School of Population Health

Use the Force: Augmenting Neural Excitability via Unexpected Acoustic Stimulation and Movement Vigour

Aaron N. McInnes

0000-0001-8104-3798

This thesis is presented for the Degree of
Doctor of Philosophy – Psychology
of
Curtin University

March 2021

DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Human Ethics (For projects involving human participants/tissue, etc) The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number HRE2018-0257

Signature: …….

Date: 15/03/2021……

PUBLICATIONS INCLUDED IN THIS THESIS

McInnes, A. N., Corti, E. J., Tresilian, J., R., Lipp, O. V., & Marinovic, W. (2020). Neural gain induced by startling acoustic stimuli is additive to preparatory activation. *Psychophysiology*, 57(3), e13493. https://doi.org/10.1111/psyp.13493

McInnes, A. N., Castellote, J. M., Kofler, M., Honeycutt, C. F., Lipp, O. V., Reik, S., Tresilian, J. R., & Marinovic, W. (2020). Cumulative distribution functions: An alternative approach to examine the triggering of prepared motor actions in the StartReact effect. *European Journal of Neuroscience, 53*(5), 1545-1568. https://doi.org/10.1111/ejn.14973

CONTRIBUTION OF OTHERS TO THE THESIS

Attribution tables by research article:

Article 1: Cumulative distribution functions: An alternative approach to examine the triggering of prepared motor actions in the StartReact effect.

	Conception and design	**Acquisition of Data**	**Data Conditioning and Manipulation**	**Analysis and Statistical Method**	**Interpretation and Discussion**	**Draft Writing**	**Critical Revisions**
Co-Author 1 **McInnes, A. N.**	12.5%		80%	45%	55%	100%	
Co-Author 1 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 2 **Castellote, J. M.**	12.5%	25%		10%	5%		10%
Co-Author 2 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 3 **Kofler, M.**	12.5%	25%		10%	5%		10%
Co-Author 3 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 4 **Honeycutt, C. F.**	12.5%	25%		10%	5%		10%
Co-Author 4 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 5 **Lipp, O. V.**	12.5%			10%	10%		25%
Co-Author 5 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							

	Conception and design	**Acquisition of Data**	**Data Conditioning and Manipulation**	**Analysis and Statistical Method**	**Interpretation and Discussion**	**Draft Writing**	**Critical Revisions**
Co-Author 6 **Riek, S.**	12.5%				5%		10%
Co-Author 6 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 7 **Tresilian, J. R.**	12.5%				5%		10%
Co-Author 7 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 8 **Marinovic, W.**	12.5%	25%	20%	15%	10%		25%
Co-Author 8 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Total	100%	100%	100%	100%	100%	100%	100%

Article 2: Neural gain induced by startling acoustic stimuli is additive to preparatory activation.

	Conception and design	**Acquisition of Data**	**Data Conditioning and Manipulation**	**Analysis and Statistical Method**	**Interpretation and Discussion**	**Draft Writing**	**Critical Revisions**
Co-Author 1 **McInnes, A. N.**	40%	60%	70%	65%	50%	100%	
Co-Author 1 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 2 **Corti, E. J.**		40%	20%				
Co-Author 2 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 3 **Tresilian, J. R.**	20%			10%	15%		30%
Co-Author 3 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 4 **Lipp, O. V.**	20%			10%	15%		30%
Co-Author 4 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Co-Author 5 **Marinovic, W.**	20%		10%	15%	20%		40%
Co-Author 5 Acknowledgement: *I acknowledge that these represent my contribution to the above research output.* *Signed:*							
Total	100%	100%	100%	100%	100%	100%	100%

ACKNOWLEDGEMENTS

I must first acknowledge my primary supervisor, Welber Marinovic, for the opportunity you have given me in embarking on this journey. I cannot express enough gratitude for the direction you have provided in helping me develop in all aspects as a scientist. The level of guidance, support, and humour you bring to all of us in the lab is boundless and it has been a privilege and a pleasure to work with you for the past few years. My endless thanks also go to my co-supervisor, Ottmar Lipp, for the invaluable help and advice you have provided over the course of my PhD. Along with my supervisors, I would like to thank the many co-authors across Australia and the globe who have contributed their ideas to the work contained in this thesis.

The time I have spent doing this PhD of course would not have been the same without the great team we have in the Curtin Behavioural Neuroscience Lab. Cheers to An Nguyen, Emily Corti, Michelle Huntley, Jaspa Favero, Kara Simpson, as well as those in the PERL-C gang.

My acknowledgements go to the many developers who have made available the range of open-access software which made this research possible.

Finally, thank you to my family. I would not be here without the love and support of Brian McInnes (Dad) and Kristen McInnes (Mum). Nor without Chrys Anastas (Grandma), who fostered the scientist in me from a young age.

FUNDING ACKNOWLEDGEMENT

I acknowledge the financial support of the Australian Government through the Australian Research Council's Discovery Project grants which funded this work (DP160102001; DP180100394) and the Australian Government Research Training Program Scholarship.

COPYRIGHT STATEMENT

Where necessary, I have obtained permission from the copyright owners to use any third-party copyright material reproduced in the thesis (e.g. questionnaires, artwork, unpublished letters), or to use any of my own published work (e.g. journal articles) in which the copyright is held by another party (e.g. publisher, co-author).

GENERAL ABSTRACT

When presented during action preparation, high intensity sensory stimuli can unintentionally trigger the prepared movement, a phenomenon referred to as the StartReact effect. Importantly, these actions occur with much shorter reaction times (RTs) and greater force than is usually produced voluntarily. Due to the substantial movement facilitation observed in this phenomenon, many have proposed that the StartReact-triggered movements recruit a unique triggering pathway which is different from that of voluntary movements. Initial accounts of the StartReact effect suggested triggering occurs through a subcortical pathway which relies on activation of startle circuits. Such a pathway would preclude any involvement of the cerebral cortex in its triggering. Later proposals suggested that a fast transcortical pathway is activated by intense sensory stimulation, or that voluntary motor pathways can be simply enhanced by the stimulation. Understanding the neural mechanisms of this phenomenon is important in appropriately making use of the StartReact effect, both in the laboratory and in applied settings. For example, the StartReact phenomenon can be used to provide insights regarding how actions are prepared and executed. It may also be used therapeutically in order to aid in retraining movement after movement control is impaired due to neurological conditions such as stroke. In this thesis, I examine the types of movements that are more easily facilitated by high intensity sensory stimuli so that mechanisms underlying the StartReact effect and motor control can be further understood. I also examine the types of movements that receive most benefit from intense sensory stimuli so that recommendations for practical applications of the StartReact effect can be made.

In chapter two, several previously published datasets were reanalysed to evaluate whether startle activity is a necessary condition for the StartReact effect to occur, as would be expected if triggering relies on subcortical startle circuits. On the basis of this assumed subcortical triggering in the StartReact effect, previous research has often compared trials with and without measurable startle activity in order to dissociate StartReact-triggered actions from voluntary actions. Using cumulative distribution functions, we observed a task-dependent association of startle activity with RT, which makes relying on startle activity as an indicator of the activation of StartReact pathways unreliable. As such, we proposed an alternative

method of trial categorisation using cumulative distribution functions of RT. Using this method, we found some evidence of certain tasks being more amenable to StartReact than others, but found little evidence of this effect in muscles which strongly differ in their cortical versus subcortical efferent connectivity. As such, we suggest that differences that can be observed between actions in their triggering by the intense sensory stimulus may be due to the functionality of the movement.

Chapter three further compared the StartReact effect in different actions which engage muscles that differ in the strength of their subcortical and cortical efferent connections. Our analysis failed to detect a significant difference in RT between movements which differ in the strength of reticulospinal versus corticospinal contributions. Peak force and vigour, however, were facilitated more by a loud acoustic stimulus (LAS) for the flexion movement, which is suggested to have stronger corticospinal connectivity. As such, we suggest this provides evidence for cortical involvement in the StartReact effect, given the production of force is highly correlated with primary motor cortex activity. Our analysis of the force enhancements provided by the LAS also indicated that the neural activity introduced to motor program circuits by the LAS is additive to the preparatory activation of the voluntary action, with these two processes summating to produce the final magnitude of motor output.

Previous research has indicated the effects of a LAS on corticospinal excitability are state-dependent. Specifically, a LAS suppresses corticospinal excitability during a light muscle contraction, but increases excitability when the level of motor preparation is high. In light of this, chapter four examines the effect of a sustained muscle contraction during action preparation (conditions which produce opposite effects of a LAS on corticospinal excitability) on the StartReact effect. We determined a sustained contraction during preparation can provide some overall benefit on RT when the contraction is maintained contralaterally to and congruently with the responding hand, at 10% of the muscle's maximum voluntary contraction. This type of contraction also provided the most benefit on peak force and vigour. Importantly, the enhancement of the StartReact effect by such contractions was muscle and laterally specific – no benefit was provided in a unilateral task or when the contraction was incongruent with the response. In this chapter we provided an

outline of how these findings may suggest cortical involvement in the StartReact effect.

Finally, chapter five examined how modulations of corticospinal excitability which occur over the course of action preparation may impact the StartReact effect. Specifically, we examined the phenomenon of preparatory inhibition, in which corticospinal excitability is suppressed during preparation for action. By increasing the urgency of an upcoming anticipatory action, we restricted the ability of the motor system to engage in preparatory inhibition. We predicted that in the absence of preparatory inhibition, the StartReact effect would be greater due to less inhibitory processes opposing the excitation of motor circuits induced by the LAS. In the absence of such preparatory inhibition, however, sensory stimuli were disruptive to motor output. Rather than an enhancement of force and vigour as is typical in the StartReact effect, acoustic stimuli reduced force and vigour when there was no detectable suppression of corticospinal excitability. In light of these unexpected findings, we provided an alternative account of preparatory inhibition, in which the suppression of corticospinal excitability may be a strategic process which acts to protect prepared responses from external interference.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AD	Anterior Deltoid
APB	Abductor Pollicis Brevis
BB	Biceps Brachii
BF	Bayes Factor
Br	Brachioradialis
CDF	Cumulative Distribution Function
CI	Confidence Interval
EDC	Extensor Digitorum Communis
EMG	Electromyogram
FDI	First Dorsal Interosseous
FDS	Flexor Digitorum Superficialis
IS	Imperative Stimulus
LAS	Loud Acoustic Stimulus
LC	Locus Coeruleus
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MVC	Maximum Voluntary Contraction
OO	Orbicularis Oris
OOc	Orbicularis Oculi
PD	Posterior Deltoid
Pe	Pectoralis
PMRF	Pontomedullary Reticular Formation
RT	Reaction Time
SCM	Sternocleidomastoid
SICI	Short-interval Intracortical Inhibition
TB	Triceps Brachii
TMS	Transcranial Magnetic Stimulation
WS	Warning Stimulus

CHAPTER ONE: GENERAL INTRODUCTION

1.0 Introduction

"Proper planning and preparation prevent piss poor performance". The British army are said to have originated these seven Ps which are echoed by military personnel around the globe. Unsurprisingly, this adage reflects one of the most fundamental neural processes engaged by the human central nervous system – movement preparation. Proper planning and preparation must precede the initiation of any goal-directed, voluntary, movement. Forewarned reaction time (RT) tasks are perhaps the most widely used method of investigating movement preparation in the laboratory. The RT is defined as the time interval between the presentation of a go-signal (referred to as the imperative stimulus; IS) and the onset of movement. The IS can occur as a visual signal, an auditory tone, or a tactile stimulus via vibration or electrotactile stimulation of the skin. Importantly, a movement plan can be prepared prior to the IS when a warning stimulus (WS), which defines the action to be initiated, is presented prior to the IS. This drastically shortens the RT, often observed in the laboratory at latencies < 200 ms (Rosenbaum, 1980).

The activation model of response initiation (also known as the accumulator model) has been proposed to explain how actions can be prepared and subsequently initiated at short latencies in RT tasks. In this model, initiation of movement occurs when a signal that represents the decision process accumulates over time to reach a threshold level (Hanes & Schall, 1996). Preparation begins by generating a movement program in the motor circuits after the goal has been defined by the WS. As preparation develops, neural activity in motor program circuits is increased to a subthreshold level in anticipation of the impending IS – referred to as temporal preparation (Lecas et al., 1986; Niemi & Näätänen, 1981; Wickens et al., 1994). When an IS is presented, neural activity is introduced to these circuits, thereby pushing activation above a threshold which is required to be met for movement initiation to begin (Hanes & Schall, 1996; Luce, 2008). Importantly, activation within motor circuits must be elevated to a sub-threshold level for the IS to trigger the motor program. As such, temporal preparation prior to a "go-signal" allows for optimisation of responses, particularly when there are tight time constraints for action. When there is no preparatory activity, the triggering stimulus cannot cause activation to surpass initiation threshold (Marinovic et al., 2013). This dictates that

preparation exists on a continuum – the higher the state of preparation, the more quickly activation can exceed initiation threshold, resulting in a shorter RT. However, RT is not the only characteristic of movement which can be influenced by the build-up of preparatory activity. The degree to which activation exceeds the initiation threshold can determine the amplitude of the descending command which produces the forcefulness of the movement (Ulrich et al., 1998). Similarly, the rate of accumulation of neural activity in motor program circuits to its peak above threshold may correspond with the vigour of the prepared movement (Ulrich et al., 1998).

It has been long known that the intensity of an IS has drastic effects on the initiation of movement (Pieron, 1914; Pins & Bonnet, 1996). In line with the activation model of response initiation, an intense stimulus can add more activity to motor program circuits and hence, can more quickly push activation above initiation threshold, resulting in reduced RT. Similarly, a more intense stimulus pushes activation to a higher amplitude and therefore, increases the magnitude of movement execution (Ulrich et al., 1998). Moreover, the presentation of sensory stimuli in multiple modalities can further increase activity in motor program circuits and produce accessory stimulation effects (Nickerson, 1973). Recently, the presentation of high intensity sensory stimuli during movement preparation, and its effects on motor actions, has been the focus of much attention.

1.1 The StartReact effect

In their pioneering work, Valls-Solé et al. (1999) demonstrated that the RT of ballistic movements can be shortened beyond the typical capabilities of the human central nervous system to an extent that was not previously thought to be possible. They demonstrated a reduction of RT from a mean of 171.4 ms (± 50.9 ms) to a mean of 77.3 ms (± 10.7 ms), when an extremely loud (> 130 dB) sound was delivered in synchrony with the IS. These reductions of RT were so substantial that pre-motor RTs as low as 65 ms could be observed in some cases (Valls-Solé et al., 1995, 1999). Moreover, high-intensity stimuli have not only been shown to substantially shorten RT, but also to increase forcefulness and vigour of movements beyond that which can typically be produced voluntarily (Anzak, Tan, Pogosyan, & Brown, 2011; Anzak, Tan, Pogosyan, Djamshidian, et al., 2011; Marinovic et al.,

2013; Marinovic, Milford, et al., 2015). This phenomenon has been referred to as the StartReact effect. Elicitation of the StartReact effect requires that a movement has been sufficiently prepared. If motor circuits have not been engaged to a sufficient sub-threshold level, the intense stimulus is likely to simply evoke a generalised skeletomotor startle response (Jones & Kennedy, 1951; Valls-Solé, Rothwell, et al., 1999). Interestingly, although RT and force are increased, the pattern of muscle activity generated when a prepared movement is elicited by an intense stimulus is not different from that of a voluntary movement (Valls-Solé et al., 1999). That is, the phenomenon does not represent a short-latency startle reflex which has an additional longer-latency voluntary motor component superimposed. Rather, the same triphasic pattern of muscle activity observed during voluntary ballistic movements can be observed when prepared ballistic movements are initiated via the StartReact effect (Valls-Solé et al., 1995; Valls-Solé, Rothwell, et al., 1999). The short RTs and increased force and vigour observed in the StartReact effect are consistent with the activation model described previously. **Figure 1.1** demonstrates the potential changes in activity in motor circuits during preparation and after stimulus presentation that would be expected to underlie the StartReact phenomenon when considering the activation model of initiation. However, the activation model of movement initiation only applies to voluntary movements. Those are actions which are voluntarily prepared and intended toward a goal. Interestingly, the traditional explanation in the literature for the StartReact effect proposes that it arises from an involuntary triggering of prepared movements through a unique circuit which is mediated through subcortical reflexive pathways (Carlsen et al., 2004a, 2004b, 2007; Valls-Solé et al., 1999). Reflexes are actions which are elicited after high intensity stimulation of sensory receptors (Yeomans & Frankland, 1995). For example, after the presentation of loud sound, there is a generalised skeletomotor startle response – the eyes produce a short-latency blink, and a cascade of movements is elicited progressively down the musculature of the body, starting at the neck, down through the trunk, and to the limbs (Brown et al., 1991; Jones & Kennedy, 1951; Yeomans & Frankland, 1995). The response occurs involuntarily through reflex circuits that are contained within subcortical areas of the central nervous system – pathways of which can conduct messages to and from the periphery much more quickly than pathways which must be routed through the cerebral cortex (Yeomans & Frankland,

1995). Considering that the StartReact phenomenon can take place at a time scale that is close to the estimated time required simply for the conduction of neural activity, it has been suggested there is little time available for sensorimotor processing at the level of the cerebral cortex (Valls-Solé et al., 1999). Therefore, the StartReact was initially suggested to represent a unique phenomenon involving the reflexive triggering of prepared voluntary actions.

