response in the upper-limb has also been demonstrated to possess similar sophistication, beyond what can be predicted from the superimposition of a triggered reaction (Pruszynski, Kurtzer, & Scott, 2011; Weiler, Saravanamuttu, Gribble, & Pruszynski,
While a simple, open-loop triggered reaction cannot account for all sophistication of rapid motor responses to a perturbation stimulus, in certain paradigms, i.e., simple RT task following upper-limb perturbations used to elicit stretch responses, there is evidence that superimposition of a voluntary response does contribute to instruction-dependent modulation of the M2 response (Forgaard et al., 2015; Lewis et al., 2006; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980). Therefore to distinguish triggered reactions in the upper-limb from other uses of the “triggered response” terminology (e.g., during speech production, whole body postural control, finger manipulation, and the StartReact effect), we appreciate the description provided by Manning et al. (2012; page 162) “in the context of the long-latency stretch reflex, triggered reactions are RT responses”.

5.4.4 Importance of correctly classifying the startle response

The SAS-Catch condition in Experiment 7 allowed for an examination of the StartReact effect which further demonstrated the importance of correctly identifying the startle response in a StartReact experiment. On SAS-CatchStartle+ trials, the pre-programmed response was elicited on 97.8% of trials, and occurred consistently at a StartReact-like latency (see figure 5.4B). By contrast, on SAS-CatchStartle- trials, the pre-programmed response was only observed on 37.0% of trials and two distinct distributions of RTs emerged. One distribution, which consisted of 36.8% of these trials, was early and at StartReact-like latency suggesting that the pre-programmed response may have been triggered by the SAS. The second distribution (consisting of 63.2% of the trials) was later and more variable (~100-200 ms). On these late RT trials, the pre-programmed response was likely voluntarily initiated to the loud sound, similar to participants in a previous study voluntarily responding to the imperative signal (Maslovat et al., 2015). These findings clearly show the importance of separating Startle+ and Startle- trials when investigating the
StartReact effect. Researchers can be very confident that when a voluntary response is pre-
programmed and a startle response is elicited, the RT will be very early. However, on SAS trials
where a startle response is not detected, the response can appear early on a small proportion of
trials (e.g., the 13.6% of SAS-CatchStartle- trials), but on the majority of trials it will either not be
initiated (63.0% of trials) or will occur late (>100 ms; 23.4% of trials). This variety of responses
to a SAS when no reflexive startle response is observed makes it difficult to draw conclusions
about response preparation (as in the current study we expected a similar preparation level for
the CatchSAS trials but observed inconsistent responses on Startle- trials – whereas the responses
were very consistent on Startle+ trials). Therefore, it is only when a startle response has been
detected that researchers can be confident that sufficient activation of startle circuitry has
occurred and can confidently make conclusions about the level or preparation of the intended
response and the presence of the StartReact effect.

5.4.5 Conclusion

A mechanical perturbation imperative stimulus can elicit a pre-programmed voluntary
response in shortened musculature at a latency of less than 100 ms. The present study
demonstrates that these perturbation “triggered reactions” can occur consistently in the absence
of an overt startle response. Through the use of a prepulse inhibition stimulus, we show that
activity in SCM, a muscle commonly used to indicate startle, was a result of a postural response
rather than the startle response. While our study provides evidence that a mechanical
perturbation may act as a subthreshold startling stimulus and therefore the perturbation could still
engage circuitry involved in the StartReact effect, important differences also exist between a
perturbation-triggered reaction and the StartReact effect. As an alternative explanation, the
perturbation imperative signal may activate supra-spinal areas involved in generation of the voluntary response resulting in consistently early RTs, even in the absence of startle.

5.5 Bridging summary

The findings of chapter 5 strongly support the hypothesis that an expected mechanical perturbation stimulus does not elicit a startle response. Taken together with findings from the earlier experiments (chapters 1-4), even though a pre-programmed voluntary response can begin during the M2b epoch, voluntary superimposition cannot account for the generic M2a modulation that has been consistently observed between the DNI instruction and various active conditions involving a compensatory reaction. Several authors that have assumed the voluntary response does not superimpose onto M2 have also been unable to demarcate the two responses (Crevecoeur et al., 2013; Pruszynski et al., 2008; Pruszynski, Kurtzer, & Scott, 2011; Selen et al., 2012; Weiler et al., 2016). It was suggested that a distinction between the reflexive and voluntary mechanisms responsible for M2 modulation may be difficult because both responses engage common supra-spinal circuitry (Scott, 2004). The M2 response reflects the first volley of activity through the neural circuits that are later engaged by the voluntary response (Pruszynski et al., 2008; Scott, 2004). Thus the same goal-dependent behaviour observed during the voluntary response is also observed at earlier latency, during the M2 response. This could explain the similarities between the M2 response and volition and why no satisfactory distinction between these two responses has been demonstrated. Despite superimposition of a voluntary response not accounting for all M2 modulation, it remains unclear whether instruction-dependent modulation of this stretch response is still reliant on the overt execution of a voluntary response. The final experiment of this dissertation addressed this issue through the use of kinesthetic motor imagery.
Chapter 6: Experiment 8

The influence of kinesthetic motor imagery on the long-latency stretch response: Overt voluntary activity is a pre-requisite for instruction-dependent modulation
6.1 Introduction

Mechanical perturbations to the arm elicit a series of reflexive responses in stretched musculature. The first (< ~50 ms; M1) response is produced by spinal circuitry and is typically only sensitive to peripheral factors such as background state of the motoneuron pool and features (e.g., magnitude) of the perturbation (Calancie & Bawa, 1985; Pruszynski et al., 2009). M1 is followed immediately by a long-latency stretch response (~50-100 ms; M2) which receives contributions from spinal (Hagbarth et al., 1981; Lourenço et al., 2006; Matthews, 1984) and supra-spinal pathways (Cheney & Fetz, 1984; Evarts & Tanji, 1976; Lourenço et al., 2006; MacKinnon et al., 2000; Omrani et al., 2016; Omrani et al., 2014; Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski et al., 2014; Spieser et al., 2013; Spieser et al., 2010). The M2 response is sensitive to both peripheral factors as well as volitional intent, thus providing the motor system with a more flexible output given the current state of the limb and the ongoing task goal (Calancie & Bawa, 1985; Kurtzer, Crevecoeur, & Scott, 2014; Pruszynski, Kurtzer, & Scott, 2011). For example, M2 is influenced by the verbal instructions on how to respond to the perturbation (Hammond, 1956) and the spatial location that the limb needs to obtain (Pruszynski et al., 2008). This stretch response also accounts for inter-segmental limb dynamics (Kurtzer et al., 2014; Kurtzer et al., 2008; Pruszynski, Kurtzer, Nashed, et al., 2011), aids in obstacle avoidance during reaching (Nashed et al., 2012, 2014), and can be flexibly expressed across limbs if it benefits the overall goal of the task (Dimitriou et al., 2012; Marsden et al., 1981; Mutha & Sainburg, 2009; Omrani et al., 2013). It has been extensively debated whether the volitional capabilities of M2 regulation are produced through feedback gain changes in the presumably supra-spinal circuitry (Hammond, 1956; Lee & Tatton, 1975; Pruszynski et al., 2008; Spieser et al., 2013), or whether the appearance of a flexible stretch response only arises
because a voluntary response is triggered early by the perturbation and superimposes onto the M2 response (Crago et al., 1976; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980).

In a number of experiments where the voluntary response was delayed with behavioural manipulations of RT, we observed the corresponding impact on M2 modulation (Forgaard et al., 2015; Forgaard, Franks, Maslovat, & Chua, 2016). In these studies, we were unable to completely dissociate the voluntary response from the end of M2. However, irrespective of the latency or magnitude of the voluntary response, we did observe a generic increase in the first half of the M2 response (50-75 ms) between a passive condition, where participants were asked to “not-intervene” with the perturbation and active conditions involving a compensatory reaction. Several other authors that have examined more sophisticated M2 regulation have also been unable to distinguish the M2 response from the voluntary response in the EMG recordings (Crevecoeur et al., 2013; Pruszynski et al., 2008; Pruszynski, Kurtzer, & Scott, 2011; Selen et al., 2012; Weiler et al., 2016). It was suggested that a distinction between the reflexive and voluntary mechanisms responsible for M2 modulation may be difficult because both responses engage common supra-spinal circuitry (Scott, 2004). The M2 response reflects the first volley of activity through the neural circuits that are later engaged by the voluntary response (Pruszynski et al., 2008; Scott, 2004). Thus the same goal-dependent behaviour observed during the voluntary response is also observed at earlier latency, during the M2 response. This could explain the similarities between the M2 response and volition and why no satisfactory distinction between these two responses has been demonstrated. Despite superimposition of a voluntary response not accounting for all M2 modulation, it remains unclear whether instruction-dependent modulation of this stretch response is still reliant on the overt execution of a voluntary response.
One way in which the motor system can be activated without physically producing a voluntary response is through kinesthetic motor imagery (Jeannerod, 2001; Stinear, Byblow, Steyvers, Levin, & Swinnen, 2006). When a participant engages in this form of mental simulation, neural activation has been shown to occur in many of the same supra-spinal regions (e.g., primary motor cortex, premotor cortex, supplementary motor area, parietal cortex, and cerebellum) as physically performing the imagined movement (Decety et al., 1994; Jeannerod, 2001; Kasess et al., 2008; Roth et al., 1996; Ryding, Decety, Sjöholm, Stenberg, & Ingvar, 1993). This is not a general activation of motor structures; rather the imagery related activity is temporally appropriate and specific to the areas involved in physical performance of the imagined action. For instance, application of TMS over the motor cortex representation for wrist flexors revealed increased motor evoked potentials (MEPs) during imagined wrist flexion and increased MEPs over the wrist extensor hotspot during imagined extension (Hashimoto & Rothwell, 1999; see also Stinear et al., 2006). Similar effects have been demonstrated in a motor imagery RT paradigm, where MEPs increased over a similar time-course as physically executing the same response (Kumru, Soto, Casanova, & Valls-Sole, 2008).

Two theories have been proposed to explain how the covert simulation of a movement can occur in the absence of overt execution (Jeannerod, 2001). One possibility is that motor activation during imagery occurs at a subliminal level, such that many motoneurons are not usually activated above firing threshold. Evidence supporting this comes from studies showing small amounts of EMG activation (partial or “leaked” responses) during some trials of imagery (Bonnet, Decety, Jeannerod, & Requin, 1997; Gandevia, Wilson, Inglis, & Burke, 1997; Guillot et al., 2007; Maslovat, Chua, & Hodges, 2013). Importantly, these “leaked” responses were restricted to the muscle(s) involved in the imagined task. An alternative explanation is that supra-
spinal regions are activated (during kinesthetic imagery) as if the individual is performing the simulated action, however a parallel inhibitory mechanism prevents the overt execution. Evidence in favour of this viewpoint comes from the case study of a patient with bilateral parietal lesions (Schwoebel, Boronat, & Coslett, 2002). This patient unknowingly produced imagined movements during an imagery task. Jeannerod (2001) suggested that both subliminal activation and parallel inhibition may operate together to prevent action execution during imagery performance.

While it is well accepted that motor imagery produces activation of various supra-spinal structures, the influence of an imagined action on the excitability of spinal circuitry remains equivocal. Some studies investigating H-reflexes, have reported small increases during imagery (Cowley, Clark, & Ploutz-Snyder, 2008; Gandevia et al., 1997; Kiers, Fernando, & Tomkins, 1997). However other groups showed motor imagery resulted in either no change to the H-reflex (Aoyama & Kaneko, 2011; Bonnet et al., 1997; Hashimoto & Rothwell, 1999), or even a decrease in H-reflex amplitude (Oishi, Kimura, Yasukawa, Yoneda, & Maeshima, 1994). Studies using mechanical stimuli to elicit spinal stretch reflexes have provided more consistent evidence of spinal (and/or peripheral) modulation with imagery. Bonnet et al. (1997) showed that ipsilateral T-reflexes during imagined isometric plantar flexion increased more for strong contractions, compared to weak contractions (the H-reflex did not modulate during the same task, see above; also see Aoyama & Kaneko, 2011). Li, Kamper, Stevens, and Rymer (2004) reported that the probability of eliciting an M1 response in a finger flexor muscle following a finger extension perturbation increased during imagery of a finger flexion action. Critically however, no previous motor imagery studies using mechanical stimuli controlled pre-perturbation muscle activation, which is known to have a strong influence on the magnitude of
the spinal stretch response (Pruszynski et al., 2009). It is likely that the observed M1 modulation by these studies resulted from subthreshold (or suprathreshold; Bonnet et al., 1997) changes in background muscle activation during imagery performance.