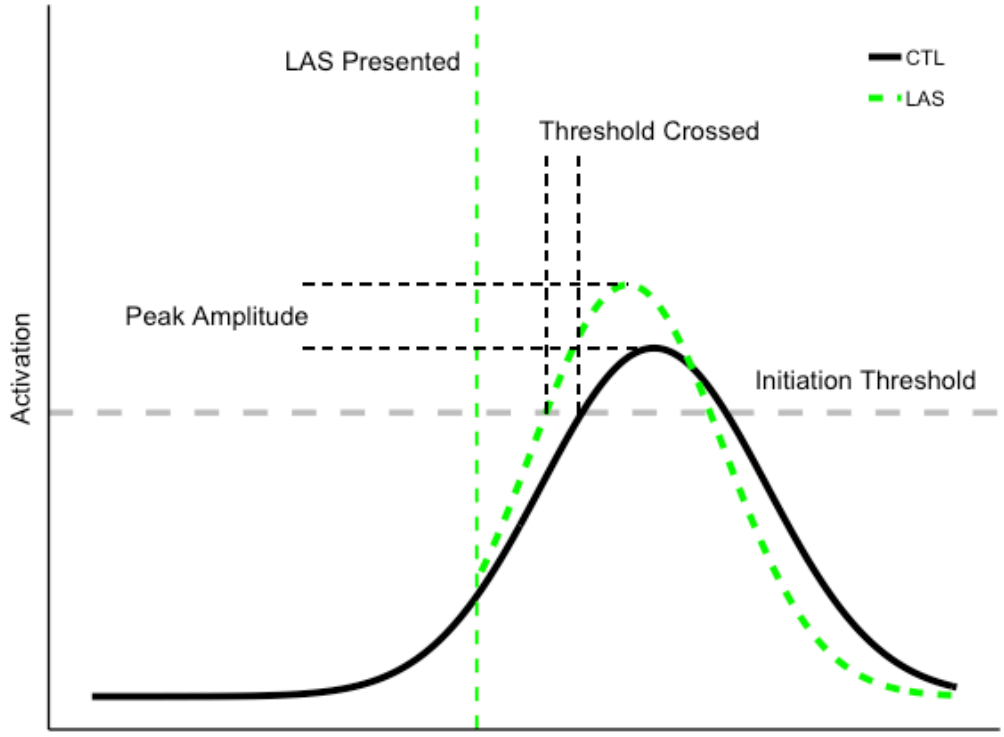


Figure 1.1. Activation model of action initiation. Neural activation in command generating circuitry builds up as movement preparation proceeds. According to this model, when the amplitude of the signal exceeds initiation threshold, the action is initiated. The extent to which amplitude exceeds initiation threshold corresponds with the force of the movement. The rate of rise above initiation threshold corresponds with the vigour of the movement. When a loud acoustic stimulus is presented, the rate of rise of activation to initiation threshold is higher, resulting in initiation threshold being crossed earlier. The amplitude also exceeds threshold to a greater extent and the rate of rise above threshold is higher, resulting in more forceful and vigorous movement. CTL = control trial, LAS = loud acoustic stimulus (probe) trial.

1.2 The startle triggering hypothesis

Unlike voluntary movements, it was initially suggested that the StartReact effect involves the storage of a motor program in subcortical circuits, which can subsequently be triggered for initiation when a high intensity stimulus evokes activity in these circuits (Carlsen et al., 2004b; Valls-Solé et al., 1999). The prime reason this differentiates startle-triggered actions from voluntary ones is because the startle-triggering hypothesis suggests this StartReact circuit is unique in that it triggers prepared volitional actions but takes no input from the cerebral cortex (Valls-Solé et al., 1999). A collection of neurons in the brainstem, known as the pontomedullary reticular formation (PMRF), was suggested to be the epicentre of the mechanism underlying this subcortical triggering (Valls-Solé et al., 1999; Yeomans & Frankland, 1995). Both voluntary and involuntary motor commands can be conducted via a descending pathway known as the reticulospinal tract (Lemon, 2008). Importantly, the reticular formation is said to give rise to the startle reflex, with reflexive activity being conducted from the reticular formation to the spinal cord via the reticulospinal tract (Brown et al., 1991; Hammond, 1973; Rothwell, 2006). Therefore, given a role of the reticular formation's association in both motor control as well as the startle reflex, it has been claimed that the StartReact effect relies on activation of the reticular formation, and in turn, the startle reflex (Carlsen et al., 2004b; Carlsen et al., 2003; Valls-Solé et al., 1999). According to this claim, when a high intensity sensory stimulus which is capable of eliciting a startle reflex evokes activity in the reticular formation, any prepared movement stored in these subcortical circuits will be evoked along with the generalised skeletomotor startle reflex. The argument for this mechanism rests on the StartReact effect in some cases occurring at latencies (~ 90 ms) which are not much longer than those usually observed for the startle reflex (~ 80 ms for muscles in the forearm) (Brown et al., 1991; Valls-Solé et al., 1999). Furthermore, voluntary actions elicited by starling sensory stimuli that are initiated at these extremely short latencies are frequently found to co-occur with startle muscle activity (Carlsen et al., 2004a).

Given the startle reflex originates from the reticular formation (Hammond, 1973; Rothwell, 2006), the activation of reflexive startle muscle activity should provide a physiological indication that circuits mediating the StartReact effect have been activated, if startle circuits do in fact mediate StartReact triggering. As such,

startle activity, measured from the sternocleidomastoid (SCM) muscle, is used as a physical indication of such startle activity (Jones & Kennedy, 1951). As described previously, it is possible to measure the startle reflex from musculature throughout the body (Brown et al., 1991; Jones & Kennedy, 1951). The orbicularis oculi (OOc), involved in the startle eyeblink reflex, as well as the SCM are most commonly measured as an indicator of the startle reflex as they are among the last muscles to habituate after repeated presentation of a startling sensory stimulus (Carlsen et al., 2007; Castellote et al., 2017; Kofler et al., 2006). However, measurement of OOc auditory-evoked startle becomes complicated as the pattern of muscle activity can be divided into two separate eyeblink components which can be difficult to distinguish from one another. The first component is a short-latency activation called the auditory eyeblink reflex, which serves as an eye protective mechanism (Brown et al., 1991). This precedes the auditory startle component which, when activated, results in a later onset OOc response which is shortly followed by the generalised skeletomotor startle reflex which progressively occurs down to the inferior musculature of the trunk and limbs (Brown et al., 1991). These two components of the reflexive eyeblink are thought to originate from circuits which are distinct from one other –mesencephalic circuits for the former (Hori et al., 1986) and bulbopontine circuits for the latter (Brown et al., 1991; Davis et al., 1982). Therefore, reliance on muscle activity in OOc to detect genuine startle activity can produce false positives, especially considering that the early component of the eyeblink reflex is more resistant to habituation than the later component (Carlsen et al., 2007). SCM has therefore become the muscle of choice for researchers measuring startle activity when investigating the StartReact effect.

1.3 Criticisms of subcortical triggering

1.3.1 Does triggering rely on activation of the startle response?

Reflexive SCM activity can indicate activation of reticular circuits which are in turn said to be responsible for the triggering of prepared actions in the StartReact effect. Therefore, actions elicited without the presence of measurable concomitant SCM activity are thought to be voluntary movements which do not represent activation of StartReact pathways (Carlsen et al., 2004a). Therefore, when SCM activity coincides with the triggering of the prepared action, the subcortical StartReact mechanism is

assumed to be activated (referred to as a SCM+ trial). In turn, when no measurable SCM activity coincides with the triggering of the prepared action, the subcortical StartReact mechanisms is assumed not to have been activated (referred to as a SCM- trial). In this situation, voluntary circuits responsible for movement initiation are assumed to have been activated, and any shortening of RT is attributed to the high intensity of the stimulus and/or the effects of accessory stimulation which were described previously. However, startle activity is not always observed when actions are elicited by the intense stimulus at short latencies which would otherwise be considered to be indicative of the StartReact effect (Marinovic & Tresilian, 2016). In addition, longer latency actions can be triggered with coinciding startle activity. Furthermore, while startle activity can be reduced with prepulse inhibition, the StartReact triggering of the prepared action does not appear to be affected by such inhibition (Valls-Solé et al., 2005). As such, Marinovic and Tresilian (2016) argued that a startle response is not necessary, nor sufficient, for the StartReact effect to occur. If sufficient activity in startle circuits is not required for the behavioural effects of the StartReact effect to occur, then this is basis to doubt whether the effect is mediated entirely by the reticular formation.

1.3.2 Plausibility of the cerebral cortex's involvement in the triggering circuit

The startle triggering hypothesis has not been unequivocally supported, and there has been a range of evidence that suggests the circuits underlying the StartReact effect do not preclude involvement of the cerebral cortex. Rather, voluntary motor pathways may be enhanced and/or a more direct transcortical pathway may be recruited by the intense sensory stimulus (Alibiglou & MacKinnon, 2012). Potential pathways which involve transmission through motor cortical areas have been proposed and the associated conduction times estimated through these pathways are compatible with the short RTs observed in the StartReact effect (Alibiglou & MacKinnon, 2012; Marinovic & Tresilian, 2016; see **Figure 1.2**). There are two known routes which have been proposed for the fast transmission of activity elicited by acoustic stimulation to motor areas of the cortex. The first, shown in **Figure 1.2A**, has been demonstrated in non-human primates (Arikuni et al., 1988; Deacon, 1992; Petrides & Pandya, 1988; Petrides & Pandya, 2006; Romanski et al., 1999). Through this potential pathway, movement initiation arises from activity evoked in

the cochlea of the inner ear, which is transmitted through the medial geniculate nucleus of the thalamus, subsequently reaching the primary auditory cortex (the classical auditory pathway; see Moller, 2003). Activity may then pass to motor areas via direct connections of the auditory cortex with the premotor cortex (Alibiglou & MacKinnon, 2012; Marinovic, Tresilian, et al., 2014; Moller, 2003; Nelson et al., 2013). This may then produce the descending volley which leads to initiation of the prepared motor program. The second potential pathway, which may mediate the StartReact effect as an alternative to or in combination with the first pathway, is shown in **Figure 1.2B**. This potential route of transmission involves activation evoked in the cochlea being sent to the reticular formation, which has dense thalamocortical interconnections directly with motor areas (Alibiglou & MacKinnon, 2012; Liang et al., 2013). Such a thalamocortical pathway which terminates on motor areas and bypasses the auditory cortex has been established in humans (Liang et al., 2013). Transmission via the reticular formation seems particularly plausible, given Alibiglou and MacKinnon (2012) observed evidence for facilitation at a subcortical level, as well as the fact that the startle reflex, but not the StartReact effect, can be reduced with prepulse inhibition (Valls-Solé et al., 2005). That is, up until the point of the reticular formation, the startle reflex and StartReact effect share a common pathway, at which point the transmission diverges, with activity being evoked in reticulospinal pathways for the startle reflex, and in corticospinal pathways for the triggering of the prepared action via the StartReact.

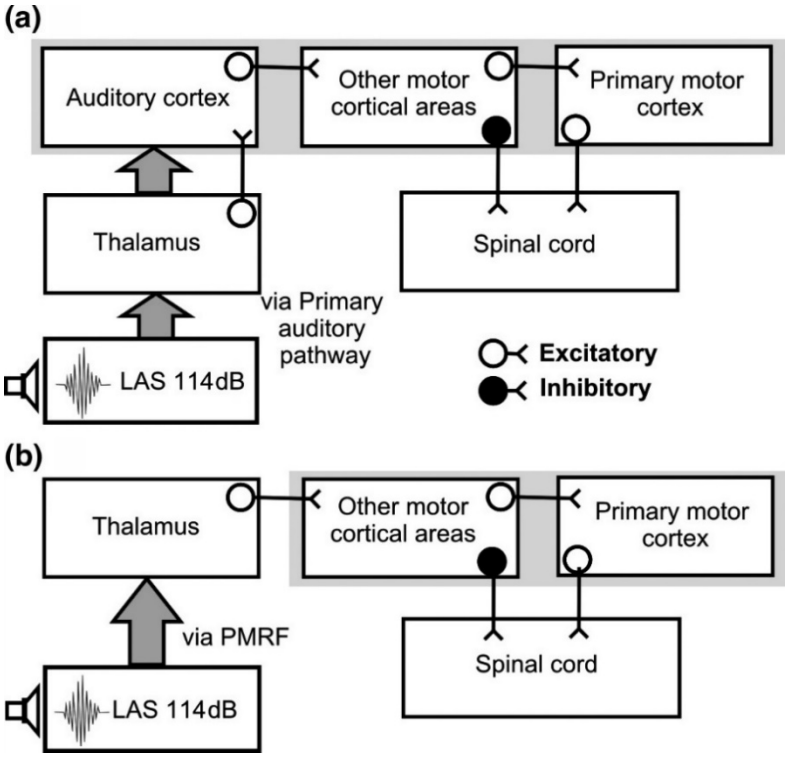


Figure 1.2. Proposed circuits mediating the triggering of motor actions by intense auditory stimulation. **A)**. Transmission of signal to the primary motor cortex via the thalamus and auditory cortex. **B)**. Transmission of signal to the primary motor cortex via the thalamus and bypassing the auditory cortex. LAS = loud acoustic stimulus. PMRF = pontomedullary reticular formation. Reprinted from "Triggering prepared motor action by sudden sounds: Reassessing the evidence for a single mechanism," by W. Marinovic and J. R. Tresilian (2016), Acta Physiologica, 217(1), p. 19. Copyright [2015] by Scandinavian Physiological Society. Reprinted with permission.

A third potential route is shown in **Figure 1.3**. In this pathway, preparatory activity from the cortex interacts with LAS-evoked activity in alpha motoneurons. This differs from the routes shown in **Figure 1.2** in that LAS-evoked activity interacts with preparatory activity at the level of the spinal cord, rather than at the cortex. Such a pathway would involve descending preparatory activity from the cortex being maintained at a subthreshold level for action initiation. At the presentation of the LAS, excitatory input through startle-circuits may reach the spinal cord and evoke the triggering of the prepared action.

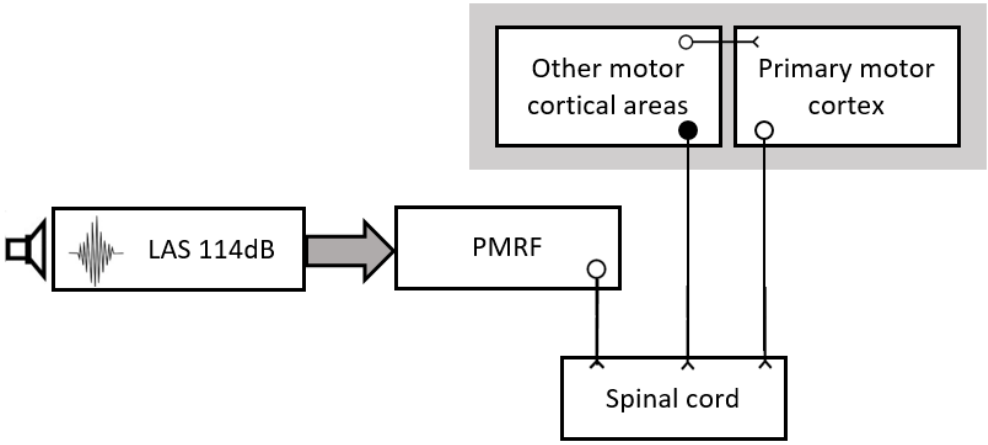
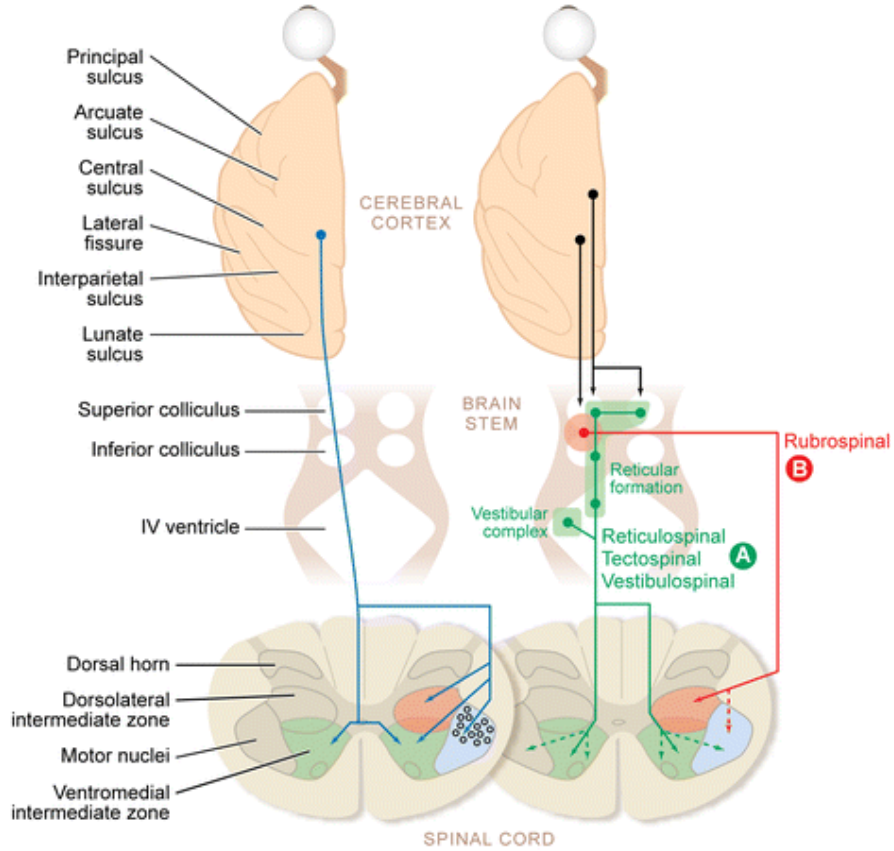


Figure 1.3. Third potential route of transmission in the StartReact effect involving the cortex, where LAS-evoked activity and preparatory activity interact at the level of the spinal cord. Adapted from Marinovic & Tresilian (2016).



Lemon RN. 2008.
Annu. Rev. Neurosci. 31:195–218.

Figure 1.4. Diagram of the descending pathways contributing to motor control. The corticospinal tract is depicted on the left (blue). These fibres project from the cortex

where some terminate bilaterally to the ventromedial areas of the spinal cord, whereas most terminate contralaterally to dorsolateral areas (red region) or directly onto motoneurons that innervate the hand and arm (blue region). On the right, Group A fibres (reticulospinal, tectospinal, and vestibulospinal tracts) are shown in green. These fibres project from brainstem areas and project predominantly bilaterally to the ventromedial intermediate zone of the spinal cord (green region). The Group B rubrospinal fibres are also depicted on the right (red), which project from the brainstem red nucleus, terminating contralaterally to the dorsolateral region of the intermediate zone and to the lateral group of motor nuclei innervating the arm and hands. The black lines depict cortical projections to brainstem areas. Reprinted from "Descending pathways in motor control" by R. N. Lemon (2008), Annual Review of Neuroscience, 31, p. 199. Copyright [2008] by Annual Reviews. Reprinted with permission.

A schematic diagram of the descending motor pathways is shown in **Figure 1.4**, with the corticospinal tract shown on the left (blue) and the Group A fibres (reticulospinal, tectospinal, vestibulospinal; green) and Group B fibres (rubrospinal; red) on the right of the figure. If the cortex is indeed involved in the triggering of actions in the StartReact effect, the most likely origin of the descending command is the primary motor cortex (M1) which gives rise to the descending pathway which is typically implicated in voluntary movement control, particularly that of the distal muscles of the limbs – the corticospinal tract (Brochier et al., 1999; Lawrence & Kuypers, 1968; Wade et al., 1983; see **Figure 1.4**). The most direct line of evidence for the involvement of M1 comes from the finding that suprathreshold, single-pulse transcranial magnetic stimulation (TMS) of M1 can delay the early triggering of motor actions by a loud acoustic stimulus (LAS) (Alibiglou & MacKinnon, 2012; Stevenson et al., 2014). However, conclusions of the transmission through pathways that pass through M1 are limited based on this evidence, given that at least in non-human primates, volleys evoked by high intensity TMS can extend to the cortico-reticular pathway and subsequently disrupt activity in the reticular formation (Fisher et al., 2012). It has been argued that this is unlikely to explain the observed disruption of the StartReact, as the authors also observed facilitation of startle circuits as indexed by a shortening of latency in SCM in five out of eight participants

(Alibiglou & MacKinnon, 2012). When discharging, TMS coils produce an auditory click and can produce a tactile sensation to the head, both of which increase with increasing TMS intensity. They argue that reticular circuits may have been facilitated by the auditory click, via cochlea input to the PMRF (Fisher et al., 2012). In addition, tactile stimulation evoked by TMS may conduct through bone to the saccule of the inner ear, which provides strong vestibular projections to the PMRF (Fisher et al., 2012). Hence, this may be evidence that TMS was unlikely to have inhibited startle circuits. As such, it is suggested the reduced StartReact is unlikely to be due to TMS suppression at a subcortical level, but rather, at a cortical level. However, the disruption of reticular activity by suprathreshold TMS has remained a concern, and contention remains regarding the involvement of M1 in the StartReact effect.

Other investigations using TMS have provided some indication that the StartReact effect is an entirely subcortically mediated process. It has been demonstrated that shortly after the presentation of loud acoustic stimuli, motor evoked potentials (MEPs) evoked by TMS, an index of corticospinal excitability, are transiently reduced in comparison to when no conditioning LAS precedes the TMS pulse (Fisher et al., 2004; Furubayashi et al., 2000; Ilic et al., 2011; Kuhn et al., 2004). Furthermore, transcranial electric stimulation (Furubayashi et al., 2000), subcortical electric stimulation of the subthalamic nucleus (Kuhn et al., 2004), and Hoffman's reflexes evoked by electric stimulation of sensory afferents (Ilic et al., 2011) have been used to measure the excitability of motoneurons that are innervated by descending pathways other than the corticospinal tract (e.g. the reticulospinal tract). As would be expected, these investigations showed a facilitation at a subcortical and spinal level after the presentation of a LAS. These studies provided strong evidence to support the subcortical triggering in the StartReact effect. However, this corticospinal suppression was observed when participants were maintaining a light muscle contraction at the time the LAS and TMS pulse were delivered. It was later discovered that the effects of loud acoustic stimuli on corticospinal excitability are not fixed, but are contingent on the background state of motor circuits (Marinovic, Tresilian, et al., 2014). The suppressive effects of loud acoustic stimuli on corticospinal excitability can be observed during early stages of movement preparation. However, in later stages of preparation closer to movement

onset, loud acoustic stimuli have an excitatory effect on corticospinal excitability (Marinovic, Tresilian, et al., 2014). This modulation of excitability was suggested to occur through a combination of intracortical disinhibition and facilitation. Furthermore, transcranial electric stimulation was used to show that the LAS-induced modulation of corticospinal excitability cannot be accounted for by a facilitation of subcortical excitability, placing the observed modulations of corticospinal excitability to likely be at the level of the cortex (Marinovic, Tresilian, et al., 2014). The early observations of cortical suppression and spinal facilitation after a LAS (Fisher et al., 2004; Furubayashi et al., 2000; Ilic et al., 2011; Kuhn et al., 2004) are incompatible with a cortically mediated StartReact effect. However, cortical facilitation is observed after acoustic stimulation in the context that the StartReact effect occurs – during high states of motor preparation. Suppression of M1 at low preparatory states may serve to inhibit voluntary actions and facilitate startle which serves to aid in survival after threatening stimuli (Furubayashi et al., 2000; Ilic et al., 2011). Facilitation at a cortical level during high states of readiness, in contrast, would aid in the engagement of prepared volitional acts which may be more suited for survival when taking into account additional sensory information associated with the threat. That is, when in a low state of arousal, it is useful for startle to disrupt voluntary motor activity, as taking in the sensory information and reprogramming actions would take too long temporally to allow an appropriate response to the threat. In higher states of arousal, the individual is more likely to have already received and processed relevant sensory information and therefore a more guided and informed set of actions that is appropriate for survival can be driven by the cerebral cortex. While these disruptive/facilitatory effects that are contingent on preparation cannot be ruled as a causal locus for the StartReact effect, it is consistent with cortical involvement through an activation model of response triggering by a LAS. These findings also have the potential to provide a neurophysiological means to explain how loud acoustic stimuli can exert their effects on prepared voluntary actions at the level of the cortex.

1.3.3 The neural control of muscles and their triggering

Some have also attempted to assert a subcortical locus of the StartReact effect based on the types of movements that are amenable to short latency triggering via the

StartReact effect. For example, distal muscles such as the fingers are likely to receive a greater degree of efferent innervation from the corticospinal tract (Baker, 2011; Lawrence & Kuypers, 1968; Lemon, 2008; Stevenson et al., 2014). In contrast, efferent innervation of proximal muscles arises more predominantly from the reticulospinal tract (Baker, 2011; Lawrence & Kuypers, 1968; Lemon, 2008). Functionality of movements can also impact the descending pathways which are preferentially used to innervate muscles; the corticospinal tract is thought to be recruited more during precision grip of the fingers, whereas more reticulospinal activation is used for grasping of the fingers (Lemon et al., 1995; see also Honeycutt et al., 2013). This may be a functionally relevant neurophysiological adaptation whereby input from the cortex can allow for a greater degree of fine control and independence (Oliveira et al., 2008; Quinn et al., 2018; Shim et al., 2007; Yu et al., 2010). In light of these differences in efferent innervation, movements which receive greater corticospinal versus reticulospinal contributions have been compared in their triggering via the StartReact effect. If the StartReact effect is indeed mediated by subcortical circuits for triggering, then movements which engage the reticulospinal tract to a greater extent would be expected to show a greater reduction of RT when triggered by a LAS. Carlsen et al. (2009) made such a comparison between the abduction of the index finger with extension of the more proximal arm. In the arm extension task, (premotor) RT was shortened from ~135 ms in control trials without a LAS, to ~85 ms in LAS trials where startle SCM activity had coincided with the triggered voluntary movement. When comparing LAS trials in this task, those that showed activation of startle circuits showed significantly shorter RTs than trials where no startle activity had been observed. However, in the index finger abduction task, RT did not significantly differ between SCM+ and SCM- trials (Carlsen et al., 2009). As discussed previously, startle activity has been thought by some to be necessary for sufficient activation to directly trigger a subcortically stored motor program. Therefore, given the finger abduction appeared to be less amenable to the StartReact effect, they argued this was consistent with a subcortical locus of the StartReact effect, since the muscles of the fingers are thought to receive more input from the corticospinal tract (Carlsen et al., 2009). Similar findings were reported in a comparison of individuated finger movements with coordinated grasping of the whole hand (Honeycutt et al., 2013). On the basis of comparing SCM+ and SCM-

trials within both tasks, Honeycutt et al. (2013) found that coordinated grasping of the hand was amenable to the StartReact effect, while individuated finger abduction was not. Consequently, they came to a similar conclusion to that of Carlsen et al. (2009) that this provided evidence for an entirely subcortical locus for the StartReact effect. Castellote and Kofler (2018) similarly found that isolated finger movement was not amenable to StartReact on the basis of a non-significant difference between SCM+ and SCM- trials. Elbow flexion, on the other hand, was amenable to the effect. However, when the finger movement was performed in combination simultaneously with elbow flexion, RT shortening consistent with the StartReact effect was observed in both movements (Castellote & Kofler, 2018). Demonstration of the StartReact effect in distal muscles only when performed in combination with a more proximal muscle seemed to provide further evidence for a subcortical StartReact origin.

However, the conclusions drawn have been under scrutiny and alternative explanations have been posed (L. A. Leow et al., 2018; Marinovic & Tresilian, 2016). For example, Carlsen et al.'s (2009) finger abduction task required only lifting the finger from a switch, the force of which required only 0.04 newtons (N). In contrast, the elbow extension required 20° of movement. In addition, due to physical distance from the spinal cord, conduction times are likely to vary between the two movements which may have imposed a lower limit to RT for finger muscles but not for the biceps brachii. As such, these are not equivalent actions for which comparison can be fairly made (Marinovic & Tresilian, 2016). Furthermore, the distribution of RTs in the distal task suggest both SCM+ and SCM- trials were often initiated close to the lower limit of RT, consequently making it difficult to detect differences between SCM+ and SCM- trials (Marinovic & Tresilian, 2016). As briefly mentioned earlier, these difficulties warrant caution when using SCM activity as a method of identifying the presence of the StartReact effect (L. A. Leow et al., 2018). As such, the findings of Carlsen et al. (2009) do not conclusively determine that distal muscles cannot be triggered by the StartReact effect. Others have also reported the StartReact effect in muscles of the hand, wrist, and face, which also limits the ability to conclude subcortical StartReact circuits, given these muscles are thought to be primarily innervated by motor circuits which receive input from the cortex (Honeycutt et al., 2013; Kirkpatrick et al., 2018; Kumru et al., 2006;

Marinovic, de Rugy, et al., 2014; Stevenson et al., 2014). The findings of Honeycutt et al. (2013) have also been questioned due to the engagement of larger, more distributed cortical network during more functionally relevant movements such as grasping (Flament et al., 1993; Graziano, 2011; Graziano & Aflalo, 2007; Kouchtir-Devanne et al., 2012). As a consequence, this may contribute to the greater magnitude of the StartReact effect reported for grasping (Honeycutt et al., 2013). The same argument can be made for the combined finger-elbow movement reported by Castellote and Kofler (2018). Therefore, the comparisons made between different muscles in the StartReact effect, to date, have not provided conclusive evidence either for or against the subcortical triggering hypothesis.

1.4 Other factors modulating the excitability of the descending motor pathways

1.4.1 The neural control of force and muscle innervation

Depending on the characteristics of the prepared movement, some actions may appear to be more amenable to RT shortening via StartReact. This may provide some evidence that certain networks within the central nervous system can be affected more greatly by acoustic stimuli. If certain networks benefit more from acoustic stimulation, the neural mechanisms underlying the StartReact effect can be inferred. The arguments presented above demonstrate that both the central nervous system's background state of preparedness, as well as certain features of the prepared action, have the potential to impact how activity associated with acoustic stimulation is introduced to motor program circuits, and how this activity can trigger the command generating circuitry. However, there are other features of selected actions and action preparation that should be considered. For example, the force of a prepared action has been overlooked in the vast majority of StartReact research which has been conducted to date. This is an important consideration for two reasons. First, the force of a prepared movement can impact its RT (MacKay & Bonnet, 1990). As such, the accumulation of neural activity to threshold may depend on the prepared force of a movement, which may subsequently interact with differences in activation accumulation that occur due to the muscle or functionality of the movement employed. Second, the production of force is highly correlated with M1 activity (Ashe, 1997). Depending on the mechanisms underlying action triggering by intense sounds, these factors related to movement preparation and the selected action have

the potential to impact the magnitude of the StartReact effect. Differences in the neurophysiological circuitry between different muscles or movement types may also play a role that can impact the StartReact magnitude. For example, early histological investigations of M1's control of force suggested that extensors receive greater contributions from the corticospinal tract, as it was observed that the slopes of regression lines of corticomotoneuronal cells against force were greater for extensor muscle groups than for flexor muscle groups. That is, the increase in static force observed for each unit increase in discharge rate of these cells was observed to be greater for extensors than that for flexors (Cheney & Fetz, 1980; Thach, 1978). It has therefore been suggested that the extensor muscle groups receive more efferent input from the corticospinal tract (Ashe, 1997). However, this finding may be interpreted differently in light of the fact that flexors are more commonly used functionally in daily tasks that require a greater degree of fine motor control (Oliveira et al., 2008; Quinn et al., 2018; Shim et al., 2007). This is particularly true for the digits – the flexor muscle is primarily recruited during actions involving fine motor coordination such as precision grip. Actions that require less precise control such as releasing a grasp, in contrast, activate the extensors primarily. As such, the lower ratio of output to firing rate of corticomotoneurons which innervate the flexors can support this finer degree of control through more precise modulations of force. Therefore the output to firing rate ratio of corticomotoneuronal cells does not necessarily implicate a greater role of these cells to the innervation of extensor muscles. Furthermore, more recent work using TMS has implicated a more functionally prevalent role of the corticospinal tract for the control of distal flexor muscles, with greater modulations of corticospinal excitability observed for flexors than extensors (Godfrey et al., 2013; Koganemaru et al., 2010; McMillan et al., 2004; Park & Li, 2013; Quinn et al., 2018; Vallence et al., 2012). Differences in the innervation between muscles may therefore be expected to impact the magnitude to which the StartReact effect takes place, if a unique triggering circuit underlies the effect. On the basis of a subcortical triggering hypothesis, actions which engage the reticulospinal tract more predominantly would be expected to show a greater magnitude of the StartReact effect in comparison to those which more strongly engage the corticospinal tract. On the other hand, if a short pathway through the cortex underlies the StartReact effect, akin to that shown in **Figure 1.2**, then

activation of M1 by the LAS would be expected to contribute more to actions which rely more predominantly on the corticospinal tract.