The purpose of this experiment was to employ kinesthetic motor imagery to determine whether the long-latency stretch response can exhibit instruction-dependent modulation in the absence of an overt voluntary response. Participants performed a passive condition (DNI) where they were instructed to “not intervene with the perturbation” and an active condition (ACT) with the instruction to “compensate against the perturbation as fast as possible”. In line with previous findings, including experiments 1-5 of this dissertation, it was hypothesized that the ACT condition would result in a larger M2 response compared to DNI and the M1 response would remain unaffected by intentional set. In the primary condition of interest (IMAGERY), participants were instructed to “physically not intervene with the perturbation, but imagine compensating against the perturbation as fast as possible, and the feeling that this would produce”. Across all three conditions, participants held a wrist extension preload prior to a perturbation, thus background EMG activity was controlled, and we did not anticipate any changes to the M1 response. However, given that kinesthetic motor imagery has been shown to activate many of the same supra-spinal regions as physical movement execution (Decety et al., 1994; Jeannerod, 2001; Roth et al., 1996; Ryding et al., 1993), and that the long-latency stretch response engages common fronto-parietal regions with the voluntary response (Omrani et al., 2016; Pruszynski & Scott, 2012; Scott, 2004), we hypothesized that IMAGERY would result in a larger M2 response than DNI. Alternatively, if the overt execution of a voluntary response is required for instruction-dependent M2 modulation to occur, IMAGERY would either not
modulate the M2 response, or modulation would be limited to a subset of trials where a partial voluntary response “leaked out” into the wrist flexor EMG recording.

6.2 Methods

6.2.1 Participants, apparatus, and procedures

Eighteen (11 female, 7 male, aged 20-33 years) healthy right handed volunteers participated in this experiment. Due to equipment malfunction, data was not available from one participant. The protocol was approved by the University of British Columbia Behavioural Research Ethics Board. Informed written consent was collected before each testing session. After participants signed the consent form, motor imagery abilities were assessed using the Motor Imagery Questionnaire (MIQ-R; see Appendix A; Gregg, Hall, & Butler, 2010). This assessment includes 12 questions related to internal visual motor imagery, external visual motor imagery, and kinesthetic motor imagery. The entire testing session lasted approximately 2 hours and participants were compensated $20 (CAD) for their time.

After completion of the MIQ-R, participants sat in a height-adjustable chair with an oscilloscope ~1 meter in front resting on a table. Both elbows were flexed at 100 degrees and hands were semi-pronated with the wrist joints aligned with the manipulanda rotational axes. Connected to the right manipulandum was a torque motor (Aeroflex TQ 82W-1C). A metal handle adjoined to the motor shaft was placed at the right metacarpophalangeal joints. Padded stops on either side of the wrists were tightened to prevent lateral wrist movement. Custom molded thermoplastic surrounded the hand and allowed movement around the right wrist while keeping the fingers relaxed. Angular position feedback of the right wrist was continuously provided on the oscilloscope. The home position was 10 degrees of wrist flexion and visually defined on the oscilloscope by arrows. The left manipulandum was immovable and positioned at
10 degrees of wrist flexion. Custom molded thermoplastic also surrounded the left wrist and kept the left metacarpophalangeal joints in contact with the manipulandum handle which allowed the monitoring of wrist tension. Prior to commencing practice of the main conditions, 3 maximum voluntary wrist flexion contractions were collected from both the right and left sides.

For the practice and testing phases of the experiment, every trial began with an auditory warning signal (80 decibels, 50 ms, 500 Hz) and a right wrist extension preload slowly increased (over 500 ms) to 0.25 Nm. Participants were instructed to “resist by lightly activating wrist flexors against the load and to hold their right wrist at the home position”. Once attaining the home position, participants closed their eyes. An evenly distributed variable foreperiod (2,500-3,500 ms) followed the warning signal and was terminated by a large wrist extension perturbation (1.5 Nm, 150 ms). To prevent active relaxation or “letting-go” (which could confound DNI and IMAGERY trials), the preload level of extension torque (0.25 Nm) remained for 1000 ms after the perturbation. A minimum of 10 seconds was given between trials.

The order of conditions was counterbalanced between participants during the testing phase but remained fixed during practice because it was essential that participants understood how to perform the DNI and ACT conditions (and the associated sensations) prior to IMAGERY. All participants began practice with 10 trials of the ACT condition where they were instructed to “compensate against the perturbation as fast as possible”. To encourage participants to respond as quickly as possible, verbal feedback was provided after every trial of either “good” if the voluntary response appeared continuous with M2 (i.e., premotor RT of ≤ 100 ms), or “go faster” if the voluntary response did not appear to overlap M2. This was followed by 10 trials of the DNI condition with the instruction to “not intervene with the perturbation”. Participants then performed 10 trials of IMAGERY where they were instructed to “physically not intervene with
the perturbation, but imagine compensating against the perturbation as fast as possible, and the feeling that this would produce”. During practice of these latter two conditions, verbal feedback of “good” was given if no obvious voluntary response (100-200 ms) was present in the wrist flexor EMG or participants were informed that “they responded” when a voluntary response was observed. After the first 30 trials of practice, participants were provided with 2 minutes of rest. Participants repeated the practice sequence a total of three times.

The testing phase of the experiment was identical to practice trials with the exception of no verbal performance feedback (and counterbalancing of the conditions). During the testing phase, participants performed 10 trials of one condition (e.g., ACT), followed by 10 trials of the next condition (e.g., IMAGERY), and 10 trials of the last condition (e.g., DNI). Two minutes of rest was given prior to beginning the same sequence. Three complete blocks of trials were performed for a total of 90 testing trials. Error trials (e.g., false starts, not responding on an ACT trial, or not beginning at the home position) were recycled online.

6.2.2 Data Collection and Analysis

EMG data were collected from the muscle bellies of right flexor carpi radialis (FCR), right extensor carpi radialis (ECR), left FCR, and left ECR using pre-amplified surface electrodes connected to an external amplifier (Model DS-80, Delsys Inc., Natick, MA). EMG signals were amplified at 3K and bandpass filtered from 20-450 Hz. Electrodermal activity (EDA) was recorded from the left palm using disposable cloth electrodes placed on the thenar and hypothenar eminences of the left hand and connected to a skin conductance module (Cambridge Electronic Design (CED) 2502, Cambridge, UK). Right wrist positional data were collected using a potentiometer (Bourns, Model 6637S-1-103, Riverside, CA) attached to the right wrist manipulandum. Signals were digitized at 2 kHz using a 1401plus data acquisition
system and Spike2 (CED) computer software. A customized LabVIEW (National Instruments, Austin, TX) program was used for offline data analysis.

At the beginning of analysis, EMG data were baseline corrected and full-wave rectified. EMG data were divided into 5 time intervals relative to perturbation onset. The first epoch occurred from -100 ms until the perturbation onset and was used to obtain a measure of baseline EMG. The second epoch occurred from 25-50 ms post-perturbation onset and captured the M1 response. The third (M2a: 50-75 ms) and fourth (M2b: 75-100 ms) epochs contained the M2 response. The final epoch provided a measure of volitional activity (100-200 ms) however the voluntary response can sometimes begin during the M2b epoch (Forgaard et al., 2015; Ravichandran et al., 2013). Integrated values for each epoch were taken from the rectified EMG traces on a trial-by-trial basis. The mean integrated baseline EMG while holding the 0.25 Nm preload (for each participant) was used to normalize the respective data from the M1, M2a, M2b, and voluntary epochs. For normalization of the M1, M2a, and M2b epochs (which were each of 25 ms duration), the baseline EMG value (based on 100 ms duration) was divided by 4. No correction was needed for normalization of the voluntary epoch (100 ms duration). A normalized value of 1.0 corresponds to integrated area equivalent to the (duration-corrected) baseline epoch.

In order to objectively determine the presence of any voluntary response “leaks” on IMAGERY trials, a .95 confidence interval (CI) was established around the mean of activity for each participant’s normalized voluntary epoch for the DNI condition. Any IMAGERY trials that had voluntary epoch activity that exceeded the value established by the upper-bound of the .95 CI for DNI were classified as IMAGERY$_{\text{Leaked}}$. It is important to note that often these voluntary response leaks were subtle and the experimenter did not notice them during data collection (they were smaller than the obvious voluntary responses observed during early practice).
One confound in a motor imagery experiment (particularly in the event of a null finding), is that the researcher does not know if imagery does not having the ability to influence a response, or if the participants were not correctly engaging in imagery (in the case of the present experiment, they could just be passive). In other words, as imagery does not typically result in the overt appearance of a voluntary response, a secondary measure that motor imagery is actually being used is needed. In order to confirm that participants were engaging in imagery, we monitored EDA, a measure of sympathetic nervous system activity. A phasic response in EDA is typically observed beginning ~2 seconds following onset of a ballistic movement and peaking at ~4 seconds (Lakhani, Miyasike-daSilva, Vette, & McIlroy, 2013). A phasic response over a similar time-course is observed following a whole body seated or standing mechanical perturbations (Lakhani et al., 2013; Sibley, Mochizuki, Esposito, Camilleri, & McIlroy, 2008). It has been hypothesized that the sympathetic nervous system does not distinguish physical movement from imagined movement (Collet & Guillot, 2010). Therefore, we expected larger phasic EDA responses in the ACT condition compared to DNI and if participants correctly engaged in IMAGERY, the phasic EDA for this condition should also appear larger than DNI. Due to technical issues, EDA data was not available from 6 participants.

EDA recordings were low-pass filtered at 10 Hz and analyzed using custom-written LabView software. Phasic responses were consistently observed in the ACT condition, however not all participants showed phasic activity in DNI and IMAGERY and so a method was required to quantify activity on trials without a phasic response. Thus we first analyzed each participants’ ACT trials. The onset of the phasic response was defined as the time at which a sustained positive slope between 0.5 and 5 seconds post-perturbation (Lakhani et al., 2011). The peak time was determined as the time at which the peak was reached. Marker placement was visually
verified and adjusted if necessary. Any ACT trials without a clear phasic response were excluded in this initial analysis. For each participant, we then calculated the mean ACT phasic response onset time and the peak time. These temporal values were subsequently used to determine an onset EDA value and a peak EDA value for every trial from all conditions within a participant. The onset value was subtracted from the peak value on a trial-by-trial basis in order to quantify the magnitude of each phasic response.

For each predefined response epoch (baseline, M1, M2a, M2b, voluntary) of wrist flexor EMG data, repeated measures ANOVA tests were used to compare the effect of Condition (ACT, DNI, IMAGERY) on the magnitude of the normalized integrated activity (with the exception of baseline epoch where raw integrated activity was used). Wrist flexor EMG data were then analyzed with IMAGERY_{Leaked} trials separated using an ANOVA to examine the effect of a leaked voluntary response on stretch response data (ACT, DNI, IMAGERY_{Leaked}). Paired samples t-test were used to compare EMG activity for DNI and IMAGERY_{Non-Leaked} trials. Phasic EDA response magnitude was analyzed using a repeated measures ANOVA comparing the effect of Condition (ACT, DNI, IMAGERY_{Leaked}). Dunn-Bonferroni corrected t-tests were used to interpret significant main effects. Mauchly’s test was used to test for any violations to the assumption of sphericity, and if necessary, Greenhouse-Geisser corrected degrees of freedom and p-values were reported. Effect sizes were quantified using partial eta squared ($\eta_p^2$) and mean values were reported (± standard deviations). Statistical significance for each test was set at $p = .05$. 

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6.3 Results

6.3.1 Motor Imagery Questionnaire

The results of the MIQ-R questionnaire revealed that all participants were capable of engaging in the three forms of assessed motor imagery. The mean score for internal visual imagery ability was 5.7 (±0.8; between “somewhat easy to see” and “easy to see”) and the mean score for external visual imagery was 5.9 (±0.8; between “somewhat easy to see” and “easy to see”). For kinesthetic imagery ability, the mean score was 5.6 (±0.8; between “somewhat easy to feel” and “easy to feel”). The lowest score was 4.25 (between “neutral” and “somewhat easy to feel”) and the highest score was 7 (“very easy to feel”).

6.3.2 The influence of kinesthetic motor imagery on the long-latency stretch response

The M1 and M2 stretch responses are influenced by background state of the motoneuron pool at the time of perturbation delivery (Pruszynski et al. 2009). For studies examining the sensitivity of stretch responses to different goals and/or instructions, it is essential that baseline EMG activity prior to the perturbation remain consistent amongst the various conditions. This was confirmed in our study by the analysis of integrated baseline EMG which showed no significant differences amongst the conditions, $F(2,32) = 0.20, p = .820, \eta_p^2 = .01$. Similarly, activity during the M1 response was not statistically different between conditions, $F(2,32) = 1.68, p = .202, \eta_p^2 = .10$. An analysis of the M2a epoch revealed significant differences, $F(2,32) = 17.13, p < .001, \eta_p^2 = .52$, and post-hoc testing confirmed that the M2a activity for the ACT (8.3 ± 3.8 NU) condition was significantly larger than DNI ($p = .001; 6.8 ± 3.6 NU$). The ACT condition was also significantly ($p = .002$) larger than IMAGERY (7.0 ± 3.5 NU) but no significant difference ($p = .522$) was observed between DNI and IMAGERY. Similar findings
were observed during the M2b epoch following the significant omnibus ANOVA, \( F(2,32) = 26.89, p < .001, \eta^2_p = .63 \). The largest response was observed for the ACT condition (9.8 ± 4.8 NU; \( p \) values < .001) followed by IMAGERY (5.3 ± 3.8 NU) and DNI (\( p = .157; 4.7 ± 3.3 \) NU). An analysis of the voluntary epoch was also statistically significance, \( F(2,32) = 46.38, p < .001, \eta^2_p = .74 \). Post-hoc testing showed that all three conditions differed. As expected, the largest response was observed for ACT (14.9 ± 8.3 NU; \( p \) values < .001), followed by IMAGERY (2.0 ± 1.6 NU), and DNI (\( p = .018; 1.3 ± 1.1 \) NU). See ensemble wrist flexor EMG data in figure 6.1.

![Figure 6.1](image_url)

**Figure 6.1.** Experiment 8 group \((n = 17)\) rectified and normalized (to baseline EMG) wrist flexor EMG data. Value of 1 equivalent to baseline EMG levels (holding a 0.25 Nm wrist extension preload). DNI condition: dashed black line. ACT condition: solid black line. IMAGERY condition: solid grey line. M1 epoch (25-50 ms); M2a epoch (50-75 ms); M2b epoch (75-100 ms); Voluntary epoch (100-200 ms).
As the present experiment was specifically concerned with the relationship between instruction-dependent M2 modulation and the overt execution of a voluntary response, we further investigated the influence of leaked voluntary responses on the modulation of stretch response activity. On average, these voluntary response “leaks” occurred on 40.9% (± 21.7%) of IMAGERY trials (see classification criteria in Methods). The number of leaked trials within a participant ranged from 0% to 90.0% of trials. See a distribution of the number of leaked response in figure 6.2.

![Box-and-whisker plot displaying the percentage of leaked voluntary responses on IMAGERY trials from the participants.]

**Figure 6.2.** Box-and-whisker plot displaying the percentage of leaked voluntary responses on IMAGERY trials from the participants.

Before examining the influence of a leaked voluntary response, we compared IMAGERY<sub>Non-Leaked</sub> and DNI differed in terms of stretch response activity. No significant differences were observed between these two conditions for the baseline, \((t(16) = 0.96, p = .352)\),
M1 \((t(16) = 0.28, p = .781)\), M2a \((t(16) = 0.95, p = .354)\), or M2b \((t(16) = -0.28, p = .978)\) epochs. However, activity during the voluntary epoch was reduced for Imagery\textsubscript{Non-Leaked} \((t(16) = 5.20, p < .001)\). See ensemble EMG comparisons in figure 6.3.

![Graph showing normalized wrist flexor EMG data for DNI and IMAGERY\textsubscript{Non-Leaked}.]

**Figure 6.3.** Experiment 8 group \((n = 17)\) rectified and normalized (to baseline EMG) wrist flexor EMG data for DNI and IMAGERY\textsubscript{Non-Leaked}. Value of 1 equivalent to baseline EMG levels (holding a 0.25 Nm wrist extension preload). DNI condition: dashed black line. IMAGERY\textsubscript{Non-Leaked} condition: solid grey line. M1 epoch (25-50 ms); M2a epoch (50-75 ms); M2b epoch (75-100 ms); Voluntary epoch (100-200 ms).

In order to objectively determine whether the leaking of a voluntary response influenced baseline EMG, the M1, or the M2 response, we reanalyzed the wrist flexor data, comparing ACT, DNI, and IMAGERY\textsubscript{Leaked}, using the 16 participants with IMAGERY\textsubscript{Leaked} trials. Kinematic and ensemble wrist flexor EMG data is presented in figure 6.4.
Figure 6.4. Experiment 8 group wrist position and normalized wrist flexor EMG data for the participants \((n = 16)\) displaying leaked voluntary responses on IMAGERY trials. DNI condition: dashed black lines/bars. ACT: solid black lines/bars. IMAGERY Leaked: solid grey lines/bars. A. Wrist angle (in degrees). Positive values denote extension and negative values flexion. B. Group normalized integrated EMG data (with inter-participant standard error bars) from the 3 stretch response epochs examined. M1 (25 to 50 ms); M2a (50-75 ms); M2b (75-100 ms). Asterisks denote statistically significant differences \((p < .05)\). C. Rectified normalized wrist flexor EMG data, along the same time course as the displacement data presented in panel A. D. Wrist flexor EMG data focused on the epochs of interest.
Similar to the preliminary analysis above, no significant difference was observed during the baseline epoch, $F(2,30) = 1.00, p = .378, \eta^2_p = .06$. Unexpectedly however, the M1 epoch approached statistical significance, $F(2,30) = 2.78, p = .079, \eta^2_p = .16$. Given that previous motor imagery studies have shown the M1 response to increase during imagery performance (Bonnet et al., 1997; Li et al., 2004), we conducted a post-hoc analysis on this epoch to examine which comparisons approached a statistical difference. Post-hoc testing showed that the IMAGERY\text{Leaked} trials (which had the largest activity during the M1 epoch; 3.6 ± 1.5 NU*ms) had a $p$ value of .120 when compared with DNI trials (which had the smallest M1 value; 3.2 ± 1.2 NU*ms). Thus, when background EMG was properly controlled prior to a perturbation, motor imagery did not significantly influence the short-latency stretch response.

As we expected, statistically significant main effects were found for the M2a ($F(2,30) = 9.79, p = .001, \eta^2_p = .40$), M2b ($F(2,30) = 18.35, p < .001, \eta^2_p = .55$), and the Voluntary epochs ($F(2,30) = 43.50, p < .001, \eta^2_p = .74$). Post-hoc analysis on the M2a data revealed that activity during this epoch was categorically sensitive to the presence of a voluntary response. For the ACT (8.3 ± 3.9 NU*ms; $p = .002$ vs. DNI) and IMAGERY\text{Leaked} (8.0 ± 3.5 NU*ms; $p = .021$ vs. DNI) trials, M2a activity was increased compared to the DNI condition where no voluntary response occurred (6.9 ± 3.6 NU*ms; see figure 6.4). ACT and IMAGERY\text{Leaked} did not differ ($p > .999$). While activity during the M2a epoch demonstrated a generic increase on trials with a voluntary response, post-hoc analysis of the M2b epoch showed that all three conditions differed significantly. The ACT condition (9.9 ± 4.9 NU*ms) was largest followed by (p < .001) IMAGERY\text{Leaked} (6.6 ± 4.0 NU*ms) and (p = .048) DNI (4.8 ± 3.3 NU*ms). Similarly, all conditions during the Voluntary epoch were statistically different (all $p$ values < .001). The
largest activity was observed for the ACT condition (15.3 ± 8.3 NU*ms), followed by IMAGERY\textsubscript{Leaked} (3.6 ± 2.2 NU*ms), and DNI (1.4 ± 1.1 NU*ms).

In summary, our findings revealed that the M2a epoch was sensitive to the presence of a voluntary response. On ACT trials where a large voluntary response always occurred, and the ~40% IMAGERY trials where a small voluntary response “leaked out” into the wrist flexor EMG, a categorical increase in M2a activity was observed compared to the passive DNI condition. The M2b epoch displayed further modulation which mirrored the activity observed during the voluntary epoch. These findings strongly suggest that the execution of a voluntary response is a pre-requisite for instruction-dependent M2 modulation.

6.3.3 The influence of kinesthetic imagery on electrodermal activity

We originally collected EDA data as secondary measure to ensure participants correctly engaged in kinesthetic motor imagery. This would have been critical to demonstrate in the event of a null finding with the stretch response data (see Methods). Overall, we found EDA responses to be highly variable (phasic activity was not even observed from 6 participants). While 11 participants displayed a phasic response on a majority ACT trials (mean onset of 1668.1 ms (± 200.7) relative to perturbation onset; mean peak time of 3826.9 ms (± 751.1)), some participants displayed no phasic response on DNI and/or IMAGERY trials or very inconsistent responses. Thus activity during the time of the expected phasic EDA response sometimes decreased (resulting in negative values). An analysis of the magnitude of phasic EDA activity revealed a significant main effect of condition, $F(2,22) = 6.33$, $p = .007$, $\eta^2_p = .37$. The post-hoc test showed that the phasic EDA response for ACT (0.38 ± 0.38 µmho) was significantly ($p = .028$) larger than DNI (0.02 ± 0.05 µmho). The IMAGERY\textsubscript{Leaked} trials (0.19 ± 0.22 µmho) approached
a statistical different with DNI ($p = 0.057$) but were not reliably different than ACT ($p = 0.400$).

See the ensemble EDA data in figure 6.5.

**Figure 6.5.** Experiment 8 group EDA data for the 11 participants displaying leaked voluntary responses on IMAGERY trials and consistent phasic EDA responses for the ACT condition. DNI condition: dashed black lines/bars. ACT condition: solid black lines/bars. IMAGERY Leaked: solid grey lines/bars. A. Ensemble EDA data. As EDA values varied considerably trial-to-trial, the baseline value (determined for 10 ms prior to a perturbation) were subtracted from each trial before averaging. B. Mean phasic response amplitudes with standard error bars.

### 6.4 Discussion

The present experiment was concerned with the relation between the presence of a voluntary response and instruction-dependent modulation of the long-latency stretch response. Although the earlier experiments in this dissertation, and work by other groups (e.g., Pruszynski, Kurtzer, & Scott, 2011), has shown that a superimposed voluntary response (or triggered reaction) cannot account for all M2 modulation, it remained unclear whether instruction-dependent facilitation of the M2 response still relies on the execution of a voluntary response. In order to test this, we instructed participants to engage in kinesthetic motor imagery of an ACT task while physically performing a DNI task. If M2 modulation could occur in the absence of a
voluntary response, we hypothesized that the IMAGERY condition would result in a larger M2 response than DNI, without concomitant changes in voluntary epoch activity. Contrasting this prediction, we found that on ~40% of IMAGERY trials, a small voluntary response “leaked” out and critically, it was only on these IMAGERY\textsubscript{Leaked} trials that the M2 response increased (compared to DNI; see figure 6.4). These findings suggest that although a superimposed voluntary response does not account for all M2 period changes, the overt execution of a voluntary response remains a pre-requisite for instruction-dependent M2 modulation to occur.