1.4.2 Modulation of the ipsilateral and contralateral corticospinal tracts' excitability

Temporal preparation and acoustic stimuli do not produce mutually exclusive modulations of neural excitability; as discussed earlier, the effects of sound on corticospinal excitability are contingent on the background state of the system (Marinovic, Tresilian, et al., 2014). This effect can further depend on the level of force at which a muscle is exerted. While the amplitude of MEPs evoked from TMS of the contralateral M1 are decreased from baseline after a LAS during light muscular contraction, at higher levels of muscle contraction, this suppression of MEP amplitude is not observed (Chen et al., 2016). Furthermore, there is a strong association of M1 activity with the level of force output - during unilateral muscle contraction, activity in the contralateral M1 is observed to increase as the amount of force exerted is increased (Ashe, 1997; Dai et al., 2001). However, these changes do not only occur in the contralateral M1. Ipsilateral M1 excitability has also been found to increase proportionally as the amount of force produced by a limb in an isometric contraction is increased (Chen et al., 2019; Perez & Cohen, 2008; Shibuya et al., 2014; Stinear et al., 2001; Uematsu et al., 2010). These effects also extend to modulations of ipsilateral M1 excitability after the presentation of loud acoustic stimuli. During isometric contractions above 10% of MVC, suppression of MEPs after a LAS is not observed in the contralateral resting biceps (ipsilateral to M1 driving the contracting limb) (Chen et al., 2016). Acoustic stimuli also have slight facilitatory effects on the corticospinal tract when the ipsilateral limb is engaged in a high state of preparation (Marinovic, Flannery, et al., 2015). As such, both contralateral and ipsilateral M1 excitability can be modulated by force production, as well as preparation. Furthermore, the enhanced background state of both hemispheres which is brought about by force production or preparation may reduce intracortical inhibitory circuitry (Chen et al., 2016), resulting in the reversal of LAS-induced corticospinal suppression that is observed when motor circuits are in a background state of low activity.

1.4.3 Premovement suppression of corticospinal excitability

The final modulation of motor circuit excitability that will be discussed here is the change in excitability that occurs in motor pathways over the course of preparation for action. It has long been established that shortly (~100 ms) prior to action onset, there is a rise in corticospinal excitability (Chen et al., 1998; Kennefick et al., 2014; Leocani et al., 2000; Marinovic et al., 2011; Rossini et al., 1988; Starr et al., 1988). This may reflect the accumulation of neural activity in motor program circuits as has been discussed throughout this chapter. However, before this ramp-up of excitability begins (~250 ms before movement onset), corticospinal excitability is transiently and paradoxically suppressed (Davranche et al., 2007; Duque et al., 2017; Duque & Ivry, 2009; Greenhouse et al., 2015; Hannah et al., 2018; Hasbroucq et al., 1997, 1999; Ibáñez et al., 2020; Lebon et al., 2016; Marinovic et al., 2011; McMillan et al., 2004; Touge et al., 1998; Van Elswijk et al., 2007). Not only does this suppression occur in the agonist muscle that is engaged in preparation, but is also observed in non-selected effectors (Duque et al., 2017; Duque & Ivry, 2009; Greenhouse et al., 2015; Klein et al., 2016). Furthermore, this inhibition can be seen in both cortical and subcortical areas of the central nervous system (Anthony & Putnam, 1985; Duque et al., 2010; Hannah et al., 2018; Marinovic et al., 2013; Nguyen et al., 2020). This phenomenon has been referred to as preparatory inhibition (also premovement suppression). The neurophysiological functional role for this intriguing inhibitory process is not clear, and several potential explanations have been posed.

One suggestion for the role of preparatory inhibition, referred to as the impulse control hypothesis, is that inhibition during preparation opposes the accumulated preparatory neural activity in motor program circuits and thereby prevents premature triggering of the action before the appropriate time (i.e. before the IS is presented) (Duque & Ivry, 2009). Duque and Ivry (2009) found evidence for this hypothesis by measuring MEPs from the left hand during a RT task that required a response from either the left, right, or both hands. The WS provided either informative information, cueing the specific action to be made, or uninformative information, where the action to be executed was not specified until the presentation of the IS. They found that MEPs in the left hand decreased by a greater extent when the WS specified that a left-hand response would, or might, be required. This suppression was weaker when the WS or IS specified a right-hand response. However, this interpretation of preparatory inhibition's neurophysiological role does

not fit all of the available data. While MEPs evoked in the majority of investigations of preparatory inhibition have reflected the summation of different descending volleys evoked by TMS, use of the Hoffman's reflex and different current directions can help discern these volleys that arise from different subpopulations of neurons (Derosiere, 2018; Hannah et al., 2018). If preparatory inhibition's neurophysiological role is to prevent activation in motor program circuits from prematurely triggering the prepared action, then any facilitatory input provided by TMS should be suppressed. Using the aforementioned methods of discerning separate subpopulations of neurons using TMS, Hannah et al. (2018) observed that there was specificity in the suppression of volleys evoked by TMS. The late I-waves showed selective suppression, while suppression of the early I-waves was not detected. The authors argue that when temporal predictability of the IS is high, there is selective suppression of certain corticospinal neurons, rather than global suppression of the entire descending pathway. They further reasoned that suppression does not occur as a direct inhibition of corticospinal neurons, but rather there is a reduced excitability in one of the excitatory input pathways that projects to corticomotoneurons (Hannah et al., 2018). The argument against the impulse control hypothesis was further corroborated by the observation that shorter RTs were associated with a greater degree of preparatory inhibition. The alternative of longer RTs would be expected if inhibition acts as a brake to initiation (Hannah et al., 2018). Finally, the impulse control hypothesis further becomes questionable when considering the fact that preparatory inhibition has been observed in self-paced movements (Ibáñez et al., 2020). In this case, there is no IS for which temporal preparation must precede and there is no need for initiation to be suppressed until the correct temporal location. Therefore, preparatory inhibition would not be expected for self-paced movements (Ibáñez et al., 2020).

An alternate proposition, referred to as competition resolution (also deselection), states that inhibition serves to suppress competing response selections from being initiated (Burle et al., 2004). Given the inhibition observed in unselected effectors, this seems an alluring explanation. However, the findings of Duque and Ivry (2009), described in the last paragraph, contradict this. If preparatory inhibition was necessary to prevent competing actions from being initiated, inhibition of the left-hand would be expected to be greater when a WS specifies a right-hand

response, in comparison to when the WS specifies a left-hand response or is uninformative. This is in contrast to the greater inhibition observed in the left hand when the WS specified a left-hand response (Duque & Ivry, 2009). Furthermore, under the competition resolution hypothesis, inhibition of the left hand should increase in the uninformative condition, after the IS indicates a right hand response is required (Duque & Ivry, 2009). It was later argued (Duque et al., 2014) that preparatory inhibition may reflect two separate inhibitory phenomena acting in a combined process of impulse control and competition resolution. For example, a general impulse control process may act earlier in preparation but eventually unfolds into a selective competition resolution as stimulus discrimination and response selection proceeds (Duque et al., 2014). However, the argument of competition resolution is weakened by the fact that preparatory inhibition occurs even in simple RT tasks, where there is no competing response to be prevented.

The final account of preparatory inhibition that will be discussed here is the spotlight hypothesis. This suggests that background activity in motor circuits can be reduced by preparatory inhibition, which increases the signal-to-noise ratio and thereby allows for easier identification of the input signal for triggering. Alternatively, if following an accumulator model of action initiation, the spotlight hypothesis would allow the preparatory activation level to be raised to a higher level, or initiation threshold could be lowered, as when there is less noise in the system there is less risk of accidental triggering (Carlsen et al., 2012). The spotlight hypothesis is in accordance with Hannah et al.'s (2018) earlier discussed observation of shorter RTs being associated with greater levels of preparatory inhibition. The association should be taken with caution though, as the directionality of the relationship cannot be confirmed. For example, higher states of preparation may simply result in concomitant short RTs and higher levels of preparatory inhibition. As discussed earlier, a similar case has been made for the relationship between motor preparation, startle activity, and early triggering of movement by a LAS (Marinovic & Tresilian, 2016). In addition, the spotlight explanation is at odds with the finding of preparatory inhibition in task-irrelevant muscles. For example, it could be argued that a reduction of background activity in the circuits of non-selected muscles would result in an increased risk of unintentionally triggering task-irrelevant actions, which does not appear to be the case.

1.5 The benefits of modulating central nervous system excitability

As has been discussed so far in this chapter, there are a wide variety of factors than can impact the amount of excitability within motor program circuits and it is clear that there is often a great deal of contention regarding the exact mechanistic underpinnings of these neural modulations. However, despite the ongoing debate regarding its underlying mechanisms, the StartReact effect has served as a useful tool for researchers looking to understand the neural correlates of action preparation. Presentation of a LAS can serve as a useful, non-invasive probe of the state of motor circuits during preparation for action. For example, the StartReact effect has been used to examine what (Carlsen et al., 2004b; Carlsen et al., 2009), when (Carlsen & MacKinnon, 2010; MacKinnon et al., 2007), and how (Marinovic et al., 2013; Marinovic, Tresilian, et al., 2017), actions are prepared in different contexts. There are also particularly intriguing applications of the StartReact effect to neurological disorders where action initiation is impaired. There is a multitude of literature demonstrating that intense sensory stimuli can restore the initiation of movement in a range of disorders where voluntary initiation is impaired (for review see Carlsen et al., 2012; Nonnekes et al., 2015), including stroke (Choudhury et al., 2019; Coppens et al., 2018; Honeycutt et al., 2014, 2015; Liu et al., 2019; Marinovic et al., 2016; Rahimi & Honeycutt, 2020a), Parkinson's disease (Anzak, Tan, Pogosyan, Djamshidian, et al., 2011; Fernandez-Del-Olmo et al., 2013; Nonnekes, de Kam, et al., 2015; Valldeoriola et al., 1998), hereditary spastic paraplegia (Nonnekes et al., 2014), cervical dystonia (Serranová et al., 2012), and multiple system atrophy (Valldeoriola et al., 1998). Hence, when used in combination with traditional rehabilitation protocols, the StartReact effect has great potential for clinical benefit (Marinovic et al., 2016).

1.6 Force and vigour: An often overlooked insight

RT has been extensively used as a measure of processes underlying our behaviour, not only in the StartReact literature, but also more generally throughout cognitive neuropsychology. However, the force and vigour of movements have frequently been overlooked in these investigations. The force of movements has previously been examined in order to quantify changes in preparatory activation during preparation (Marinovic et al., 2013), and the facilitation of movement force via the

StartReact effect has been shown to be a potential therapeutic target in Parkinson's disease, along with movement initiation (Anzak, Tan, Pogosyan, Djamshidian, et al., 2011). Furthermore, the utility of movement vigour has recently been highlighted as an important index of decision making processes (Yoon et al., 2018). However, the examination of movement vigour has been somewhat neglected in investigations of the StartReact effect to date. As described earlier, the force of a prepared action may impact the accumulation of preparatory activity and subsequently interact with the effects of a LAS on motor output. As such, this should be an important component to consider when evaluating the types of movements that can receive more benefit from a LAS. Furthermore, movement force and vigour can be informative indices of the neural processes underlying action preparation and execution (Marinovic et al., 2013). A range of kinematic and kinetic features of movement can be used in order to quantify the effects of acoustic stimulation on motor output (see **Figure 1.5**). Acquiring data from force sensors is particularly useful in capturing these features of movement. RT, representing the processing and conduction time of the sensorimotor system, can be determined from force time-series data by subtracting the time of IS presentation from the time of movement onset. This represents the initiation process of movement. There are many difficulties associated with accurately detecting the time of onset of an analogue signal. Teasdale et al. (1993) have proposed a well-defined and accurate method of estimating the time of movement onset from force time series data which has been used throughout the research contained in this thesis. This algorithm consists of multiple steps. First, the peak amplitude of the time-series data is determined, and 10% of this amplitude is calculated, referred to as the tolerance range (1). Next, an initial starting point is determined by finding the first time point at which amplitude is greater than or equal to the tolerance range (1). A new tolerance range (2) is calculated at 10% of the starting point's amplitude. The algorithm then works backward, and finally onset is determined as the time point at which the difference between the amplitude of the current sample and the amplitude of the initial starting point is greater than or equal to the tolerance range (2). It is also useful to measure the magnitude of movement execution through the variables peak force and peak vigour. Peak force can be determined from the signal by calculating the peak amplitude and time to peak force is determined by subtracting the sample of movement onset from the sample at which the peak amplitude occurs. The term

movement vigour refers to the peak rate of force development (velocity). This is calculated as the peak of the derivative of the force signal over time. Time to peak rate of force development is calculated by subtracting the sample of movement onset from the sample at which the peak rate of force development occurs. These variables are depicted visually in **Figure 1.5**. Given the lack of attention previously directed to these action characteristics, a particular emphasis on the magnitude of motor output is given throughout the work conducted in this thesis.

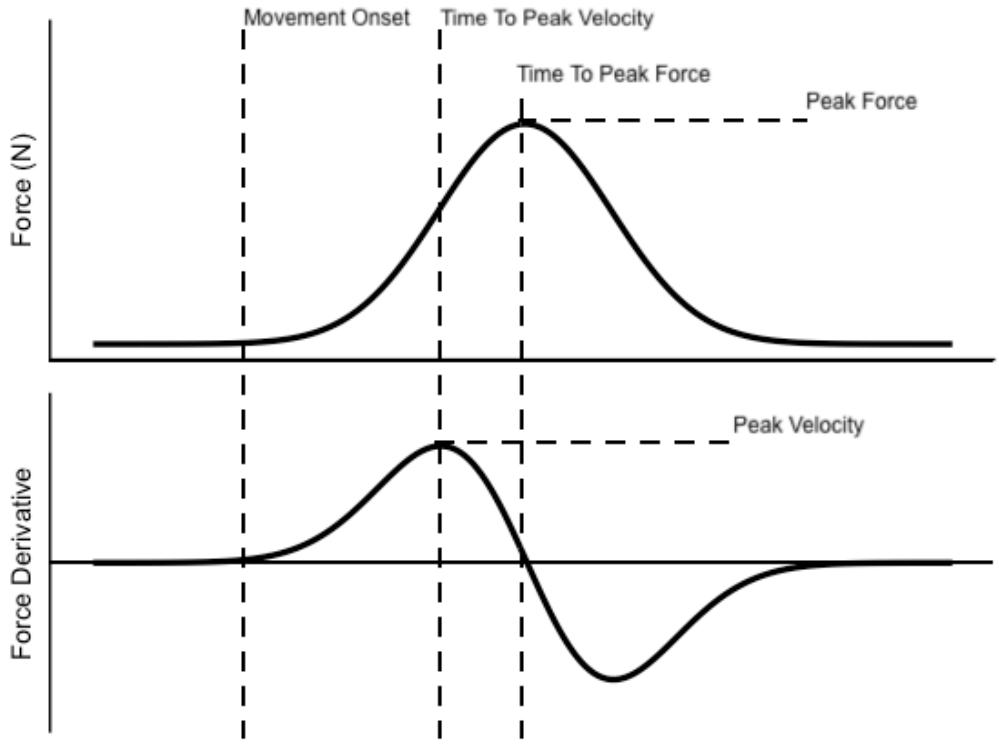


Figure 1.5. Force time series (top) and time derivative (bottom). Movement onset is determined from the force data using Teasdale's (1993) algorithm. Peak velocity is the maximum rate of change over time determined from the force time derivative. Peak force refers to the maximum amplitude of the force data over the recording period. Time to peak force and time to peak velocity are determined from the samples at which each of the variables occur in relation to movement onset.

1.7 General purpose and outline.

The overall aim of this thesis is to outline what types of movements are more easily facilitated by a LAS for two reasons.

1. The types of actions that are more easily facilitated by a LAS can provide informative insights as to the neural mechanisms underpinning the StartReact effect, as well as movement control in general.
2. Identifying the types of actions that receive more benefit from a LAS can be useful in determining therapeutic targets when using the StartReact effect to aid in rehabilitation after neurological conditions which impair the voluntary control of movement.

This thesis comprises six chapters. The first chapter has provided a summary of the issues which will be addressed throughout as well as the theoretical approach taken during this research. Chapters two through five each comprise a standalone scientific work. Chapter two in more detail examines the issue of relying on startle activity to identify cases of the StartReact effect, in which several datasets from the literature are re-analysed. An alternative method of data categorisation and analysis is proposed, and this new method is used to identify the types of movements that may receive more benefit from intense sensory stimulation. In chapter three, the issue of efferent muscle connectivity and force level of movements triggered by a LAS is addressed. Furthermore, in this chapter I examine whether the introduction of neural activity to motor program circuits by a LAS is additive to, or multiplicative of, the preparatory signal in these circuits. Chapter four examines how the effects of acoustic stimuli on movement initiation and execution may change, depending on the amount of force that is maintained in a sustained contraction during action preparation – situations that produce paradoxically different effects of a LAS on M1 excitability. Finally, in chapter five, I examine how the process of preparatory inhibition may impact the enhancements of motor performance gained from the StartReact effect. I further propose a new account of the neurophysiological role of M1 suppression during action preparation. Finally, in chapter six, I summarise the main findings of this thesis and provide overall conclusions in the context of the theoretical background introduced in chapter one.

CHAPTER TWO: CUMULATIVE DISTRIBUTION FUNCTIONS: AN ALTERNATIVE APPROACH TO EXAMINE THE TRIGGERING OF PREPARED MOTOR ACTIONS IN THE STARTREACT EFFECT

This is the peer reviewed version of the following article:

McInnes, A. N., Castellote, J. M., Kofler, M., Honeycutt, C. F., Lipp, O. V., Riek, S., Tresilian, J. R., & Marinovic, W. (2020). Cumulative distribution functions: An alternative approach to examine the triggering of prepared motor actions in the StartReact effect. *European Journal of Neuroscience*, *53*(5), 1545-1568.

which has been published in final form at https://doi.org/10.1111/ejn.14973.
Permission has been obtained from the copyright holder to reproduce this article.