6.4.1 Voluntary response leaks; Evidence of subliminal activation or failed inhibition?

Motor imagery is defined as the internal simulation of an action in the absence of overt motor output (Decety, 1996). Previous studies have reported small amounts of EMG activity in task relevant muscles during motor imagery performance (e.g., Guillot et al., 2007; Maslovat et al., 2013). Consistent with the definition of imagery, the leaked EMG activity reported in these studies was not of sufficient magnitude to generate observable movement (i.e., physical limb displacement). In the present experiment, the mechanical perturbation produced substantial wrist motion, even on DNI trials (see figure 6.4A). Therefore we could not determine if the leaked voluntary responses were sufficient to overcome the inertial properties of the limb and generate wrist movement, if investigated with the wrist in an unperturbed state. The IMAGERY\textsubscript{Leaked} trials did appear to have a reduced peak extension amplitude and greater peak flexion amplitude than DNI (see figure 6.4A). The larger M2 response and increased activity during the voluntary epoch on IMAGERY\textsubscript{Leaked} trials both likely contributed to this modified joint motion.

While it was suggested that the leaking of a voluntary response into task-relevant musculature during motor imagery performance is evidence for subliminal activation of the motor system (Maslovat et al., 2013), or that imagery lies on a continuum between rest and
movement execution (Guillot et al., 2010, pg. 88), it was also proposed that a parallel inhibitory mechanism prevents the overt appearance of an action during imagery (Jeannerod, 2001). The exact locus of this movement inhibition remains subject to debate, but one possibility is that it involves a fronto-parietal network inhibiting primary motor cortex (Jeannerod, 2001; Kasess et al., 2008; Schwoebel et al., 2002). Alternatively, inhibition may occur via descending inputs onto spinal interneurons which prevents activation of alpha motoneurons (Jeannerod, 2001). This latter mechanism has not been confirmed in the imagery literature but was demonstrated in non-human primates performing an instructed-delay task (Prut & Fetz, 1999).

The present study was not designed to address the neural mechanisms underlying kinesthetic imagery, but interpretation of our findings in the context of what is known about the circuitry involved in the long-latency stretch response, leads us to believe that spinal interneurons were not inhibiting alpha motoneurons during the IMAGERY condition. It is well accepted that a transcortical pathway involving primary sensorimotor cortex and the corticospinal tract contributes to the M2 response (Cheney & Fetz, 1984; Evarts & Tanji, 1976; Pruszynski et al., 2014). The efferent limb of this pathway is believed to be shared with voluntary activation of the same muscle. Because of this common transcortical route, if a spinal inhibition mechanism prevented activation of alpha motoneurons during IMAGERY, we would expect suppression of the M2 response compared to DNI. Contrary to this, the M2 response was identical for IMAGERYNon-Leaked and DNI conditions (figure 6.3) and was increased on IMAGERYLeaked trials (figure 6.4).

6.4.2 Task engagement and the long-latency stretch response

Primary motor cortex is the most cited candidate region contributing to modulation of the long-latency stretch response (Evarts & Tanji, 1976; Kimura et al., 2006; Omrani et al., 2014;
This area receives substantial inputs from motor associative areas and sensory regions and an estimated 30-40% of corticospinal tract neurons originate here (Rizzolatti & Strick, 2013). A TMS silent period of primary motor cortex can suppress M2 modulation in some conditions (Kimura et al., 2006; Spieser et al., 2013) and single-cell recordings from neurons in primary motor cortex display goal-dependent changes at an appropriate time-course to account for stretch response changes in muscle (Evarts & Tanji, 1976; Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski et al., 2014). The dentate nucleus was also implicated in M2 modulation possibly via connections with primary motor cortex (Strick, 1983). However, the task used by Strick (1983) always involved the animals actively moving towards a target, there was no passive control (similar to Evarts & Tanji, 1976). Moreover, cerebellar-primary motor cortex connections were proposed to mediate the M3 response (Lee & Tatton, 1975), which corresponds closely to the M2b epoch using our nomenclature.

Supplementary motor area (SMA) (Hummelsheim, Wiesendanger, & Bianchetti, 1986; Spieser et al., 2013) and posterior parietal cortex (PPC; Omrani et al., 2016) are other candidate regions that may mediate M2 modulation, either via intra-cortical connections with primary motor cortex or direct corticospinal projections. For example, SMA stimulation influenced the firing rates of neurons in primary motor cortex activated by a mechanical perturbation (Hummelsheim et al., 1986), and a TMS induced silent period of SMA attenuated instruction-dependent M2 modulation between let-go and resist instructions (Spieser et al., 2013). This frontal area has an integral role in motor planning and movement initiation (Cunnington, Windischberger, Deecke, & Moser, 2002; Haith, Pakpoor, & Krakauer, 2016) and also in the inhibition of a planned action (Haydук-Costa, Drummond, & Carlsen, 2013; Tanji & Kurata,
1985; Tanji, Kurata, & Okano, 1985). Not surprisingly, SMA is highly active during motor imagery (Kasess et al., 2008). Kasess and colleagues (2008) found strong inhibition of primary motor cortex via connections with SMA which the authors proposed was to prevent movement execution during imagery performance. Given that SMA has been implicated in pre-setting primary motor cortex for instruction-dependent M2 modulation (Spieser et al., 2013), and this region inhibits primary motor cortex during imagery (Kasess et al., 2008), it is reasonable to assume that SMA may have contributed to the generic M2a increase observed in our study.

In a recent study by Omrani and colleagues (2016), neural recordings were taken from various fronto-parietal regions (but not SMA) while non-human primates performed different perturbation tasks. When the animals switched from a non-engaged (watching a movie) condition to a task involving postural control (i.e., maintaining the limb at the home position), the earliest change following the onset of a perturbation was found in PPC (~23 ms after perturbation onset). Primary motor cortex neurons displayed an increased firing rate in the posture task, ~15 ms later (than PPC). A different pattern of results was obtained from a target selection task, where the animals were always engaged in responding to the perturbation, but required different magnitude motor responses in the arm depending on the target location. The first change between targets was observed in primary motor cortex at ~66 ms, followed ~30 ms later by dorsal premotor cortex. PPC neurons did not display modified firing rates until ~150 ms post-perturbation. We believe that in our study, the generic M2a increase on trials with a voluntary response was reflective of task engagement and was in part, behaviourally analogous to the differences between the movie and posture tasks reported by Omrani et al. (2016).

Even though many authors have cited primary motor cortex as a candidate region involved in long-latency stretch response modulation (Evarts & Tanji, 1976; Omrani et al., 2014;
Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski et al., 2014; Spieser et al., 2010), the majority of evidence suggests that this area does not play a role in facilitating the M2 increase on ACT trials (compared to DNI). For example, sensory-evoked cortical potentials consistent with the afferent volley through sensorimotor cortex were not found to modulate between these verbal instructions (MacKinnon et al., 2000). Likewise, no differences were found in short-interval intra-cortical inhibition within primary motor cortex (Lewis et al., 2006), and a TMS-induced silent period of primary motor cortex had no influence on instruction dependent M2 modulation between DNI and ACT conditions (Shemmell et al., 2009). It was suggested that the site of instruction-dependent modulation occurs downstream of primary motor cortex (Lewis et al., 2006; MacKinnon et al., 2000; Shemmell et al., 2009). Although the role of descending inputs on the modulation of spinal excitability during the long-latency stretch response has not been examined (to our knowledge), the generic M2a increase on trials with a voluntary response (ACT and IMAGERY\text{Leaked}) could have been produced by descending inputs influencing one or more spinal mechanisms. For example, in a standard (non-perturbation) RT task, H-reflexes increase (~40 ms) prior to the onset of a voluntary response which was suggested to be due to removal of pre-synaptic inhibition of Ia afferents (Hasbroucq, Akamatsu, Burle, Bonnet, & Possamaï, 2000). Perhaps in our study, prior to the onset of a voluntary response on ACT trials and on IMAGERY\text{Leaked} trials, descending inputs from various higher centres such as PPC or SMA reduced pre-synaptic inhibition of the Ia afferents, and this occurred at an appropriate time-course to result in a generic facilitation of the long-latency stretch response.

While the M2a epoch seems to only be categorically sensitive to the presence of a voluntary response (between DNI and ACT), other goal-dependent behaviours have been
observed during the M2b epoch, including the selection to move between different targets while reaching (Nashed et al., 2014) and the scaling based on target location (Pruszynski et al., 2008). While the first portion of the long-latency stretch response receives contributions from reverberating Ia afferents acting over a spinal pathway (Hagbarth et al., 1981) and a transcortical route involving fronto-parietal circuits and the corticospinal tract (Cheney & Fetz, 1984; Omrani et al., 2014; Pruszynski et al., 2014), a cerebellar-primary motor cortex pathway may make contributions during the latter half of the response (Lee & Tatton, 1975; Strick, 1983). Lee and Tatton (1975) were the first to describe two separate reflex peaks during the long-latency stretch response period which were named M2 (~50 ms) and M3 (~85 ms). Although we did not specifically examine the presence of two separate stretch responses during the M2a and M2b epochs, we did observe the largest amount of activity during the M2b epoch on ACT trials, followed by IMAGERY\_Leaked, and the smallest amount of activity on DNI trials. These M2b epoch differences could be due to varying levels of input from the pathway that contributes to the M3 response. Alternatively, this is also the time period in which a voluntary response can superimpose onto the long-latency stretch response (Crago et al., 1976; Manning et al., 2012; Ravichandran et al., 2013), thus the M2b differences could be due to varying amounts of voluntary superimposition.

6.4.3 Conclusion

Modulation of the long-latency stretch response is inherently linked with volition (Pruszynski & Scott, 2012). Many studies over the past 60 years (and especially over the past decade) have shown that this stretch response demonstrates features traditionally associated with voluntary motor behaviour. In this study, we employed kinesthetic motor imagery to examine whether instruction-dependent M2 modulation relied on the overt execution of a voluntary
response. During IMAGERY performance, the long-latency stretch response only increased on trials where a partial voluntary response leaked out into the EMG recording. Interestingly, this facilitation during the early portion of the long-latency stretch response appeared similar to trials where participants actively compensated against the perturbation. On IMAGERY trials without a leaked voluntary response, the long-latency stretch response appeared identical to the control (passive) condition. Thus our findings revealed that the overt execution of a voluntary response is required for instruction-dependent modulation of the long-latency stretch response.
Chapter 7: General Discussion

The overall goal of this dissertation was to examine the contributions of a voluntary response on instruction-dependent modulation of the long-latency stretch response. While it is well accepted that a mechanical perturbation can elicit short- and long-latency responses in stretched muscle (Hammond, 1956; Lee & Tatton, 1975; Pruszynski et al., 2008), the onset latency of a proprioceptive triggered voluntary response has remained a topic of contention (Crago et al., 1976; Day et al., 1983; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980). Given that the voluntary response often appears continuous with the long-latency stretch response, and because premotor RT cannot be accurately determined when a voluntary response is preceded by a non-quiescent baseline, this matter has remained unresolved. In addition, as EMG activity during the long-latency response modulates based on the voluntary intention of the performer, it has been disputed whether the appearance of a modified stretch response reflects gain modulation of the underlying neural circuitry (Hammond, 1956; Lee & Tatton, 1975; Pruszynski et al., 2008), or rather is an artefact of a superimposed voluntary response (Crago et al., 1976; Manning et al., 2012; Ravichandran et al., 2013; Rothwell et al., 1980). In other words, researchers have been trying to determine if the long-latency stretch response displays a level of “intelligence” like a voluntary response, or the appearance of volitional characteristics only occur because a voluntary response begins during the time period associated with the stretch response.

We addressed these inter-related issues with a number of different techniques. First, behavioural manipulations that are known to influence the latency and magnitude of a voluntary response in a traditional RT task were employed in a perturbation protocol and we examined the corresponding changes to EMG activity during the long-latency stretch response (chapters 2 &
3). This was followed with an investigation of a potential startle/StartReact mechanism of voluntary response triggering (chapters 4 & 5) including the role it may play in instruction-dependent M2 period modulation. In the final experiment (chapter 6), we employed kinesthetic motor imagery to determine whether the overt execution of a voluntary response was required for instruction-dependent modulation of the long-latency stretch response.