2.0 Abstract

There has been much debate concerning whether startling sensory stimuli can activate a fast-neural pathway for movement triggering (StartReact) which is different from that of voluntary movements. Activity in sternocleidomastoid (SCM) electromyogram is suggested to indicate activation of this pathway. We evaluated whether SCM activity can accurately identify trials which may differ in their neurophysiological triggering and assessed the use of cumulative distribution functions (CDFs) of reaction time (RT) data to identify trials with the shortest RTs for analysis. Using recent datasets from the StartReact literature, we examined the relationship between RT and SCM activity. We categorised data into short/longer RT bins using CDFs and used linear mixed effects models to compare potential conclusions that can be drawn when categorising data on the basis of RT versus on the basis of SCM activity. The capacity of SCM to predict RT is task-specific, making it an unreliable indicator of distinct neurophysiological mechanisms. Classification of trials using CDFs is capable of capturing potential task- or muscle-related differences in triggering whilst avoiding the pitfalls of the traditional SCM activity-based classification method. We conclude that SCM activity is not always evident on trials that show the early triggering of movements seen in the StartReact phenomenon. We further propose that a more comprehensive analysis of data may be achieved through the inclusion of CDF analyses. These findings have implications for future research investigating movement triggering as well as for potential therapeutic applications of StartReact.

2.1 Introduction

Large reductions of reaction time (RT) can be observed when an intense sensory stimulus is presented during movement preparation (Valls-Solé, Rothwell, et al., 1999), a phenomenon termed the StartReact effect. These observations of remarkably short RTs have led to the proposal that triggering mechanisms separate to those responsible for voluntary movements are activated by an intense sensory stimulus which is capable of producing a startle response (Carlsen et al., 2007; Carlsen et al., 2012; Valls-Solé et al., 1999). That is, prepared movements may be released when a startling stimulus excites subcortical structures, bypassing the usual cortical circuits involved in voluntary motor control (Carlsen et al., 2004b; Valls-Solé, Rothwell, et al., 1999). Initial investigations of the benefit of startling stimuli on RT compared trials with and without an accompanying intense sensory stimulus (Carlsen et al., 2000; Valldeoriola et al., 1998; Valls-Solé et al., 1995; Valls-Solé et al., 1999). However, it was later proposed (Carlsen et al., 2004; Carlsen et al, 2007) that it is necessary to observe a startle reflex to differentiate the StartReact effect from other phenomena that can cause (usually less extensive) reductions in RT, such as the well-documented stimulus intensity and accessory stimulus effects (Bernstein et al., 1969; Pieron, 1914; Pins & Bonnet, 1996). As such, comparing trials with and without a startle response was later adopted as a standard practice to differentiate these assumedly separate phenomena which can shorten RTs. The justification for this praxis relies on the assumption that excitation of subcortical structures associated with the startle response can lead to the engagement of a distinct StartReact pathway for movement triggering. Thus, motor responses have typically been defined as StartReact movements on the basis of activity in surface electromyography (EMG) of the sternocleidomastoid (SCM) muscle, which is said to indicate startle (Carlsen et al., 2007). When no SCM activity is recorded in a trial, it is assumed that the specific mechanism responsible for the StartReact effect was not activated, and the less dramatic reductions of response time that are typically observed are attributed to stimulus intensity and/or accessory stimulus effects through the pathway used for volitional motor control (Carlsen et al., 2011; Kohfield, 1971).

While, on average, movements in the presence of SCM activity usually occur with shorter RTs than those in absence of SCM activity, it has not been unequivocally demonstrated that observation of a startle response is a necessary condition for the vast reductions of RT which are indicative of the StartReact effect. There are several lines of evidence which suggest startle may not be (directly) linked to these RT savings. For example, surface SCM activity is not always present when eliciting movements with latencies short enough to be indicative of a StartReact effect (Marinovic & Tresilian, 2016; Maslovat et al., 2015), and several studies failed to detect RT differences between SCM+ and SCM- trials (Campbell et al., 2013; MacKinnon et al., 2007; Marinovic, Cheung, et al., 2014; Nonnekes et al., 2013). The impaired reliability of using SCM as a marker of neurophysiological circuitry is further demonstrated by the finding that SCM activity can be reduced with pre-pulse inhibition without modifying RT shortening in the StartReact (Castellote et al., 2017; Lipp et al., 2006; Maslovat et al., 2012; Valls-Solé et al., 2005), and unlike startle, the StartReact effect does not appear to be prone to habituation (Castellote et al., 2017; Valldeoriola et al., 1998). As such, even if activity associated with the intense stimulus reaches startle-related circuits, this may not always be indicated by SCM activity. Therefore, making inferences about the circuitry used for fast movement triggering based on surface SCM activity may be rather unreliable. Furthermore, the available data do not preclude cortical involvement in the StartReact effect. As an alternative view to this triggering through subcortical areas, the shortening of RT seen in the StartReact effect may be a product of an enhancement of voluntary motor pathways via an engagement of a more wide-spread cortical-subcortical network when an intense sensory stimulus is presented (Alibiglou & MacKinnon, 2012; Stevenson et al., 2014; see Marinovic & Tresilian, 2016 for a review). The difficulties in determining neurophysiological mechanisms underlying the early triggering of motor responses using the presence or absence of SCM activity has been outlined previously (Dean & Baker, 2017; L. A. Leow et al., 2018; Marinovic & Tresilian, 2016; McInnes et al., 2020) and it seems SCM activity can be an unreliable indicator of distinct mechanisms that can be activated by intense sensory stimuli. Rather, determination of the presence of a specific StartReact mechanism may be more feasible when trials are separated based on their response latency (L. A. Leow et al., 2018; McInnes et al., 2020).

Here, we evaluate the utility of separating RT trials on the basis of SCM activity to investigate mechanisms underlying the StartReact phenomenon and further examine an alternative approach, cumulative distribution functions (CDFs) that separate trials based on response latency (L. A. Leow et al., 2018; McInnes et al., 2020). CDFs allow an examination of how trials with the fastest RTs differ from those with slower RTs which would be considered unrepresentative of the StartReact effect, whilst avoiding the pitfalls of relying on SCM activity as an indicator of StartReact mechanisms which have been outlined previously (Marinovic & Tresilian, 2016). We re-analysed data from seven studies (Castellote & Kofler, 2018; Honeycutt et al., 2013, 2014; Marinovic, de Rugy, et al., 2014; Marinovic, Milford, et al., 2015; Ossanna et al., 2019; Tresch et al., 2014) which have investigated differences in response times across trials in the presence and absence of SCM activity. We used our method to evaluate the utility of separating trials on the basis of SCM activity by examining the distribution of SCM activity across the spectrum of RTs and evaluating the relationship between RT and the presence of SCM activity. We further analysed these datasets in order to define a common method of separating trials on the basis of response latency. Lastly, we used our method of trial categorisation to evaluate the hypothesis that separate mechanisms contribute to StartReact and voluntary movements.

2.2 Methods

Data comparing responses which occur in the presence and absence of SCM activity were provided from the authors of seven studies reported in recent literature and subject to statistical analyses. Note that the Tresch et al. (2014) dataset includes data collected from participants with stroke (n = 4) which were reported separately in Honeycutt et al. (2014). For the sake of brevity, we have limited the report within the main body to the analysis of a single dataset provided by Castellote and Kofler (2018). This task recorded EMGs from the biceps brachii (BB) in a flex-only task, first dorsal interosseous (FDI) in a pinch-only task, and both BB and FDI in a combined pinch-flex task. Extended analyses for the individual datasets from the remaining studies, which differed in tasks used and muscles from which EMGs were recorded in addition to SCM (summarised in **Table 2.1**), are reported in the appendices of this report.

Table 2.1. Overview of studies included in analyses.

Authors (year)	*N*	*Task*	*Muscles recorded*
Castellote & Kofler (2018)	11	Elbow flexion Finger pinch Combined pinch-flex	Biceps brachii (BB) First dorsal interosseous (FDI) BB and FDI
Honeycutt et al. (2013)	10	Finger abduction Grasp	FDI
Marinovic et al. (2014)	7	Lip press Button press with thumb	Orbicularis oris (OO) Abductor pollicis brevis (APB)
Marinovic et al. (2015)	10	Arm supination	BB
Ossanna et al. (2019)	10	Five-direction arm reaching	Anterior deltoid (AD) BB Brachioradialis (Br) Posterior deltoid (PD) Pectoralis (Pe) Triceps brachii (TB)
Honeycutt et al. (2014); Tresch et al. (2014)	34	Hand flexion, extension	Extensor digitorum communis (EDC) Flexor digitorum superficialis (FDS) FDI

All analyses were conducted using R software (*v3.6.0*; R Core Team, 2019). StartReact experiments typically employ "control" trials in which the participant performs a predetermined movement in response to an imperative stimulus (IS). In a subset of trials, an intense sensory stimulus (probe) is delivered in addition to the IS. Data used for our analyses of each individual dataset were limited to (premotor; time to EMG onset) RTs in probe trials for which an intense sensory stimulus was delivered (i.e. control trials were removed). Movements made in response to probes

for which SCM activity was recorded are defined as SCM+ responses. Responses not accompanied by SCM activity were defined as SCM- responses. If the responses of the target muscles in a given task that occur after an intense stimulus differ in terms of neurophysiological pathways, i.e. are either short latency SCM+ movements or longer latency SCM- movements, then RTs from those target muscles should fit a bimodal distribution. Alternatively, if a common mechanism underlies both SCM+ and SCM- movements, the data should fit a unimodal distribution. Data were separated for each task type and/or muscle type and we tested for the modality of each distribution with Hartigan's (1985) dip test, using the *dip.test* function from the *diptest* package (*v0.75*). Due to the skewness commonly observed in RT data, we conducted a natural logarithmic transformation of all data for each movement type to assess whether skewness had any significant impact on the results of the dip tests (Whelan, 2008).

For all movements within each experiment, we calculated each participant's median RT for SCM+ and SCM- responses. We conducted paired sample t-tests on these median values to examine the difference in RT between SCM+ and SCM- trials. This test allowed us to examine what muscles or movement types are identified as being amenable to StartReact in accordance with the SCM based method used to categorise responses to the intense probe stimulus. These results were later used for comparison with our analyses using the classification of responses on the basis of response latencies via CDFs.

For each individual participant, CDFs were calculated for the response time data of all trials in which an intense probe was delivered for each movement recorded, using the *quantile* function (Hyndman & Fan, 1996) from the *stats* package (*v3.6.0*). Quantiles were calculated for each participant's RTs at the 5^{th}, 15^{th}, 25^{th}, 35^{th}, 45^{th}, 55^{th}, 65^{th}, 75^{th}, 85^{th}, and 95^{th} percentiles of RT. We then calculated the mean RT across subjects for each of the quantiles within the CDFs (Ratcliff, 1979), giving ten values which represent the average response times of participants at each percentile for all CDFs we conducted. We further calculated the mean of our subject medians of SCM+ and SCM- responses to determine the mean SCM+ and SCM- latencies. Once these were calculated, these average SCM+ and SCM- latencies were used to estimate the latencies of responses that may differ in their triggering mechanisms and compared these to our calculated quantiles. Therefore,

given SCM+ and SCM- trials have been assumed to differ in their triggering mechanisms, for a given movement type within each experiment, the mean percentile closest in terms of RT to the mean SCM+ trial latency across participants was deemed the SCM+ percentile. Similarly, the mean percentile latency that was closest to the mean latency of SCM- trials for a given movement type was deemed the SCM- percentile. These percentiles allowed us to approximate the short and long RTs that may occur as a product of the potentially different neurophysiological pathways contributing to RTs in response to the intense probe stimulus.

Once percentiles approximating responses with and without SCM activity were calculated, we used these percentiles to group data into "startle" and "non-startle" categories (see **Figure 2.1**). If distinct mechanisms are activated for StartReact versus voluntary movements, splitting trials on the basis of latency should separate those movements which are thought of as being distinct, with trials at the shortest latencies representing the StartReact triggered movements and those at the longer latencies representing voluntarily triggered movements. Trials were placed into the startle category if their RT was equal to or shorter than the SCM+ percentile latency that was calculated for a given movement type within an experiment. Similarly, trials were placed into the non-startle category if their RT was equal to or longer than the SCM- percentile latency for that movement/muscle. We then calculated the percentage of trials within each category that occurred with SCM activity. This was calculated as $(\frac{n(SCM+\ responses\ in\ category)}{n(Total\ responses\ in\ category)} \times 100)$. If SCM activity is a critical criterion for the considerable reductions of RT in the StartReact effect, then SCM+ responses should primarily occur in the startle category and SCM- responses should primarily occur in the non-startle category. To test this, we conducted a series of Bayesian tests of association using the *contingencyTableBF* function from the *BayesFactor* package (*v0.9.12*), with the joint multinomial sampling method (Albert, 1997; Gunel & Dickey, 1974; Morey et al., 2018). This test assesses the degree to which the data provide evidence for the dependence of SCM activity (SCM+/SCM-) on startle categorisation (startle/non-startle). If the presence of SCM activity does indeed depend on startle or non-startle categorisation – that is, SCM activity is predominantly found for responses in the startle category – this test would provide decisive evidence against the null hypothesis. This result would provide support for the use of SCM activity as an indicator of the activation of

a fast-neurophysiological pathway. If, however, SCM+ responses are distributed across both startle and non-startle categories, and these variables are independent of one another, we expect to observe weaker evidence to support their dependence. BF_{10} values are reported, which describe the degree to which the data provide evidence against the null hypothesis.

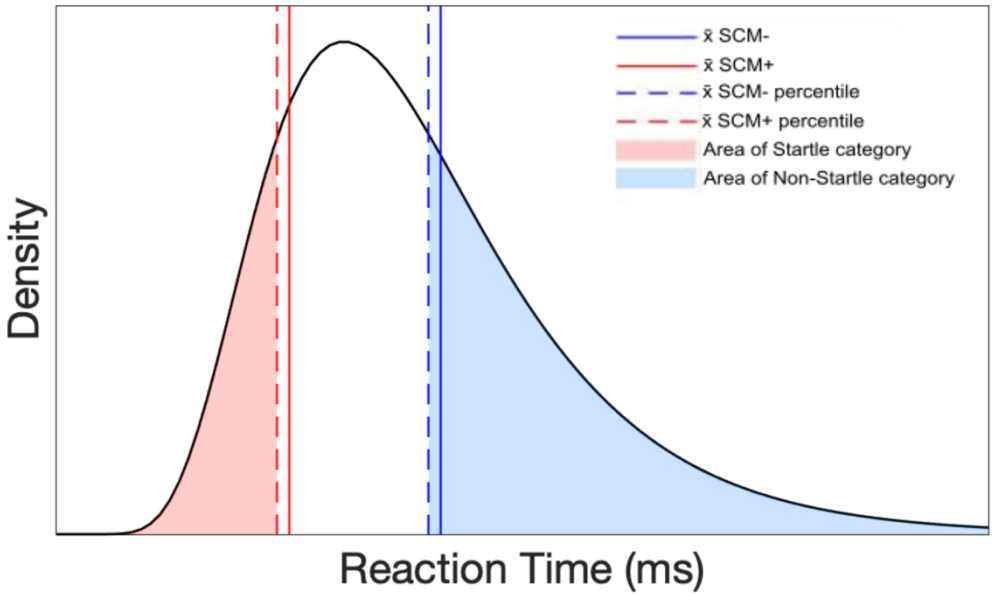


Figure 2.1. Illustration of the method used to categorise trials. Mean latencies of SCM+ and SCM- responses were used to determine the equivalent SCM+ and SCM- percentiles. Responses were categorised into the startle category if their latency was equal to or shorter than the mean latency of the SCM+ percentile. Responses were categorised into the non-startle category if their latency was equal to or longer than the mean latency of the SCM- percentile. Note this is a modelled exemplary RT dataset with x and y values not to scale.

We further used our percentiles across all data analysed to determine a common percentile ranking which may be used to categorise responses across all data sets. Across all tasks and muscles over all datasets analysed, the 45th percentile was the latest percentile that was approximated to SCM+ responses, and the 55th percentile was the earliest percentile approximated to SCM- responses. For each movement type in each experiment, all participants RTs at the 45th percentile or earlier were therefore determined to be equivalent to (fast onset) SCM+ responses,

and RTs at the 55th percentile or later were determined to be equivalent to (slower onset) SCM- responses.

With our categorised data, we conducted a linear mixed-effects model with Kenward-Roger approximation for degrees of freedom using the *lmer* function (*lmerTest* package; *v2.0-36*; Kuznetsova et al., 2017) on the Castellote and Kofler (2018) data as a representative dataset. Percentile categorisation (fast onset/slower onset) and task type were set as fixed-factors in the model and participants were set as a random factor. We examined the interactions of percentile and task/muscle type to assess whether the shortening of RT by the probe stimulus differs between movements which likely have distinct connectivity to different brain regions. Such an interaction would support separate pathways being recruited for StartReact versus voluntarily initiated movements. Post hoc analyses were conducted using the *emmeans* function (*emmeans* package; *v1.3.4;* Lenth, 2019) using the Tukey correction for multiple comparisons.