Similar to other studies that have examined modulation of upper-limb stretch activity, the short-latency response remained immutable to instruction and the long-latency stretch response displayed “voluntary-like” characteristics. When we compared a passive control condition to various active conditions involving a voluntary intervention in the stretched muscle, the M2a epoch consistently displayed an increase in activity (chapters 2, 3, 4, & 6). We have referred to this as “generic modulation” because it was uninfluenced by voluntary response latency and/or magnitude (chapters 2 & 3), and was present even in the imagery experiment when a partial voluntary response “leaked out” into the wrist flexor musculature (chapter 6). This generic M2a facilitation only appeared to be sensitive to the presence of a voluntary response in stretched muscle. We also demonstrated that M2a modulation could occur consistently in the absence of a startle response and was thus unlikely to be produced by the StartReact effect (chapters 4 & 5). Modulation during the M2b epoch was more sophisticated than a categorical on/off switch between passive and active conditions, with activity tending to mirror the patterns observed during the voluntary response epoch (chapters 2, 3, 4, 5, & 6). For example, in chapter 2 we found the largest M2b epoch when the voluntary response was largest (and earliest), and in chapter 3 we observed aging foreperiod effects where M2b epoch activity on active conditions increased with increased temporal resolution of perturbation delivery. We also showed that a
voluntary response was consistently elicited at a latency that began during the M2b epoch, without the need to elicit a startle reaction (chapter 5).

The general conclusion from this dissertation is that instruction-dependent M2 modulation results from a hybrid of voluntary response superimposition and gain modulation of the underlying stretch response circuitry. We have shown that even though a hastened voluntary response cannot account for all changes to activity during the M2 response, the overt execution of a voluntary response remains a pre-requisite for instruction-dependent modulation to occur. Despite addressing the issue of M2 modulation primarily from a feedforward (i.e., voluntary superimposition) perspective, we agree with the suggestion by Scott and colleagues that a distinction between the reflexive and voluntary mechanisms underlying M2 modulation have been difficult to ascertain because both the M2 response and the voluntary response engage overlapping neural circuitry (Pruszynski et al., 2008; Scott, 2004). With the current consensus in the literature that multiple sources contribute to long-latency response period (Kurtzer, 2015; Matthews, 1991; Pruszynski & Scott, 2012; Shemmell et al., 2010), it is important to remember that one of these contributors is the beginning of a voluntary response. In this final chapter, I review the major conclusions from the studies in this dissertation and draw extensions to broader findings in the literature. Topics that will be discussed include M1 response immutability, why some groups have not found generic M2a modulation, and the different mechanisms that can contribute to the M2b epoch including a triggered reaction and the M3 response. I will also discuss the unresolved issue of choice RT in a perturbation task and different regions in the central nervous system that may contribute to stretch response modulation.
7.1 Is the M1 response completely immutable?

Throughout this dissertation, we replicated the finding that the spinally generated M1 response is immutable to the verbal instruction on how to respond to the mechanical perturbation (Hammond, 1956; Lee & Tatton, 1975; Manning et al., 2012; Rothwell et al., 1980). We also showed that the M1 response was uninfluenced by perturbation predictability (chapter 3), the presentation of a PPI stimulus prior to the perturbation (chapter 5), delivery of a SAS stimulus simultaneous with the perturbation (chapters 4 & 5), and engagement in kinesthetic motor imagery (chapter 6). There have been very few examples of M1 modulation in the literature and in nearly all of the documented cases (c.f., Mutha, Boulinguez, & Sainburg, 2008; c.f., Weiler, Gribble, & Pruszynski, 2018b; see below), either muscle activation levels changed prior to the perturbation (e.g., Bonnet et al., 1997; Mortimer, Webster, & Dukich, 1981; Sciarretta & Bawa, 1990) or a preload was not used and therefore subthreshold changes in motoneuron excitability could not have been ruled out (Aoyama & Kaneko, 2011; Li et al., 2004). For example, in a design that was similar to our experiments, Sciarretta and Bawa (1990) showed that the M1 response modulated when the same verbal instruction was given in a block of trials but not when the instruction was randomized trial-to-trial. Counterintuitive to what one may expect, the M1 response was reduced on compensate trials (compared to DNI), a pattern that was opposite to these authors’ M2 findings. However, baseline EMG was also reduced on compensate trials, even for the same preload torque level.

There are two examples of goal-dependent M1 response modulation in spite of matched pre-perturbation muscle activity (Mutha et al., 2008; Weiler et al., 2018b). Mutha and colleagues instructed participants to perform voluntary elbow extension movements towards a target. On random trials the target would jump forwards or backwards and this was occasionally followed
by a perturbation which displaced the limb forwards or backwards. Both the M1 and M2 responses were appropriately tuned to the new target location such that a perturbation moving the limb towards the new target reduced the amplitude of both stretch responses but delivery of the same perturbation when subjects intended to correct the limb to the other target location produced increased M1 and M2 responses. Recently, Weiler et al. (2018b) reported M1 response modulation while participants performed a postural control task (i.e., return the limb to the home position following a perturbation). The elbow was always perturbed into flexion (stretching triceps), but on random trials the wrist was also perturbed into flexion, extension, or was unperturbed. When the wrist was extended (and the hand was closer to the target), the M1 response in triceps was inhibited (even though this combination of multi-joint perturbations produced the largest amount of elbow flexion). By contrast, perturbing the wrist into flexion resulted in an increased M1 response (in spite of the least amount of elbow flexion). Again, this response was appropriate to aid the performer in returning the hand to the target. These authors also demonstrated that verbally instructing participants to “not intervene” or “compensate” following the same perturbation combinations did not produce goal-dependent EMG modulation until the long-latency response. Thus it would be appear that the M1 response can modulate in a goal-dependent manner under a finite set of conditions in which background muscle activation is properly controlled and targets (but not verbal instructions) are used to define behaviour.

More consistently documented changes to the M1 response occur following extended periods of operant conditioning (Norton & Wolpaw, 2018). When non-human primates were trained for many months with use of the tested limb restricted to a perturbation task, the animals could increase or decrease magnitude of the M1 response (Wolpaw, Braitman, & Seegal, 1983). Similar findings have been demonstrated with the M1 response (Evatt, Wolf, & Segal, 1989) and
the H-reflex in humans (Thompson, Chen, & Wolpaw, 2009). In the first study to use an IN/OUT task (Pruszynski et al., 2008) it was stated that two of the authors practiced for thousands of trials, but were unable to modulate the M1 response. By contrast, as observed in the present dissertation experiments, as well as studies by many other groups (Calancie & Bawa, 1985; Hammond, 1956; Kurtzer et al., 2008; Lee & Tatton, 1975; Pruszynski et al., 2008), participants can modulate the M2 response in a task appropriate manner with little-to-no training.

7.2 “Generic” M2a modulation

Across all experiments where we compared a DNI control condition to various active conditions involving a voluntary intervention in stretched muscle, we observed an increase in M2a activity (chapters 2, 3, 4, & 6). As this modulation was too early (50-75 ms post-perturbation onset) for voluntary response superimposition and because it appeared insensitive to the latency or magnitude of a voluntary response, we consider it as indirect evidence for gain modulation along one or more of the neural pathways contributing to the long-latency stretch response. Modulation during the M2a epoch is in line with findings from other groups that have used similar verbal instructions to examine the long-latency stretch response in wrist flexors (Lee & Tatton, 1975; MacKinnon et al., 2000). An increase in M2a activity on compensate trials was also observed by a study that provided arguably the strongest support for the voluntary superimposition hypothesis (Lewis et al., 2006). These authors employed very short duration perturbations that did not produce an M2 response (similar to previous studies by Lee & Tatton, 1982; Lewis, Perreault, & MacKinnon, 2005). Instruction-dependent M2 period modulation (between DNI and compensate conditions) was still demonstrated on these short perturbation trials, even in the absence of the M2 response. While this is strong evidence that a voluntary response can superimpose onto the long-latency stretch response, Lewis et al. (2006) reported a
further increase in the M2 period activity on compensate trials following a long-duration perturbation. This additional increase on trials where the M2 response was present was potentially a result of an increase in the early portion of the M2 response (i.e., gain modulation) in addition to the superimposition of a voluntary response. However, the authors also suggested that the motoneuron pool was in a disfacilitated state following the short-duration perturbation versus a facilitated state following the long-duration perturbation.

While we have consistently demonstrated instruction-dependent modulation early in the long-latency stretch response, it is important to mention that not all studies using verbal instructions have found modulation during this epoch (e.g., Capaday et al., 1994; Ravichandran et al., 2013). Some studies have even reported complete immutability of the entire long-latency response by instruction (e.g., Marsden et al., 1978). For example, the M2 response in flexor pollicus longus either does not modulate with instruction (Capaday et al., 1994; Marsden et al., 1978) or shows minimal flexibility compared to other muscles (e.g., biceps brachii) (Rothwell et al., 1980). Conceivably (and considered by these authors; e.g., Capaday et al., 1994; Rothwell et al., 1980) the perturbations used on the thumb were large enough to saturate the long-latency response, thus preventing further modulation with voluntary intent. Rothwell et al. (1980) went on to show that M2 in flexor pollicus longus can exhibit instruction-dependent modulation when smaller (non-saturating) perturbations were used.

The work by Ravichandran and colleagues (2013), cited extensively throughout this dissertation, did not observe instruction-dependent M2a modulation in triceps brachii. This study used a very large servo perturbation and from their EMG figures, it would appear that minimal activity was elicited during the early portion of the M2 response. This servo perturbation also produced a large short-latency response. It is known that motor units activated during the M1
response have a reduced firing probability during the M2 response (Calancie & Bawa, 1985). Thus it is plausible that these large servo perturbations activated nearly all motor units in triceps during the M1 response and these units became refractory during the M2a epoch thus rendering the stretch response incapable of modulation.

Even though the present dissertation was concerned with instruction-dependent modulation of the long-latency stretch response, more sophisticated modulation during the M2a epoch has been demonstrated by studies examining OFC. For example, M2a activity can modulate in a manner that appears to reflect knowledge of inter-segmental limb dynamics (Gielen et al., 1988; Kurtzer et al., 2014; Kurtzer et al., 2008; Pruszynski, Kurtzer, Nashed, et al., 2011; Soechting & Lacquaniti, 1988). Activity during the M2a response also begins to scale with intended movement distance (although this does not become prominent until the M2b epoch) (Pruszynski et al., 2008), and aids in obstacle avoidance during reaching (Nashed et al., 2012; Nashed et al., 2014). M2a activity is also known to scale with the urgency to respond to the perturbation and mirrors the patterns of activity observed in the voluntary epoch (Crevecoeur et al., 2013). However, this latter finding is counter to our results in chapter 2, 3, and 6, where we found M2a activity was insensitive to the latency or magnitude of a voluntary response.

If we compare our findings to the study by Crevecoeur and colleagues (2013), we can qualify the differences by arguing that participants adopted different strategies of responding based on altered task demands. In the motor control literature, it is known two distinct strategies can be implemented to perform voluntary movements. These were termed the “speed-sensitive” and “speed-insensitive” strategies (Corcos, Gottlieb, & Agarwal, 1989; Gottlieb, Corcos, & Agarwal, 1989a, 1989b). Participants adopt a speed-insensitive strategy if instructed to respond “as fast as possible” or “quickly and accurately”. All movements performed with this strategy
were hypothesized to be generated by supra-spinal centers delivering pulses of maximal excitation to the alpha motoneurons in the spinal cord. Movement amplitude is varied by changing the duration of these rectangular pulses (and onset latencies of subsequent pulses). By contrast, the speed-sensitive strategy is used when participants are provided temporal or speed constraints to reach a target. Movements controlled by this strategy are altered by varying the height of the rectangular pulses. In the study by Crevecoeur et al. (2013), urgency was manipulated, both explicitly with verbal timing constraints and implicitly with changes in target size, thus participants likely adopted the speed-sensitive strategy. By contrast, in the present dissertation experiments (for all ACT conditions), we instructed participants to compensate for the perturbation “as fast as possible” (chapter 2, 3, 4, 5, & 6) or “as fast and as accurately as possible” (chapter 2). Even in the motor imagery experiment (chapter 6), participants were instructed to “imagine compensating against the perturbation as fast as possible”. We can assume that our participants adopted a speed-insensitive strategy and thus the intention was to respond with maximum (initial) vigour, and we consistently observed a similar increase in M2a activity compared to DNI. Although a direct comparison of the impact of speed-sensitive versus speed-insensitive strategies on the long-latency response has not been made, it is a topic that is worth exploring in the future as it could further reveal further insights into the relation between M2 and volition. Related to this, because we observed a generic M2a increase even on Imagery\textsubscript{Leaked} trials in chapter 6, we could interpret this finding as indirect evidence that participants were mentally simulating the performance of a speed-insensitive (i.e., compensate) movement.