In order to encourage future use of CDFs when investigating triggering mechanisms in the StartReact effect, we have provided an R script which runs all analyses used in this report on a simulated dataset. The code can be obtained at http://doi.org/10.5281/zenodo.3760340. The data from the studies analysed in this report have been published elsewhere and may be obtained at the request of the original authors.

2.3 Results

2.3.1 Unimodality versus bimodality of data

Hartigan's (1985) dip test failed to reject the null hypothesis of unimodality for the elbow flexion (flex-only; $p = .715$), the finger pinch (pinch-only; $p = .095$), the combined task BB latency (BB pinch-flex; $p = .093$), or the combined task FDI latency (FDI pinch-flex; $p = .277$) reported by Castellote and Kofler (2018). This suggests all tasks analysed produced a unimodal distribution of data. Extended analyses of the remaining datasets are presented in **Table 2.3**. The analysis of the logarithmically transformed data was consistent with that of the original data and as such, we have reported the analyses of untransformed data.

2.3.2 Differences between SCM+ and SCM- trials

Paired sample t-tests of the difference between each subject's median SCM+ and SCM- trial latencies for all movement types in the representative dataset indicated a significant difference in RT between SCM+ and SCM- responses in BB for the flex-only task (mean difference = -30.2 ms, CI = -38, -22.4), in BB for the combined pinch-flex task (mean difference = -53.5 ms, CI = -66.3, -40.7), in FDI for the combined pinch-flex task (mean difference = -54.6 ms, CI = -67.7, -41.6), but not in FDI for the pinch-only task (mean difference = -19 ms, CI = -38.5, 0.5). Extended analyses can be found in **Appendix B**.

2.3.3 Determining SCM+ and SCM- percentiles

For all tasks analysed, we have indicated the equivalent SCM+ and SCM- percentiles in Table 2. The percentage of responses within each category after splitting the data into startle and non-startle categories (see **Figure 2.1**) are also presented in **Table 2.2**. The CDFs calculated for the Castellote and Kofler (2018) data are plotted along with the mean latency of SCM+ and SCM- responses in **Figure 2.2**, and the distribution of SCM+ responses within the startle and non-startle categories can further be seen in **Figure 2.3**. Extended analyses are presented in **Appendix C**.

Table 2.2. Overview of percentiles closest matching the mean latency of SCM+ and SCM- responses, along with the percentage of responses within the SCM+ and SCM- categories which occurred with SCM activity for each muscle and task analysed.

Authors (year)	*Task*	*Muscle*	*SCM+ percentile*	*% of responses in startle category occurring with SCM activity*	*SCM- percentile*	*% of responses in non-startle category occurring with SCM activity*
Castellote & Kofler (2018)	Elbow flexion	BB	15th	52.2	65th	0.9
	Finger pinch	FDI	35th	36.2	55th	21.8
	Combined finger-pinch and elbow flexion	BB	15th	87.3	65th	4.0
		FDI	15th	71.4	65th	5.3
Honeycutt et al. (2013)	Finger abduction	FDI	55th	62	45th	56.3
	Grasp	FDI	35th	66.7	65th	37.2
Marinovic et al. (2014)	Lip press	OO	45th	40.8	65th	26.7
	Button press with thumb	APB	45th	41.4	65th	37.0
Marinovic et al. (2015)	Arm supination	BB	35th	45.2	65th	24.4
Ossanna et al. (2019)	5D Arm reaching task	AD	35th	70.1	75th	32.9
		BB	35th	69.2	75th	28.3
		Br	35th	67.3	75th	29.1
		PD	45th	67.9	65th	33.0
		Pe	35th	71.4	55th	35.8
		TB	45th	65.1	75th	33.5
Honeycutt et al. (2014); Tresch et al. (2014)	Hand flexion	FDS	25th	72.6	75th	19.0
		EDC	35th	72.6	75th	23.0
		FDI	25th	78.6	75th	17.4
	Hand Extension	FDS	25th	72.7	75th	29.2
		EDC	35th	77.1	75th	20.1
		FDI	25th	73.8	75th	29.3

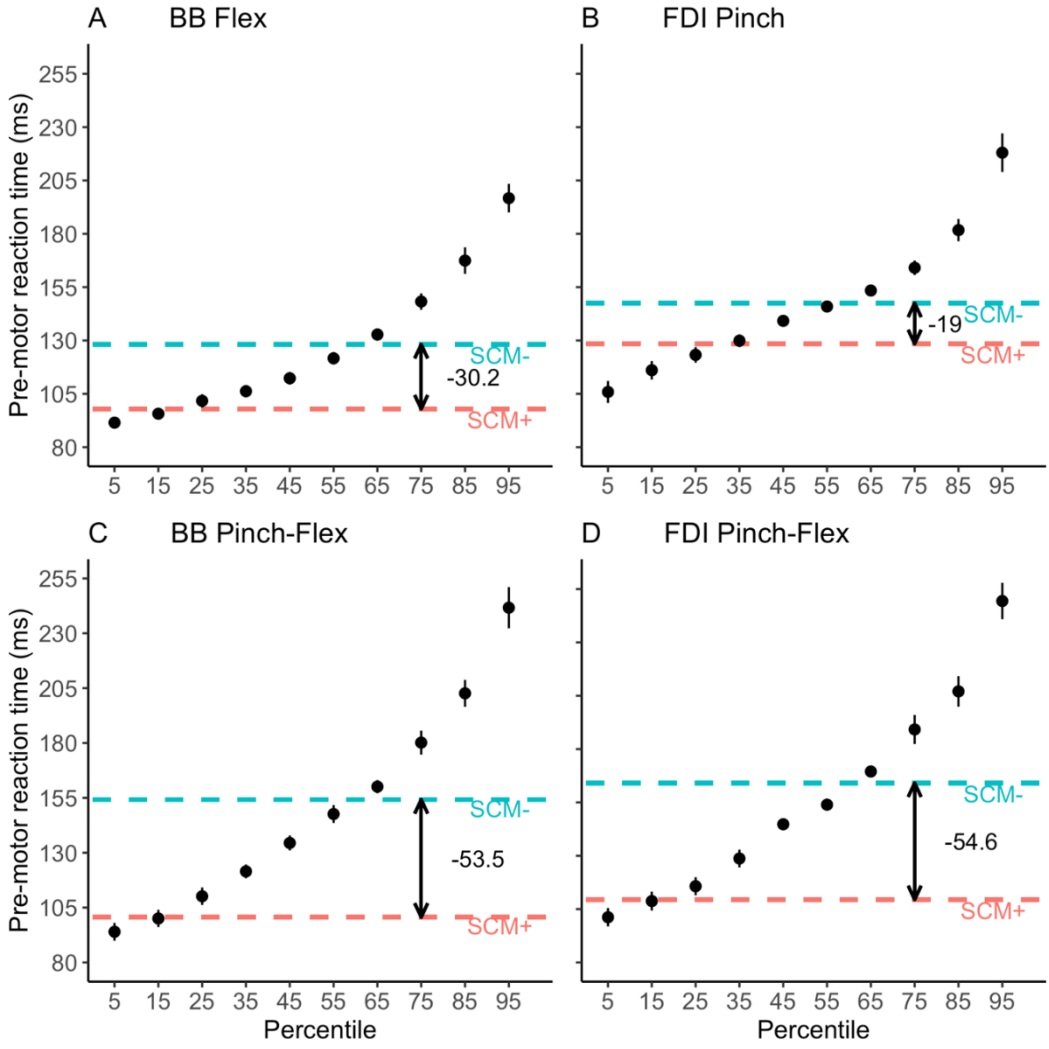


Figure 2.2. Cumulative distribution function of Castellote and Kofler's (2018) data. **A)**. Biceps brachii (BB) latency for flexion task **B)**. First dorsal interosseous (FDI) latency for pinch task **C)**. BB latency in combined task **D)**. FDI latency in combined task. The mean latency of responses in the presence (SCM+) and absence (SCM-) of sternocleidomastoid activity are shown by the dotted lines. Error bars represent standard error of the mean.

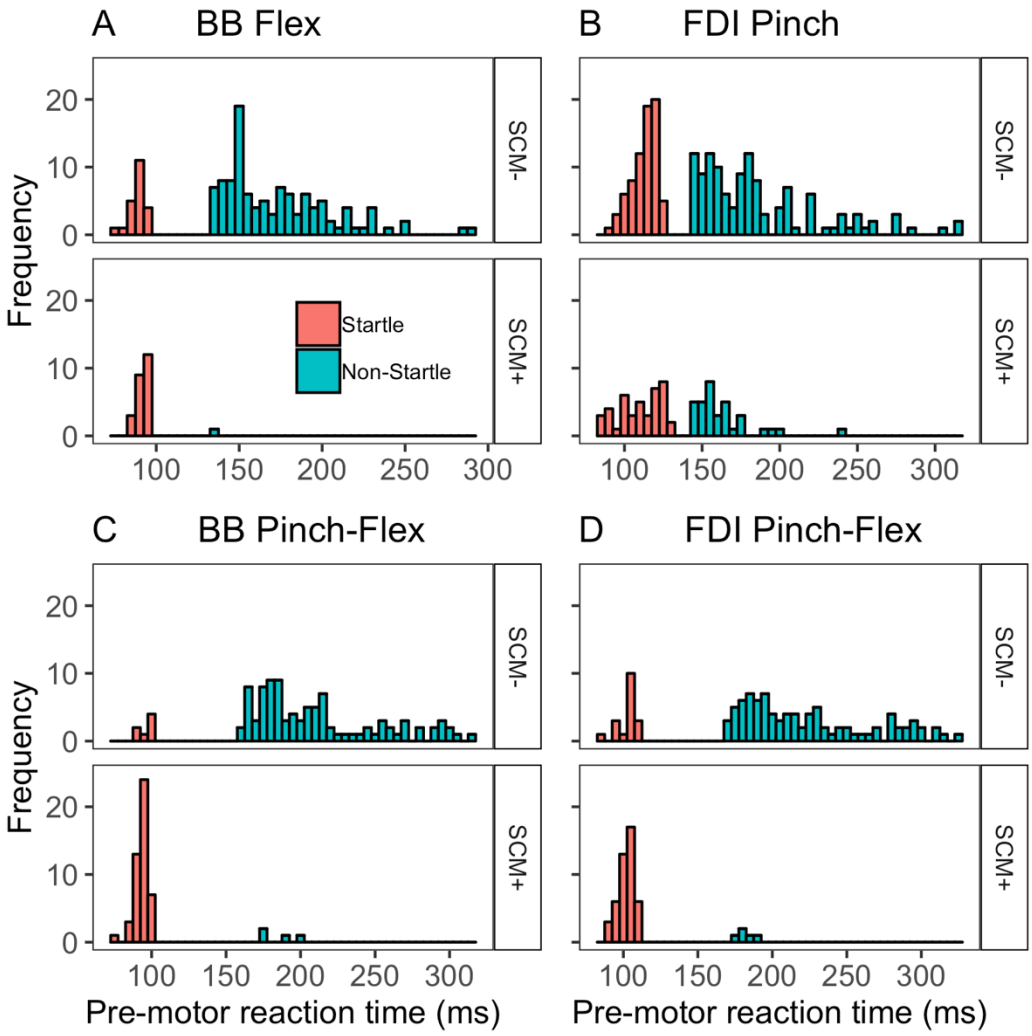


Figure 2.3. Histogram displaying response times of SCM+ and SCM- responses across startle and non-startle percentile categories for Castellote and Kofler's (2018) data. **A)**. Biceps brachii (BB) latency in flex-only task. **B)**. First dorsal interosseous (FDI) latency in pinch-only task. **C)**. BB latency in combined pinch-flex task. **D)**. FDI latency in combined pinch-flex task.

2.3.4 Presence of sternocleidomastoid activity in shorter and longer latency reaction times

Given some movement types showed a large proportion (max = 56.3%; see Table 2) of trials in the non-startle categorisation of RT which occurred with SCM activity, we conducted a Bayesian test of association (Albert, 1997) to examine whether the presence of SCM activity differs across our startle and non-startle RT categories. The analysis of Castellote and Kofler's (2018) data resulted in BF = 2.5×10^{12} for

BB in the flex-only task, BF = 6 for FDI in the pinch-only task, BF = 2 x 10^{25} for BB in the combined pinch-flex task, and BF = 8.2 x 10^{16} for FDI latency in the combined pinch-flex task. BFs > 100 indicate decisive evidence against the null hypothesis (Jeffreys, 1961) and as such, the flex-only, BB pinch-flex, and FDI pinch-flex tasks show decisive evidence for the dependence of percentile categorisation on SCM activity. These results indicate FDI latency in the pinch-only task is the only task within the dataset for which decisive evidence for the percentile-SCM dependence failed to be found. Extended analyses are shown in **Appendix D**.

2.3.5 Examining triggering mechanisms via faster onset and slower onset categorisation

Our analyses indicated that SCM activity does not always co-occur with shortened RT and also suggested that this relationship may be task-dependent. That is, for some tasks, a significant proportion of SCM+ responses are not only found in the startle category, but also within the non-startle category of RTs which approximates the longer latency SCM- RTs. Therefore, we examined an alternative approach to investigate triggering mechanisms of responses via intense sensory stimuli: categorisation via percentiles of RT. With the Castellote and Kofler (2018) dataset as a representative example, we conducted a linear mixed-effects model analysis to examine the appropriateness of this method of distinguishing responses at long (>= 55th percentile) and short (<= 45th percentile) latencies (see **Figure 2.4**). As expected, the main effect of percentile categorisation (fast onset/slower onset) was statistically significant, $F_{(1, 422)} = 533.67$, $p < .001$. More importantly, the interaction of percentile categorisation with muscle type (BB/FDI) was not statistically significant, $F_{(1, 422)} = 0.05$, $p = .814$, however, the interaction of percentile categorisation with task type (combined/single) was found to be statistically significant, $F_{(1, 422)} = 18.63$, $p < .001$.

If separate mechanisms contribute to the fastest RTs – as a result of a modulated effect of the probe stimulus between muscles or tasks which differ in their neurophysiological contributions, then differences in RT should be observed between muscles or tasks in the fast-onset percentiles. Therefore, we ran a linear mixed model on the fast onset data to test the hypothesis that differences across tasks/muscles may be observed in trials at the shortest RTs. Our analysis found a

statistically significant interaction of task type with muscle type, $F_{(1, 206)} = 10.9$, $p = .001$. Post-hoc analysis indicated a significant difference in RT between BB latency in the combined pinch-flex task and BB latency in the flex-only task, $p = .002$. This difference was not significant between FDI in the combined pinch-flex task and FDI in the pinch-only task, $p = .725$. The results of these analyses using our categorisation method via RT are consistent with those in the original report (Castellote & Kofler, 2018). Extended analyses can be seen in **Appendix E**.

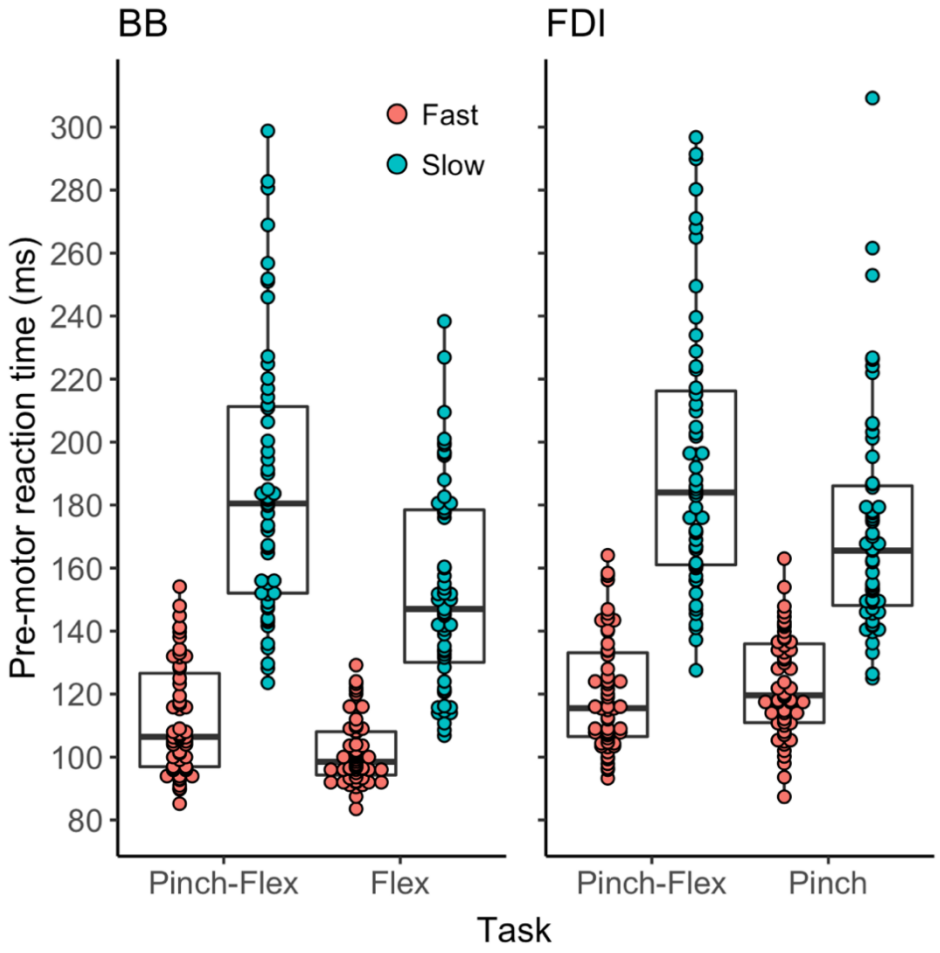


Figure 2.4. Box plots displaying median reaction times and first and third quartiles across subjects for all tasks reported by Castellote and Kofler (2018). These data have been categorised into fast onset and slower onset response times. **A)**. Biceps brachii (BB) latencies. **B)**. First dorsal interosseous (FDI) latencies.