Across all of our experiments, we used the same preload and perturbation magnitudes. This was critical because both the short-latency and early portion of the long-latency response are highly sensitive to background muscle activation, a feature known as “automatic gain
scaling” (Pruszynski et al., 2009). Using an IN/OUT task and varying preload combinations, it was shown that the “automatic” component of M2 is functionally independent from the “target-dependent” component (Pruszynski, Kurtzer, & Scott, 2011). These authors suggested that because the long-latency response is produced by both spinal and supra-spinal pathways, and given that the automatic component is also present during the M1 response (which is mediated entirely by spinal circuits) it is likely the automatic component of M2 is also of spinal origin. In a follow-up study, Kurtzer et al. (2014) investigated whether both the automatic and target-dependent components of the long-latency stretch response display knowledge of inter-segmental limb dynamics. These authors did not use IN/OUT targets, instead employing a very large target (requiring minimal intervention) and a small target requiring a vigorous corrective response. With this set of target combinations, it was shown that both the automatic and the target-dependent components of the long-latency stretch response incorporate knowledge of intersegmental limb dynamics. We believe that because the target combinations used by Kurtzer et al. (2014) were closer (than IN/OUT) to the verbal instructions of DNI versus compensate, an avenue of future research may be to examine the role that the automatic component of the long-latency stretch response has on the generic M2a modulation we have consistently observed.

7.3 **M2b modulation, the M3 response, and triggered reactions**

Delineating potential sources of instruction- and/or goal-dependent activity during the M2b epoch becomes increasingly difficult. Whereas the M2a epoch receives a (presumably spinal) “automatic” component and a (presumably transcortical) “task-dependent” component, at ~75 ms, the automatic contributions begin to fade, and the number of supra-spinal task-dependent contributors increase (Pruszynski et al., 2009; Pruszynski, Kurtzer, & Scott, 2011). For example, some authors have described the presence of a third stretch response, named M3,
which was believed to involve a cerebellar to primary motor cortex pathway and begins at ~85 ms (Lee & Tatton, 1975; Tatton, Forner, Gerstein, Chambers, & Liu, 1975). It was also suggested that a triggered reaction (Crago et al., 1976; Houk, 1978) or a startle elicited pre-programmed voluntary response (Koshland & Hasan, 2000; Lewis et al., 2006; Ravichandran et al., 2013; Shemmell et al., 2009) may superimpose onto the end of the long-latency stretch response. Causing further (or maybe fewer) complications, some authors have questioned the existence of an M3 response, postulating instead that it is the first agonist burst of the voluntary response (Marsden et al., 1978). While we did not specifically examine the M3 response, the data in chapters 2, 3, 5, and 6, provided evidence that a voluntary response can contribute to the M2b epoch. Importantly, we also produced convincing evidence that the activation of startle circuitry by a mechanical perturbation was not required to cause an early trigger of the voluntary response (chapters 4 and 5). We cannot rule out that the StartReact effect did not contribute to voluntary response triggering in some paradigms (e.g., Ravichandran et al., 2013), but we can confidently conclude that the StartReact effect does not underlie all instruction-dependent changes to EMG activity during the long-latency stretch response.

While a non-startle triggered voluntary response can superimpose onto the end of the long-latency stretch response, the simple hastening of a pre-programmed voluntary response does not account for all modulation during the M2b epoch. This issue was examined by Pruszynski, Kurtzer, and Scott (2011), in which participants were provided advance knowledge of target location and the perturbation always displaced the limb away from the target. The critical manipulation was randomly varying the perturbation magnitude trial-to-trial. The authors reasoned that if a triggered reaction contributed to the M2b epoch, the same response would be elicited irrespective of perturbation intensity. However, the authors found that the evoked
activity was highly sensitive to sensory input, being increased following the large perturbation and decreased following the small perturbation. This modulation of response output was appropriate for participants to always reach the target quickly and accurately. Thus rather than the simple open-loop release of a pre-programmed voluntary response, the target-dependent behaviour of the long-latency stretch response produced a more flexible output, with an appropriately scaled motor response given the unpredictable sensory input and the constant task goal.

Another unresolved issue in the stretch response literature that could impact the M2b epoch is whether RT differences occur between simple and choice perturbation tasks. In traditional RT paradigms (to auditory or visual imperative signals) it is well accepted that RT increases as the number of potential stimulus-response alternatives increases. RT values typically increase by ~50 ms or more per doubling of alternatives (Carlsen et al., 2004a). However in a perturbation choice task, similar increases in RT with stimulus uncertainty have not always been reported. For example, one study suggested that a “triggered reaction” did not increase in latency when perturbation direction was unpredictable (Jaeger et al., 1982). Other groups reported minimal increases, ranging from ~10-50 ms (Crago et al., 1976; Evarts & Vaughn, 1978; Glencross & Koreman, 1979; Manning et al., 2012). It is possible that the rapid choice RTs resulted from the perturbation directly engaging neural circuitry involved in producing a voluntary response in stretched muscle. Indeed, Glencross and Koreman (1979) reported much larger RT increases (~70 ms) when participants had to respond with the unperturbed limb. In all of the perturbation studies cited in this paragraph (with the exception of the contralateral conditions used by Manning et al. 2012; Glencross & Koreman, 1979), either the authors attempted to determine RT directly from the EMG profiles of stretched muscle (Evarts &
Vaughn, 1978; Jaeger et al., 1982) or from deviations in acceleration (Crago et al., 1976). As mentioned repeatedly throughout this dissertation, premotor RT cannot be accurately determined when the voluntary response occurs in a muscle that has been stretched. The only method that may provide an accurate estimate of RT in a compensate task (in both simple and choice paradigms) is to use a very short duration perturbation (Lee & Tatton, 1982; Lewis et al., 2006; Lewis et al., 2005) where the M2 response will not confound the RT determination.

Operating under the assumption that voluntary response onset latencies are typically greater than 100 ms (as estimated by Pruszynski et al., 2008; Selen et al., 2012; Yang et al., 2011), many OFC studies have demonstrated goal-dependent M2 modulation, even when key features of the perturbation (e.g., direction, magnitude, likelihood of occurrence) could not be predicted in advance. These studies cannot however, be considered a true choice perturbation RT task, because participants were always provided with advance knowledge of the spatial target with which they were to move the arm. For example, Pruszynski et al. (2008) included a random perturbation direction condition in the IN/OUT task. Modulation was still observed during the M2a, M2b, and voluntary epochs, but the magnitude of differences between IN and OUT conditions were substantially reduced compared to the blocked condition where the perturbation direction was known in advance. The studies examining reaching movements typically delivered the perturbation on unexpected trials (e.g., 20%) with perturbation direction (and sometimes also magnitude) randomized (Lowrey et al., 2016; Nashed et al., 2012, 2014). The remarkably consistent finding is that as long as the spatial goal is known a minimum of ~90 ms before the perturbation (Yang et al., 2011), the long-latency stretch response will modulate in a goal-dependent manner that will aid the performer in reaching the target.
7.4 Neural substrates underlying modulation of the long-latency stretch response

With the exception of contributions from startle/StartReact circuitry, this dissertation did not specifically examine neural structures or pathways involved in mediating the long-latency stretch response. Based primarily on studies conducted by other groups, in this section, I speculate on different regions of the central nervous system that could have contributed to the stretch response modulation observed in the present experiments.

Neural recordings in non-human primates (Cheney & Fetz, 1984; Evarts & Tanji, 1976; Omrani et al., 2014; Pruszynski, Kurtzer, Nashed, et al., 2011; Pruszynski et al., 2014), human patient studies (Capaday et al., 1991; Matthews et al., 1990), cortical surface recordings (MacKinnon et al., 2000; Spieser et al., 2010), and TMS studies (Day et al., 1991; Lewis et al., 2004; Palmer & Ashby, 1992; Pruszynski, Kurtzer, Nashed, et al., 2011), all support the existence of a transcortical pathway contributing to the long-latency stretch response. Many studies have highlighted primary motor cortex as a probable site where M2 modulation could occur. Indeed, this region has been implicated in some forms of M2 modulation including multi-joint integration (Pruszynski, Kurtzer, Nashed, et al., 2011), and limb stability regulation in a compliant environment (Shemmell et al., 2009). When monkeys were given a precue signal to push or pull the arm towards a target following an upcoming perturbation, ~75% of recorded neurons in primary motor cortex showed anticipatory modulation within 200 ms of the precue (Tanji & Evarts, 1976). Following a perturbation that pushed or pulled the limb towards the target (i.e., compensate vs. assist), an initial response (at ~20 ms) was seen in pyramidal tract neurons that depended only on the direction of the perturbation. This was followed by a second response (at ~40 ms) that was modulated by prior instruction (Evarts & Tanji, 1976). Similar perturbation-evoked responses have been obtained in an IN/OUT task without specifically
classifying pyramidal tract neurons (Pruszynski et al., 2014). These authors also reported less preparatory activity than Tanji and Evarts (1976), which was suggested to be a result of the animals having to modulate muscle activity within the same muscle (between targets), as opposed to categorically activating antagonistic muscles.

While primary motor cortex does appear to contribute to some forms of long-latency stretch response modulation, evidence is equivocal on whether it plays a role in the instruction-dependent modulation observed in humans. For example, cortical potentials localized over sensorimotor cortex demonstrated modulation between let-go and resist instructions (Spieser et al., 2010), but not between DNI and compensate (MacKinnon et al., 2000). Similarly, a TMS-induced silent period of primary motor cortex attenuated instruction-dependent modulation between let-go and resist in the wrist flexors (Spieser et al., 2013), but not between DNI and compensate in biceps brachii (Shemmell et al., 2009). When comparing DNI and compensate conditions following a short-duration perturbation (M2 absent) and a long-duration perturbation (M2 present), corticospinal tract excitability was shown to increase on compensate trials, irrespective of the presence of M2 (Lewis et al., 2006). These authors also found no differences in short interval intra-cortical inhibition within primary motor cortex between instructions, thus proposing that the corticospinal tract excitability increase on compensate trials was a result of an additional input onto the corticospinal tract (i.e., not from primary motor cortex) (Lewis et al., 2006). Thus while primary motor cortex may play a role in inhibiting the long-latency stretch response when participants are instructed to let-go, this region does not appear to contribute to modifying the stretch response between a true passive (DNI) task and a compensate condition. It was proposed that the site of instruction-dependent M2 modulation occurs downstream from primary motor cortex (Lewis et al., 2006; MacKinnon et al., 2000).
Before discussing downstream (subcortical) contributions, I briefly describe other cortical areas that might contribute to mediation of the long-latency stretch response. One candidate region is supplementary motor area (Dick et al., 1987; Hummelsheim et al., 1986; Spieser et al., 2013). For example, SMA stimulation influenced the firing rates of perturbation-evoked single neuron responses in primary motor cortex (Hummelsheim et al., 1986) and a lesion of SMA produced an increased duration of the M2 response (Dick et al., 1987). It was recently shown that a TMS-induced silent period of SMA attenuated instruction-dependent M2 modulation between let-go and resist instructions (Spieser et al., 2013). Unfortunately, the role SMA may play in modulating muscle activity between DNI and compensate conditions has not been examined.

Posterior parietal cortex is another candidate cortical region implicated in M2 modulation. Recall (from chapter 6) that when monkeys were tested in a movie task (behaviourally analogous to DNI), Omrani et al. (2016) reported very different responses in PPC compared to when the animal had to make a correction to the limb perturbation. Neurons in PPC produced an increased firing rate at ~23 ms following perturbation onset in the posture task. However it is not clear whether PPC can influence the long-latency stretch response indirectly via intra-cortical connections with primary motor cortex or directly through corticospinal tract transmission. For example, PPC could have provided the additional input onto the corticospinal tract suggested by Lewis and colleagues (2006).

One candidate subcortical region implicated in long-latency stretch response modulation is the cerebellum (Kurtzer et al., 2013; Strick, 1983; Vilis, Hore, Meyer-Lohmann, & Brooks, 1976). In a push/pull task (similar to Evarts and Tanji; see above), cerebellar cooling was shown to have no influence on the initial perturbation evoked response in primary motor cortex, but it
attenuated magnitude of the second response phase that was sensitive to prior instruction (Vilis et al., 1976). Using a similar behavioral paradigm, Strick (1983) showed that neurons in both the interpositus and dentate nuclei responded to the direction of a perturbation, but only the dentate neurons displayed activity that varied based on the instruction of how to respond to the perturbation. Recently, it was demonstrated that multi-joint integration during the M2 response was still present in individuals with cerebellar damage, however the magnitude of differences between conditions was reduced (Kurtzer et al., 2013). In a review paper, Kurtzer (2015) suggested that the cerebellum may be involved in scaling the magnitude of the long-latency stretch response, but it is not necessarily the structure involved in generating the stretch response. To my knowledge, no study has examined the role cerebellum may play in instruction-dependent modulation between DNI and compensate conditions.

The reticular formation is another subcortical region that may contribute to instruction-dependent modulation. Perreault and colleagues have proposed that reticular formation and the reticulo-spinal tract is the main source of instruction-dependent M2 modulation between a DNI and compensate condition (Ravichandran et al., 2013; Shemmell et al., 2009). This evidence is indirect, with Shemmell et al. (2009) demonstrating that instruction-dependent M2 modulation was not impacted by a TMS-induced silent period over primary motor cortex and Ravichandran et al. (2013) providing evidence that servo perturbations applied to the elbow produced a startle response (which is known to be mediated by neurons in the reticular formation; Yeomans & Frankland, 1995; Yeomans et al., 2002). These authors (Ravichandran et al., 2013; Shemmell et al., 2009) proposed that instruction-dependent changes to the M2 response resulted from the StartReact effect, or the subcortical triggering of a pre-programmed voluntary response via activation of startle circuitry (see also Shemmell, 2015; Shemmell et al., 2010). However, even if
this was the mechanism underlying the M2 period modulation that these authors observed, it cannot account for generic M2a modulation, or even the M2b modulation that we have consistently found in the absence of a startle response. This StartReact explanation also cannot explain modulation of the long-latency stretch response in tasks where a corrective voluntary response cannot be pre-programmed in advance of perturbation delivery (e.g., Lowrey et al., 2016; Nashed et al., 2012; Pruszynski, Kurtzer, & Scott, 2011).

While we have provided strong evidence that an expected torque perturbation applied at the wrist does not readily activate startle circuitry (chapters 4 & 5), it is still possible that the reticular formation contributed to instruction-dependent M2 modulation observed in chapters 2, 3, 4, and 6. Indeed, neural recordings from the feline pontine reticular formation have shown that many neurons respond at short-latency following a platform perturbation (Stapley & Drew, 2009). Indirect evidence from humans has shown that pre-programming of a voluntary movement increases excitability of reticular circuits which results in an increase incidence and amplitude of the startle response (Maslovat, Carlsen, & Franks, 2012). Thus, assuming that non-startling mechanical perturbations in humans can also activate neurons in the reticular formation, and that the excitability of reticular circuits increases when participants pre-program a voluntary response on ACT trials (compared to DNI), this subcortical region could have contributed to the instruction-dependent modulation observed in the present dissertation experiments.

We had a surprising result in chapter 4 that highlighted potential contributions from the reticular formation on stretch response activity. Using data combined from both experiments 4 and 5, there was a clear supra-linear interaction (beyond what could be predicted by the summation of these two stimuli presented in isolation) between a SAS and a mechanical perturbation on wrist flexor EMG during the M2a and M2b epochs (figure 4.7). Furthermore,
this finding was independent of volitional intent, being observed for both the DNI and ACT conditions. While this supra-linear interaction could have occurred in the reticular formation, as mentioned in chapter 4, the time-course was also appropriate for a reticulo-thalamo-cortical pathway interacting with the perturbation evoked afferent volley at the level of primary motor cortex (Alibiglou & MacKinnon, 2012; Carlsen et al., 2012; Stevenson et al., 2014).

One must not forget that spinal circuitry can also contribute to the long-latency stretch response. The potential spinal afferent contributors to the M2 response include continued activation of primary spindle afferents (responsible for the M1 response), activation of slower conducting afferents from secondary spindle endings, as well as joint and cutaneous receptors. However, evidence suggests that group II spindle afferents do not have a substantial role in mediating the long-latency response in the upper-limbs of humans (Kurtzer et al., 2018; Matthews, 1989). Similarly, anesthetization of joint and cutaneous receptors had no impact on the M2 response (or voluntary response latency) in a compensate task involving wrist flexors (Bawa & McKenzie, 1981). Thus, reverberation of primary spindle afferents is the most probable spinal contributor (Hagbarth et al., 1981). However, to my knowledge, it has not been tested whether task-dependent descending inputs can influence Ia afferents during the long-latency stretch response. For example, supra-spinal inputs could theoretically reduce presynaptic inhibition of Ia afferents prior to the onset of a voluntary response on ACT trials. It is known that H-reflexes increase immediately prior to the onset of a voluntary response in a non-perturbation RT task (Hasbroucq et al., 2000) and it is plausible that similar spinal modulation could happen early in the long-latency stretch response.
7.5 Postural responses to a perturbation and the need to distinguish from startle

As discussed in detail in chapters 4 and 5, SCM is the most reliable muscle to determine the presence of a startle response for researchers investigating the StartReact effect (Carlsen et al., 2009; Carlsen et al., 2007; Carlsen et al., 2011; Honeycutt, Kharouta, & Perreault, 2013). However, neck musculature can also be activated for non-startle related reasons such as stabilizing the head during upper-limb movements (Carlsen et al., 2004b; Dean & Baker, 2017). Typically this is not an issue for StartReact researchers, because non-startle RTs are usually >120 ms, and postural SCM follows onset of a voluntary response in the upper-limb (Carlsen et al., 2004b). However actively responding to a mechanical perturbation is a unique scenario in which voluntary RT can consistently be less than 100 ms (e.g., MacKinnon et al., 2000; Manning et al., 2012; Ravichandran et al. 2013; chapter 5 of this dissertation), and thus postural SCM activity can be advanced within the startle time criteria (<120 ms). This can result in the interpretation that the StartReact effect underlies the rapid release of a pre-programmed voluntary response by a mechanical perturbation. Through the use of a PPI stimulus (chapter 5) and by also recording from a startle indicator not involved in postural control (OOC; chapters 4 and 5), we provided convincing evidence that a majority of SCM activity elicited by an expected mechanical perturbation imperative signal was the result of early postural activity.

Recent work has demonstrated that postural responses to a mechanical perturbation can occur in many (unperturbed) muscles throughout the body (Lowrey et al., 2016). In this study, participants performed upper-limb reaching movements towards targets of different shapes (large rectangle or a small circle) while standing upright. A previous study by this group showed larger feedback corrections were evoked when a perturbation was delivered while participants aimed towards the circle (Nashed et al., 2012). Lowrey and colleagues replicated these findings in the
upper-limb, but critically, they also showed that corrective responses were evoked in the lower-limbs at a latency of ~75 ms. These were not generic responses; rather they showed appropriate tuning to aid performance of the ongoing reaching task. While aiming for the circle, larger responses were observed in lower-limb muscles compared to when aiming for the rectangle target. Moreover, if the perturbation displaced the arm towards the participant, larger responses were evoked in tibialis anterior and rectus femoris. By contrast, perturbations away from the body evoked larger responses in gastrocnemius and bicep femoris. Although this is conjecture, I believe that if these authors had also recorded from SCM, goal-dependent postural activity (larger responses for perturbations towards the body) would also be observed in the neck and this would occur at a startle-like latency.

7.6 Limitations

This dissertation was primarily concerned with instruction-dependent M2 modulation in the wrist flexor muscles of human participants. A general limitation of this work is whether similar findings (specifically modulation of M2 and voluntary RTs of <100 ms in the absence of startle) would also be observed following perturbations to other joints in the body. As I alluded earlier, our findings likely would not generalize to stretch responses in muscles of the hand. Instruction-dependent M2 modulation in hand musculature often does not occur because the perturbations that were used saturated the M2 response (Capaday et al., 1994; Rothwell et al., 1980). This is not to say that the M2 response in hand musculature cannot modulate. For instance, instruction-dependent changes following a small perturbation were demonstrated when participants were given an additional electric shock to the contralateral limb to minimize RT (Rothwell et al., 1980). Hand musculature is also capable of flexible goal-dependent modulation similar to what has been demonstrated in muscles surrounding the elbow and shoulder (Gielen et
al., 1988; Kurtzer et al., 2008). For example, in an object manipulation task, a thumb perturbation produced an appropriately scaled M2 response in both thumb and forefinger muscles, suitable for rapidly compensating for the applied load (Cole et al., 1984). Even if our findings may not directly generalize to instruction-dependent changes following finger perturbations, our results likely would extend to perturbations applied at the elbow and/or shoulder. Many studies have shown that muscles of the upper arm can modulate in an instruction- or goal-dependent manner (Hammond, 1956; Pruszynski et al., 2008; Rothwell et al., 1980) and can also result in voluntary RTs of <100 ms (Evarts & Granit, 1976; Evarts & Vaughn, 1978; Ravichandran et al., 2013).

In these dissertation experiments (c.f., some conditions in Experiments 1 and 2), we always used verbal instructions to specify participant behaviour. It has been suggested that the use of verbal instructions can result in ambiguities and different interpretations of how to respond to the perturbation (Pruszynski et al., 2008). Thus many recent studies have used targets rather than instructions to ensure all participants perform similarly (Kurtzer et al., 2008; Omrani et al., 2013; Pruszynski et al., 2008; Weiler et al., 2018a). Likely in all ACT conditions tested here, different participants responded with varying degrees of flexion following the perturbation and this is known to influence activity during the M2b epoch and magnitude of the voluntary response (Pruszynski et al., 2008). Potentially more troubling is performance in the DNI condition. We were concerned that participants may perform a “let-go” response, which involves actively relaxing stretched muscle (Calancie & Bawa, 1985). In the experiments that followed chapter 2, we added a post-load after the perturbation (equivalent to the preload level of torque). Thus we ensured that if participants were actively relaxing wrist flexors, the wrist would end up at the extension endpoint. Although this behaviour did occur on occasion during early practice, it
never occurred during the testing phase. We do agree with Scott and colleagues that targets are superior to clearly define and control participant behaviour. However, to specifically address the questions of interest in this dissertation, it was critical that we used verbal instructions as no clear target analogue of a DNI condition exists (i.e., no true passive condition can be specified with targets).

One limitation from Experiment 8 is the issue of whether participants actually engaged in kinesthetic imagery. Recall that in this experiment, participants were asked to physically perform the DNI task but to imagine compensating against the perturbation and the associated sensations. If we had demonstrated instruction-dependent M2 modulation in the absence of a voluntary response, it would be clear evidence that kinesthetic imagery could modulate M2. However, we only found M2 modulation when a voluntary response leaked out into the wrist flexor musculature. This nicely answered our research question, but on the non-leaked trials, we were left with the issue of whether participants actually engaged in imagery. We initially considered applying single-pulse TMS over primary motor cortex to examine the time-course of corticospinal excitability changes. Corticospinal tract excitability is known to increase in a simple RT imagery task, however the magnitude of MEP changes during imagery performance is ~3 times smaller than the magnitude of change during physical movement performance (Kumru et al., 2008). Given the current issues with TMS (Héroux, Taylor, & Gandevia, 2015), especially when trying to replicate a small effect size, we chose to not use this technique in our perturbation protocol. Another option was to examine various EEG measures such as the CNV which are known to change during imagery performance (Eagles, Carlsen, & MacKinnon, 2015). However, given that EEG potentials do not modulate between and ACT and a DNI task (MacKinnon et al., 2000), we did not believe scalp potentials would be sensitive enough to detect changes with
imagery in our paradigm. Thus we cannot make any conclusions about whether kinesthetic imagery influences M2, but we can confidently state that the overt execution of a voluntary response is required for instruction-dependent M2 modulation.