In order to compare our CDF method of analysis with the traditional SCM categorisation method, we further analysed the Castellote and Kofler (2018) dataset

using the traditional classification of responses on the basis of SCM activity. The two methods produced similar results. Correspondingly with the previous main effect of percentile categorisation, the main effect of SCM activity (SCM+/SCM-) using the traditional method was statistically significant, $F_{(1, 1263.9)} = 252.53$, $p < .001$. Furthermore, in line with the observed statistically significant interaction of percentile categorisation with task type in our previous analysis, the interaction of SCM activity with task type was statistically significant, $F_{(1, 1260.8)} = 25.49$, $p < .001$, but the interaction of SCM activity with muscle type was not, $F_{(1, 1256.2)} = 2.66$, $p = .103$. Further examination of the SCM+ data showed an interaction of task type with muscle type, $F_{(1, 352.6)} = 25.93$, $p < .001$, consistent with that of the fast-onset data. However, post hoc tests indicated RTs of FDI in the pinch-only data ($M = 132.95$ ms, $SD = 23.87$) were significantly longer than those for BB in the flex-only data ($M = 103.87$ ms, $SD = 17.66$; $p < .001$), BB in the combined pinch-flex data ($M = 108.56$ ms, $SD = 24.56$; $p < .001$), and FDI in the combined pinch-flex data ($M = 117.16$ ms, $SD = 24.47$; $p < .001$). Importantly, these differences may be explained by our previous finding that for FDI latency in the pinch-only task there were a larger percentage (21.8%) of trials in the longer-latency percentiles of RT which occurred with SCM activity in comparison to the other tasks (see Table 2). Potentially these SCM+ trials at longer RTs would have had an impact on average latencies of FDI in the pinch-only data and as such, result in the significantly longer RTs for FDI in the pinch-only task as compared to the other tasks when categorising via SCM. This may lead to alternative interpretations of the data when analysing on the basis of SCM activity in comparison to our method of categorising trials on the basis of RT. Although categorisation of trials via RT appears to provide a more comprehensive view of the data, the CDF method of analysis has additional benefits in that the entire RT distribution can be examined. For example, when all percentiles are analysed, changes in the RT distribution between movement types can indicate differences in triggering across the RT spectrum. Further analyses using all RT percentiles, including percentiles as a fixed factor in a linear mixed-effects model are presented in **Appendix F**. These analyses provided insights regarding which movements may be less prone to triggering delays, as well as the role of potential RT floor effects (see **Appendix F**; **Figure 2.16**). This highlights the additional benefits that can be gained from incorporating CDFs in analyses.

2.4 Discussion

EMG activity of orbicularis oculi (OOc) and SCM are the most commonly used indicators of the presence of a startle response, being among the last to habituate (Carlsen et al., 2007; Castellote et al., 2017; Kofler et al., 2006). However, OOc responses are characterised by an early-onset component (the eye-protective auditory or somatosensory blink reflex) which is more resistant to habituation and takes a separate route to the brainstem as opposed to the later occurring startle component, which is more amenable to habituation and is associated with the generalised skeletomotor response to startle (Brown et al., 1991; Valls-Solé et al., 2008). It is difficult to distinguish the acoustic/somatosensory eyeblink response from the startle response in OOc EMG records (Brown et al., 1991), and as such, SCM has been argued to provide a key indication of the presence of a "true" startle. On the basis of the assumption that startle activity is a necessary condition for the StartReact effect, the presence of SCM activity when prepared movements are triggered by an intense sensory stimulus has thus been used in the literature to make inferences about the potential mechanisms underlying the StartReact effect which may rely on activation of startle circuits (Carlsen et al., 2007; Valls-Solé et al., 1999). Movements made in response to the intense stimulus which occur without measurable surface EMG activity in SCM have therefore been deemed to be voluntarily initiated movements and unrepresentative of the StartReact effect. Analysis of data on the basis of SCM activity has traditionally examined the difference between SCM+ trials and SCM- trials to determine what types of movements are amenable to StartReact and those which are not. When a statistically significant difference cannot be found between SCM+ and SCM- trials for a particular muscle or task, that particular muscle or task is deemed to be unamenable to StartReact (Carlsen et al., 2007; Carlsen et al., 2009; Honeycutt et al., 2013). Differences in the neurophysiological efferent connectivity between muscles which are or are not amenable to StartReact in accordance with this method of analysis are then used to assert the involvement of different brain regions in the StartReact effect. Our analyses suggest a flaw in this interpretation of data. Firstly, analysis of probe trials failed to confirm that RT data are bimodally distributed, which may be expected if triggering differs for StartReact versus volitional movements. Furthermore, when percentiles within a CDF are approximated to response times on trials with and without SCM activity, and RTs

are split into these SCM+ and SCM- percentiles, for some movement types a large proportion of responses with long RTs which would otherwise be considered to be indicative of slower, voluntarily triggered responses, can be seen to occur in the presence of SCM activity. A number of SCM- responses are also present in the group of responses with shorter latencies that are equivalent in terms of RT to responses otherwise recognised as typical StartReact triggered movements. While some of these short latency movements may have been anticipatory, or SCM activity may have gone undetected by surface EMG, this finding along with the presence of SCM+ responses in late RTs clearly demonstrates that SCM activity is neither always necessary, nor always sufficient, to identify the short response times which are a hallmark of StartReact movements (Marinovic & Tresilian, 2016). While SCM activity tends to be more prominent for the shortest latency movements, this is likely a product of SCM activation being more probable when levels of motor preparation are high (L. A. Leow et al., 2018; MacKinnon et al., 2013; Marinovic & Tresilian, 2016). Therefore, SCM activity may not be a product of the engagement of a unique triggering circuit, but rather a by-product, along with short response latency, of elevated preparatory activity.

Examination of our Bayesian tests of association (**Appendix D**) may provide a means to interpret why differences are observed between SCM+ and SCM- responses for some tasks, and not for others. A statistically significant difference was observed between SCM+ and SCM- trials for Castellote and Kofler's (2018) BB latency in the flexion task, BB latency in the combined pinch-flex task, and FDI latency in the combined pinch-flex task, but not for FDI latency in the pinch task. Similarly, for BB latency in the flexion task and combined pinch-flex task, and FDI latency in the combined pinch-flex task, our Bayesian test of association provided decisive evidence (Jeffreys, 1961) to support the dependence of SCM activity and percentile categorisation – indicating the presence of SCM activity was most often found for responses within the fastest percentiles of RT. However, for the pinch-only task, the Bayesian test of association provided weaker evidence to support the dependence of SCM activity on percentile categorisation. This suggests that for this task, SCM activity was not significantly more likely to occur with responses which had the fastest RTs and could occur across both short and long latency movements. This finding of weak evidence to support the dependence of SCM activity and

percentile categorisation holds true for all muscles and tasks we have analysed which failed to indicate a statistically significant difference between SCM+ and SCM- responses (see **Appendix B** and **Appendix D**). We may therefore conclude that this difference depends on the distribution of SCM+ responses across the spectrum of RTs.

It has been previously proposed that a lack of RT difference between SCM+ and SCM- trials indicates that the StartReact effect could not be elicited in a certain muscle or task (Carlsen et al., 2007; Carlsen et al., 2009; Honeycutt et al., 2013). Our analyses here suggest that the failure to find a statistically significant RT difference between SCM+ and SCM- responses for a given response type does not indicate a specific mechanism has failed to be activated by the intense stimulus, but rather, a larger proportion of SCM+ responses at late RTs, or SCM- responses at short RTs, is likely to have obscured this difference. Therefore, the presence of SCM activity is an unreliable method to indicate whether a distinct StartReact mechanism which produces the shortest response latencies has been activated; regardless of whether this pathway acts through the bypassing of cortical circuits or through an engagement of a larger and more functionally relevant brain network (Alibiglou & MacKinnon, 2012; Carlsen & Maslovat, 2019; Marinovic & Tresilian, 2016; Stevenson et al., 2014). Making inferences about the underlying circuitry of StartReact responses is therefore likely to be unreliable when using surface EMG activity in SCM as a sole criterion for the classification of responses. Furthermore, studies which classify responses on the basis of SCM activity are prone to the loss of large amounts of data. For example, when SCM activity is required to classify responses, participants for whom no measurable SCM activity can be consistently observed must be excluded entirely from analyses. This leads to a reduction of statistical power, unnecessary burden to the participant, and the loss of time and resources. On the basis of the unreliability of SCM activity as a criterion to determine the triggering mechanisms of responses and the loss of data associated with using this neurophysiological indicator, we therefore propose the mechanisms underlying the StartReact effect may be further examined when responses are categorised via their latency.

We deemed responses at or below the 45th percentile to be representative of responses at the shortest latencies which most often occur with SCM activity, and

subsequently categorised responses at the 45th percentile of RT or earlier into our fast onset response category for analysis. Those at the 55th percentile or later were similarly categorised into our slower onset response category for analysis, representative of voluntarily triggered responses. Our analysis of a representative dataset (Castellote & Kofler, 2018) showed a significant interaction of percentile categorisation with task type, indicating responses from the target muscles may have differed depending on the task that they were engaged in. Examination of our extended analyses (**Appendix E**) however, does not consistently show this interaction of percentile categorisation with task type or muscle type across datasets, even in muscles which are thought to strongly differ in their efferent connectivity to subcortical brain areas (e.g. Marinovic et al., 2014). This may warrant further examination of a modulated benefit of the intense sensory probe on the triggering of movements which differ in their neurophysiological connectivity. Furthermore, our Bayesian test of association analyses presented in **Appendix D** suggests there may be task-related factors that influence the dependence of SCM activity on RT. The percentile-SCM dependence that we observed for some tasks but not for others may be a consequence of high levels of motor preparation. Alternatively, the task specificity we observed in these analyses may also suggest that it is possible for differences in the neural circuitry used in the control of a muscle or movement type to influence the distribution of SCM activity across RTs. As a result, this task-specific percentile-SCM dependence may influence interpretations that can be made regarding the presence of the StartReact phenomenon when determining responses as StartReact or volitional on the basis of SCM activity. The task-specific effects we observed may relate to the use of SCM as part of a proximal stabilisation pattern in startle. Potentially, SCM and other trunk muscles may be activated to stabilise the body and head before rapid muscle activity in a proximal effector. This pattern of stabilisation may not be required as prominently for rapid activity in a more distal effector, which may provide some explanation for why the RT-SCM dependence was weaker for Castellote and Kofler's (2018) distal pinch-only task, but was decisive for the remaining tasks which recruited the proximal BB.

CDFs have been employed in a similar context previously, for example, comparing RT distributions for unisensory versus bimodal stimuli (Ulrich et al., 2007), evaluating the dynamic nature of the relationship between RT and force

production (Marinovic, Poh, et al., 2017), as well as between RT and movement direction (Marinovic, Poh, et al., 2017; Marinovic, Tresilian, et al., 2017). Here, we have shown multiple benefits of including CDF analyses in the StartReact context. For example, this method can be used to evaluate the effectiveness of SCM categorisation and offer an alternative method to categorise data into the fast and slower onset trials. Our RT categorisation also has benefits over the SCM categorisation in that all trials can be analysed, without having to exclude participants. This is beneficial in maximising the return from the data that is collected (Whelan, 2008). In addition, the CDF method is not affected by the apparent task-specific RT-SCM dependence which we have shown can influence interpretations that may be made regarding the presence of the StartReact effect. Finally, by analysing all percentiles within a CDF, entire RT distributions can be examined to potentially provide insights regarding differential triggering between movements or potential RT floor effects which may limit the ability to detect differences between movement types. As such, we suggest CDFs are a suitable tool to investigate the triggering of prepared motor actions via intense sensory stimuli.

2.5 Conclusions

Overall, inferences made about the presence of a distinct triggering mechanism for StartReact responses based on the presence or absence of SCM activity require careful consideration. The findings here suggest there are task- and muscle-specific responses to the probe stimulus that may influence both the manifestation of the StartReact as well as the ability to detect StartReact on the basis of SCM activity. Furthermore, while our analyses here cannot confirm nor rule out distinct triggering mechanisms for prepared motor responses via intense sensory stimuli, we suggest these underlying mechanisms for the StartReact effect should be further examined on the basis of response latency of the target muscle, rather than surface EMG activity of the SCM alone.

2.6 Appendix A

We conducted Hartigan's (1985) dip test to test the multimodality of all datasets. The test failed to reject the null hypothesis of unimodality for all datasets we analysed. This suggests responses to intense sensory stimuli tend to fit a unimodal distribution. Resulting *p* values of the tests are reported in **Table 2.3**

Table 2.3. *p* values returned from our Hartigan's (1985) dip test. We tested the null hypothesis of unimodality for each muscle/task. Statistical significance is determined at $\alpha = 0.05$.

Authors (year)	*Task*	*Muscle*	*p value*
Castellote & Kofler (2018)	Elbow flexion	Biceps brachii (BB)	.715
	Finger pinch	First dorsal interosseous (FDI)	.095
	Combine flex-pinch	BB	.093
		FDI	.277
Honeycutt et al. (2013*)*	Finger abduction	FDI	.976
	Grasp	FDI	.434
Marinovic et al. (2014)	Lip press	Orbicularis oris (OO)	.964
	Button press with thumb	Abductor pollicis brevis (APB)	.239
Marinovic et al. (2015)	Arm supination	BB	.439
Ossanna et al., (2019)	Five-direction arm reaching	Anterior deltoid (AD)	.988
		BB	.902
		Brachioradialis (Br)	.990
		Posterior deltoid (PD)	.827
		Pectoralis (Pe)	.994
		Triceps brachii (TB)	.872
Honeycutt et al. (2014); Tresch et al. (2014)	Hand flexion	Flexor digitorum superficialis (FDS)	.929
		Extensor digitorum communis (EDC)	.990
		FDI	.851
	Hand Extension	FDS	.994
		EDC	.995
		FDI	.990

2.7 Appendix B

We conducted paired samples t-tests of each subject's median SCM+ and SCM- trial RT for each movement type across datasets to examine the difference in response latency between SCM+ responses and SCM- resonses. Mean differences and confidence intervals for each movement type are reported in **Table 2.4**.

Table 2.4. Difference between SCM+ and SCM- trials for each task and muscle analysed.

Authors (year)	*Task*	*Muscle*	*Mean difference (ms) [95% CI]*
Castellote & Kofler (2018)	Elbow flexion	Biceps brachii (BB)	-30.2 [-38.0, -22.4]
	Finger pinch	First dorsal interosseous (FDI)	-19.0 [-38.5, 0.5]
	Combine flex-pinch	BB	-53.5 [-66.3, -40.7]
		FDI	-54.6 [-67.7, -41.6]
Honeycutt et al. (2013)	Finger abduction	FDI	3.5 [-6.9, 14.0]
	Grasp	FDI	-8.8 [-13.3, -4.3]
Marinovic et al. (2014)	Lip press	Orbicularis oris (OO)	-14.5 [-50.3, 21.3]
	Button press with thumb	Abductor pollicis brevis (APB)	-22.1 [-62, 17.8]
Marinovic et al. (2015)	Arm supination	BB	-13.1 [-27.8, 1.5]
Ossanna et al. (2019)	5D Arm reaching task	Anterior deltoid (AD)	-41.9 [-64.4, -19.4]
		BB	-33.3 [-55.1, -11.6]
		Brachioradialis (Br)	-43.0 [-61.3, -24.6]
		Posterior deltoid (PD)	-25.6 [-43.1, -8.0]
		Pectoralis (Pe)	-43.1 [-64.9, -21.2]
		Triceps brachii (TB)	-27.9 [-47.4, -8.5]
Honeycutt et al. (2014); Tresch et al. (2014)	Hand flexion	Flexor digitorum superficialis (FDS)	-23.1 [-30.5, -15.7]
		Extensor digitorum communis (EDC)	-18.3 [-23.1, -13.6]
		FDI	-27.7 [-36.9, -18.6]
	Hand Extension	FDS	-23.1 [-30.4, -15.9]
		EDC	-25.7 [-38.3, -13.1]
		FDI	-24.0 [-34.7, -13.3]

2.8 Appendix C

We calculated CDFs for each muscle and task across all datasets analysed. For each movement type, the mean latency across participants of the percentile that closest matched the mean latency of SCM+ responses was deemed the SCM+ percentile for that task. Similarly, the SCM- percentile was determined as the percentile within the CDF that closest matched the mean latency of SCM- responses for that task. The CDFs for each movement type are plotted along with the mean latency of SCM+ and SCM- responses in **Figure 2.5** - **Figure 2.9**. Responses were placed into Startle and Non-Startle categories based on SCM+ and SCM- percentile latency (see **Figure 2.1**), and we subsequently calculated the percentage of responses within each category that occurred with SCM activity to determine the distribution of SCM+ responses between our categories. The distribution of SCM+ responses within the Startle and Non-Startle categories is displayed in **Table 2.2** and **Figure 2.10** - **Figure 2.14**.