7.7 Implications and future directions

One outstanding issue surrounding M2 is whether primary motor cortex is the structure responsible for scaling this feedback response. Given that a transcortical pathway involving primary sensory and motor cortex is a major contributor to the M2 response (Cheney & Fetz, 1984; MacKinnon et al., 2000; Matthews, 1991), primary motor cortex has been suggested as a strong candidate to mediate feedback response modulation (Pruszynski et al., 2008; Scott, 2004). This region plays a role in target-dependent modulation (Pruszynski et al., 2014; Tanji & Evarts, 1976), limb stability regulation (Shemmell et al., 2009), and multi-joint integration (Pruszynski, Kurtzer, Nashed, et al., 2011). It has also been shown to inhibit the M2 response when participants are instructed to “let-go” (Spieser et al., 2013; Spieser et al., 2010). However, no evidence supports an involvement of primary motor cortex in facilitation of the M2 response when participants transition from a DNI to an ACT task (Lewis et al., 2006; MacKinnon et al., 2000; Shemmell et al., 2009). The exact role of primary motor cortex and other cortical and subcortical regions in instruction-dependent modulation of M2 remains unresolved. Uncovering the structures and mechanisms that contribute to stretch response modulation is an important topic to address in the future.

The ability to modulate the long-latency stretch response is often impaired in various clinical populations such as stroke (Trumbower, Finley, Shemmell, Honeycutt, & Perreault, 2013; Trumbower, Ravichandran, Krutky, & Perreault, 2010) and Parkinson’s disease (Lee & Tatton, 1975; Rothwell, Obeso, Traub, & Marsden, 1983; Tatton & Lee, 1975). Developing a
method to re-train these individuals to scale stretch responses may greatly aid their recovery. Recent work has shown that the long-latency stretch response can modulate following motor learning in a force field adaptation task (Cluff & Scott, 2013; Kimura et al., 2006). In the study by Kimura et al. (2006), participants learned to upregulate or downregulate the M2 response prior to entering a known force field. Perhaps a similar force field adaptation protocol may prove valuable in training individuals with Parkinson’s disease or stroke to modulate the long-latency stretch response. As an example, Parkinson’s patients often display enhanced M2 responses, even in a DNI condition (Lee & Tatton, 1975; Tatton & Lee, 1975). Teaching these individuals to downscale M2 may be possible if they adapt to a leftward force field while making reaching movements with the right arm to a target. Healthy individuals show a reduced M2 response in shoulder flexor muscles in such a task (Kimura et al., 2006). Force field or visual motor adaptation may prove as a valuable therapy in helping patient populations regain the ability to modulate the long-latency stretch response.

7.8 Conclusion

Humans and non-human primates are capable of generating remarkably fast and accurate goal-directed motor responses to correct for perturbations applied to the upper-limbs. While it has been debated for many years whether flexibility of the “reflexive” motor output results from modifications in feedback gains or is produced entirely through superimposition of a voluntary response, this dissertation has provided evidence that both mechanisms play an important and symbiotic role. In order for instruction-dependent modulation of the long-latency stretch response to occur, at least between the different verbal instructions tested in this dissertation, a voluntary response must be generated in the stretched muscle. For the reason that this “reflexive” motor response taps into the volitional control system and affords the sensorimotor system with a
remarkably fast goal-directed error corrective mechanism, I believe the long-latency stretch response remains an important topic of study.
Bibliography


Shemmell, J. (2015). Interactions between stretch and startle reflexes produce task-appropriate rapid postural reactions. *Front Integr Neurosci, 9*(2).


Appendix A: Movement Imagery Questionnaire

Full Questionnaire with Instructions

Instructions

This questionnaire concerns two ways of mentally performing movements which are used by some people more than by others, and are more applicable to some types of movements than others. The first is attempting to form a visual image or picture of a movement in your mind. The second is attempting to feel what performing a movement is like without actually doing the movement. You are requested to do both of these mental tasks for a variety of movements in this questionnaire, and then rate how easy/difficult you found the tasks to be. The ratings that you give are not designed to assess the goodness or badness of the way you perform these mental tasks. They are attempts to discover the capacity individuals show for performing these tasks for different movements. There are no right or wrong ratings or some ratings that are better than others.

Each of the following statements describes a particular action or movement. Read each statement carefully and then actually perform the movement as described. Only perform the movement a single time. Return to the starting position for the movement just as if you were going to perform the action a second time. Then depending on which of the following you are asked to do, either (1) form as clear and vivid a visual image as possible of the movement just performed from an internal perspective (i.e., from a 1st person perspective, as if you are actually inside yourself performing and seeing the action through your own eyes), (2) form as clear and vivid a visual image as possible of the movement just performed from an external perspective (i.e., from a 3rd person perspective, as if watching yourself on DVD), or (3) attempt to feel yourself making the movement just performed without actually doing it.

After you have completed the mental task required, rate the ease/difficulty with which you were able to do the task. Take your rating from the following scale. Be as accurate as possible and take as long as you feel necessary to arrive at the proper rating for each movement. You may choose the same rating for any number of movements “seen” or “felt” and it is not necessary to utilize the entire length of the scale.

RATING SCALES

Visual Imagery Scale

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<tr>
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<td>Very hard to see</td>
<td>Hard to see</td>
<td>Somewhat hard to see</td>
<td>Neutral (not easy nor hard)</td>
<td>Somewhat easy to see</td>
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Kinesthetic Imagery Scale

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<td>Very easy to feel</td>
</tr>
</tbody>
</table>
1. **STARTING POSITION:** Stand with your feet and legs together and your arms at your sides.

**ACTION:** Raise your right knee as high as possible so that you are starting on your left leg with your right leg flexed (bent) at the knee. Now lower your right leg so you are once again standing on two feet. The action is performed **slowly**.

**MENTAL TASK:** Assume the starting position. Attempt to **feel** yourself making the movement just observed without actually doing it. Now rate the ease/difficulty with which you were able to do this mental task.

Rating: ______

2. **STARTING POSITION:** Stand with your feet and legs together and your arms at your sides.

**ACTION:** Bend down low and then jump straight up in the air as high as possible with both arms extended above your head. Land with both feet apart and lower your arms to your sides.

**MENTAL TASK:** Assume the starting position. Attempt to **see** yourself making the movement just observed from an **internal perspective**. Now rate the ease/difficulty with which you were able to do this mental task.

Rating: ______

3. **STARTING POSITION:** Extend the arm of your non-dominant hand straight out to your side so that it is parallel to the ground, palm down.

**ACTION:** Move your arm forward until it is directly in front of your body (still parallel to the ground). Keep your arm extended during the movement, and make the movement **slowly**.

**MENTAL TASK:** Assume the starting position. Attempt to **see** yourself making the movement just observed from an **external perspective**. Now rate the ease/difficulty with which you were able to do this mental task and the angle the image was observed from (see additional sheet provided for full list of different angles)

Rating: ______
<table>
<thead>
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<th>STARTING POSITION:</th>
<th>ACTION:</th>
<th>MENTAL TASK:</th>
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<tr>
<td>4.</td>
<td>Stand with your feet slightly apart and your arms fully extended above your head.</td>
<td><strong>Slowly</strong> bend forward at the waist and try and touch your toes with your fingertips (or, if possible, touch the floor with your fingertips or your hands). Now return to the starting position, standing erect with your arms extended above your head.</td>
<td>Assume the starting position. Attempt to <strong>feel</strong> yourself making the movement just observed without actually doing it. Now rate the ease/difficulty with which you were able to do this mental task.</td>
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<tr>
<td>5.</td>
<td>Stand with your feet and legs together and your arms at your sides.</td>
<td>Raise your right knee as high as possible so that you are starting on your left leg with your right leg flexed (bent) at the knee. Now lower your right leg so you are once again standing on two feet. The action is performed <strong>slowly</strong>.</td>
<td>Assume the starting position. Attempt to <strong>see</strong> yourself making the movement just observed from an <strong>internal perspective</strong>. Now rate the ease/difficulty with which you were able to do this mental task.</td>
</tr>
<tr>
<td>6.</td>
<td>Stand with your feet and legs together and your arms at your sides.</td>
<td>Bend down low and then jump straight up in the air as high as possible with both arms extended above your head. Land with both feet apart and lower your arms to your sides.</td>
<td>Assume the starting position. Attempt to <strong>see</strong> yourself making the movement just observed from an <strong>external perspective</strong>. Now rate the ease/difficulty with which you were able to do this mental task and the angle the image was observed from (see additional sheet provided for full list of different angles)</td>
</tr>
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Rating: __________
7. STARTING POSITION: Extend the arm of your non-dominant hand straight out to your side so that it is parallel to the ground, palm down.

ACTION: Move your arm forward until it is directly in front of your body (still parallel to the ground). Keep your arm extended during the movement, and make the movement slowly.

MENTAL TASK: Assume the starting position. Attempt to feel yourself making the movement just performed without actually doing it. Now rate the ease/difficulty with which you were able to do this mental task.

Rating: __________

8. STARTING POSITION: Stand with your feet slightly apart and your arms fully extended above your head.

ACTION: Slowly bend forward at the waist and try and touch your toes with your fingertips (or, if possible, touch the floor with your fingertips or your hands). Now return to the starting position, standing erect with your arms extended above your head.

MENTAL TASK: Assume the starting position. Attempt to see yourself making the movement just observed from an internal perspective. Now rate the ease/difficulty with which you were able to do this mental task.

Rating: __________

9. STARTING POSITION: Stand with your feet and legs together and your arms at your sides.

ACTION: Raise your right knee as high as possible so that you are starting on your left leg with your right leg flexed (bent) at the knee. Now lower your right leg so you are once again standing on two feet. The action is performed slowly.

MENTAL TASK: Assume the starting position. Attempt to see yourself making the movement just observed from an external perspective. Now rate the ease/difficulty with which you were able to do this mental task and the angle the image was observed from (see additional sheet provided for full list of different angles)

Rating: __________
10. **STARTING POSITION:** Stand with your feet and legs together and your arms at your sides.

ACTION: Bend down low and then jump straight up in the air as high as possible with both arms extended above your head. Land with both feet apart and lower your arms to your sides.

MENTAL TASK: Assume the starting position. Attempt to feel yourself making the movement just performed without actually doing it. Now rate the ease/difficulty with which you were able to do this mental task.

Rating: __________

11. **STARTING POSITION:** Extend the arm of your non-dominant hand straight out to your side so that it is parallel to the ground, palm down.

ACTION: Move your arm forward until it is directly in front of your body (still parallel to the ground). Keep your arm extended during the movement, and make the movement slowly.

MENTAL TASK: Assume the starting position. Attempt to see yourself making the movement just observed from an *internal perspective*. Now rate the ease/difficulty with which you were able to do this mental task.

Rating: __________

12. **STARTING POSITION:** Stand with your feet slightly apart and your arms fully extended above your head.

ACTION: Slowly bend forward at the waist and try and touch your toes with your fingertips (or, if possible, touch the floor with your fingertips or your hands). Now return to the starting position, standing erect with your arms extended above your head.

MENTAL TASK: Assume the starting position. Attempt to see yourself making the movement just observed from an *external perspective*. Now rate the ease/difficulty with which you were able to do this mental task and the angle the image was observed from (see additional sheet provided for full list of different angles)

Rating: __________
Response Form Only
(if Instructions and Items are read to participants)

After you have completed the mental task required, rate the ease/difficulty with which you were able to do the task in the space provided below. Take your rating from the provided scale. Be as accurate as possible and take as long as you feel necessary to arrive at the proper rating for each movement. You may choose the same rating for any number of movements “seen” or “felt” and it is not necessary to utilise the entire length of the scale.

RATING SCALES

Visual Imagery Scale

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Kinesthetic Imagery Scale

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1) Knee lift  Rating: ____
2) Jump  Rating: ____
3) Arm movement  Rating: ____
4) Waist Bend  Rating: ____
5) Knee lift  Rating: ____
6) Jump  Rating: ____
7) Arm movement  Rating: ____
8) Waist Bend  Rating: ____
9) Knee lift  Rating: ____
10) Jump  Rating: ____
11) Arm movement  Rating: ____
12) Waist Bend  Rating: ____
# Movement Imagery Questionnaire-3

## Instructions for Scoring

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<tr>
<td>External Visual Imagery</td>
<td>Item 3 + Item 6 + Item 9 + Item 12/4</td>
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<td>Kinesthetic Imagery</td>
<td>Item 1 + Item 4 + Item 7 + Item 10/4</td>
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