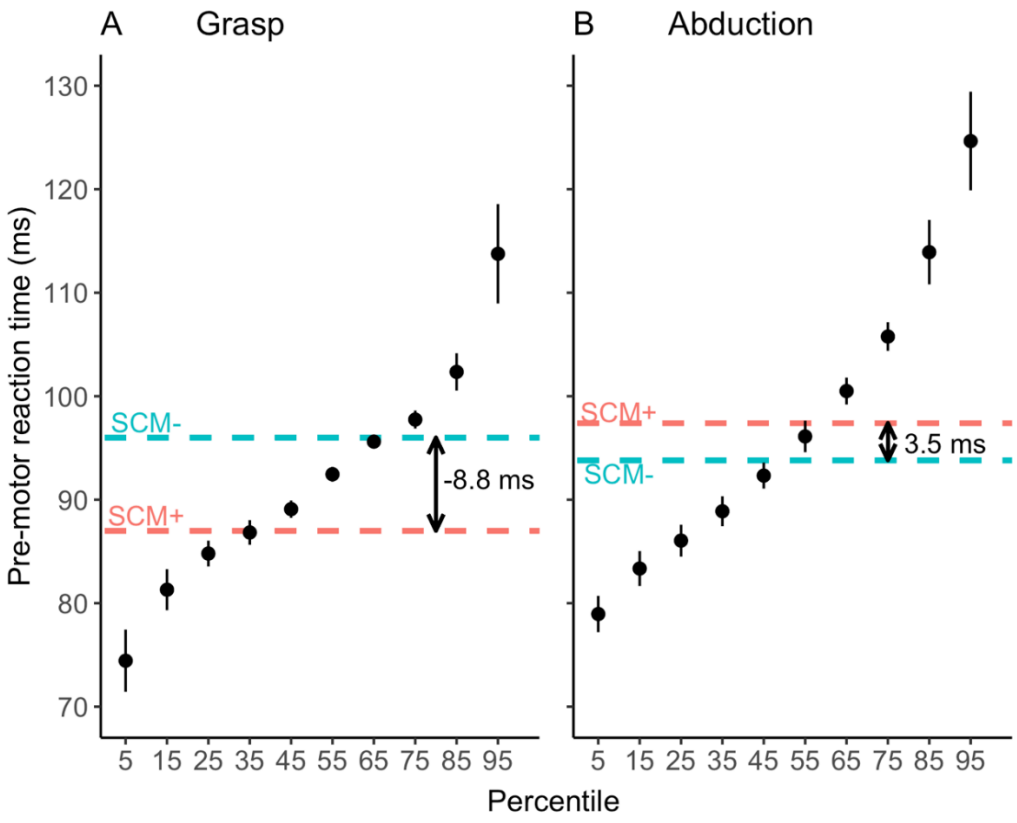


Figure 2.5. Cumulative distribution function of first dorsal interosseous latency in Honeycutt et al.'s (2013) data. **A)**. Mean first dorsal interosseous (FDI) latency at

each percentile of grasp task. **B)**. Mean FDI latency at each percentile of finger abduction task

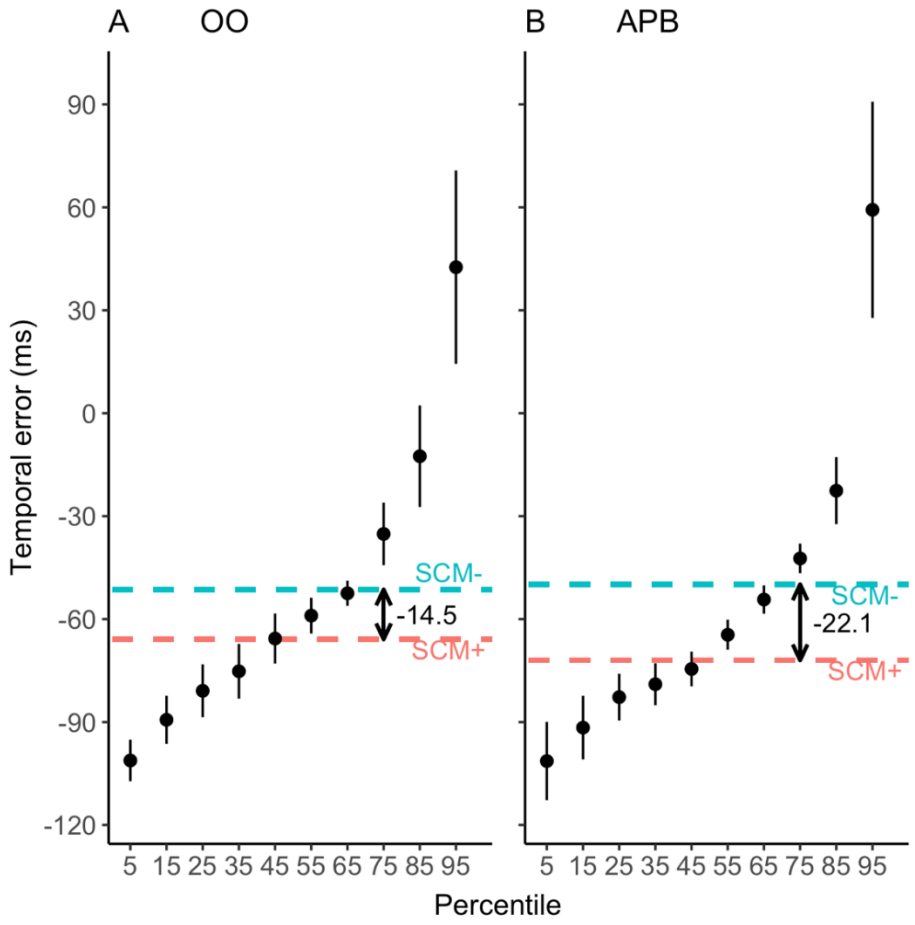


Figure 2.6. Cumulative distribution function of temporal error in Marinovic et al.'s (2014) anticipatory timing tasks. **A)**. Mean temporal error of orbicularis oris (OO) at each percentile **B)**. Mean temporal error of abductor pollicis brevis (APB) at each percentile. Mean latency of responses in the presence (SCM+) and absence (SCM-) of sternocleidomastoid activity are shown by the dotted lines. Error bars represent standard error of the mean.

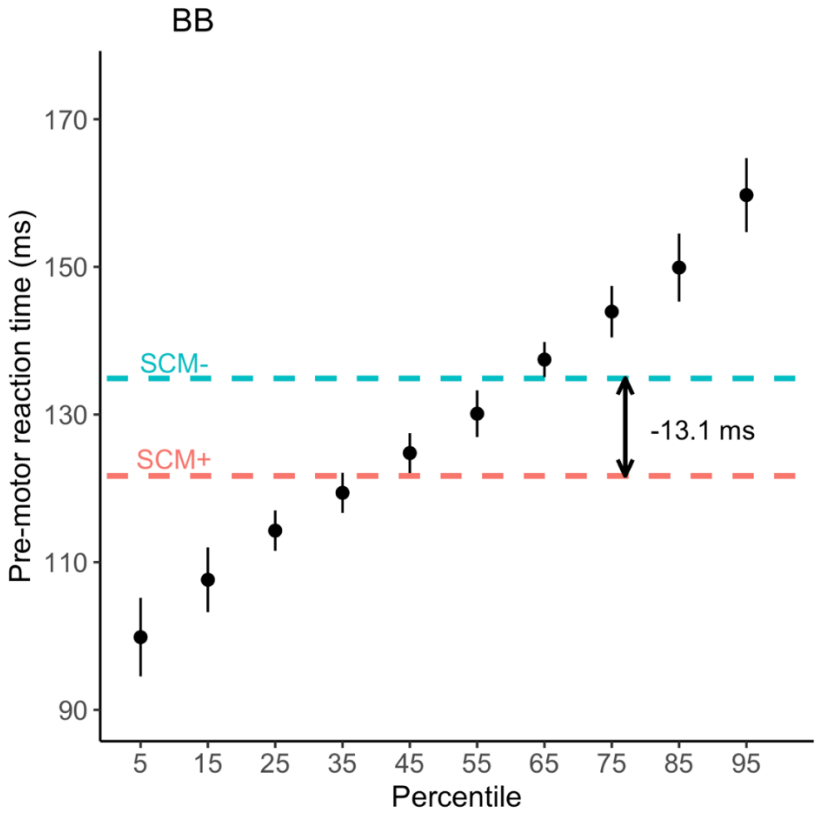


Figure 2.7. Cumulative distribution function of biceps brachii latency in Marinovic et al.'s (2015) arm supination task. Mean response times of biceps brachii (BB) at each percentile are displayed. Mean latency of responses in the presence (SCM+) and absence (SCM-) of sternocleidomastoid activity are shown by the dotted lines. Error bars represent standard error of the mean.

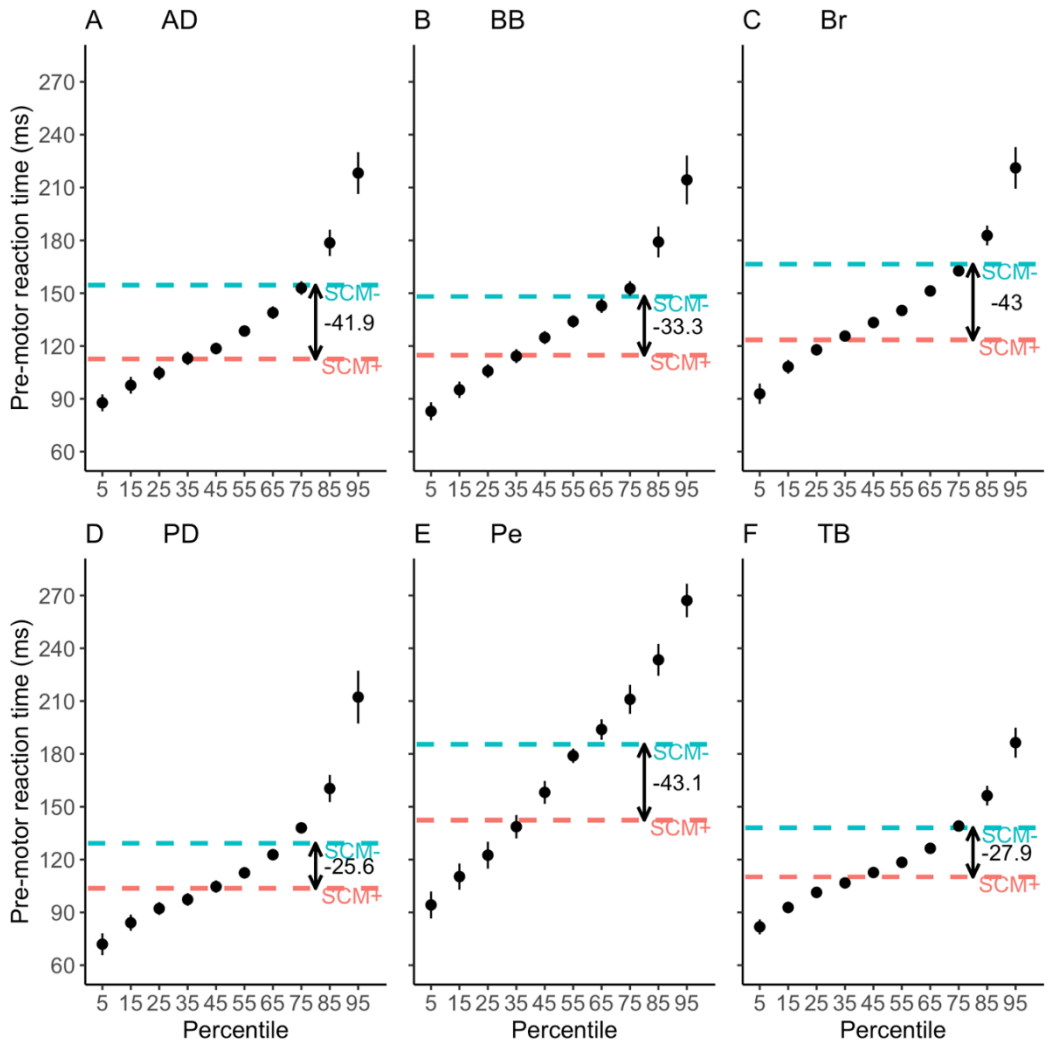


Figure 2.8. Cumulative distribution function of response latencies in Ossanna et al.'s (2019) reaching task. **A)**. Mean latency of anterior deltoid (AD) at each percentile **B)**. Mean latency of biceps brachii (BB) at each percentile **C)**. Mean latency of Brachioradialis (Br) at each percentile **D)**. Mean latency of posterior deltoid (PD) at each percentile **E)**. Mean latency of pectoralis (Pe) at each percentile **F)**. Mean latency of triceps brachii (TB) at each percentile. Mean latency of responses in the presence (SCM+) and absence (SCM-) of sternocleidomastoid activity are shown by the dotted lines. Error bars represent standard error of the mean.

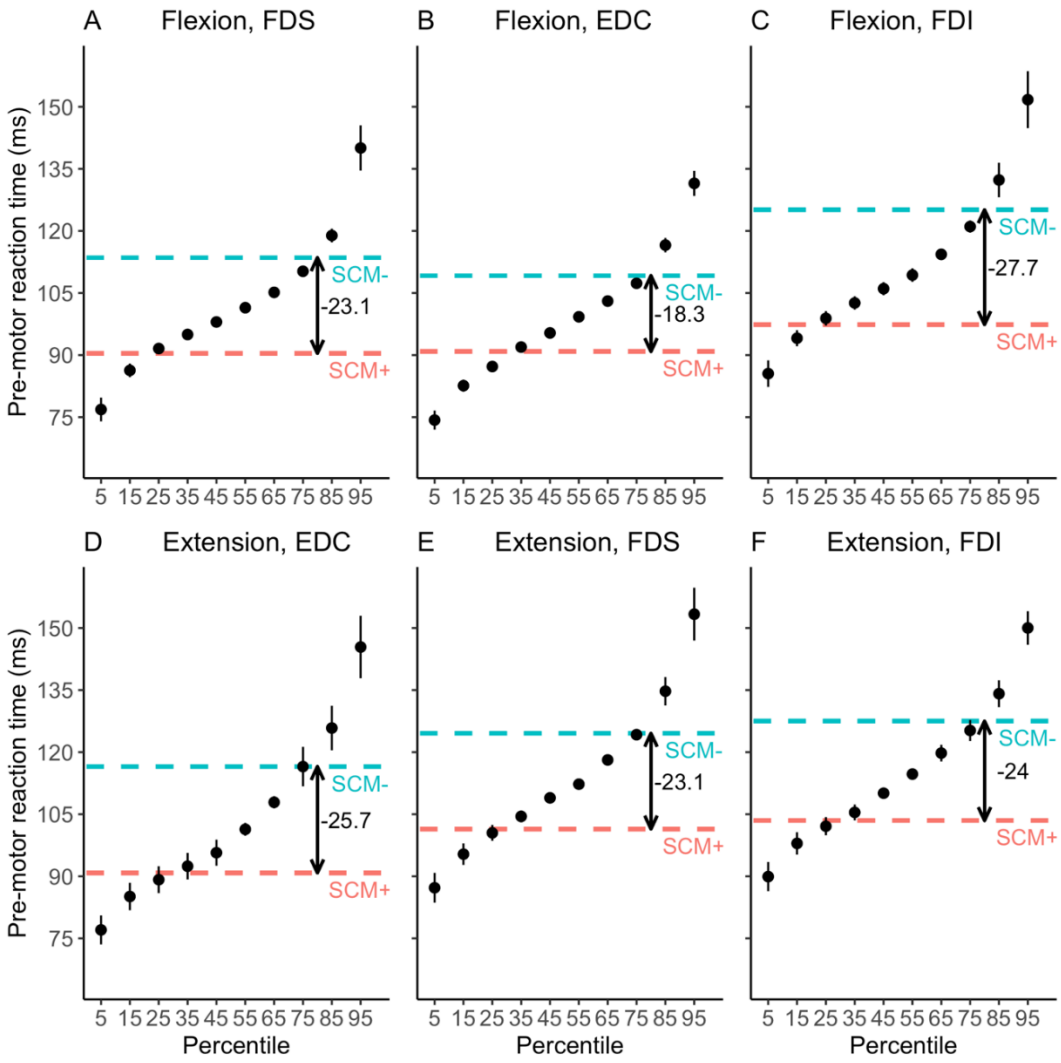


Figure 2.9. Cumulative distribution function of response latencies in Honeycutt et al. (2014); Tresch et al. (2015). **A)**. Mean latency of flexor digitorum superficialis (FDS) at each percentile of the hand flexion task. **B)**. Mean latency of extensor digitorum communis (EDC) at each percentile of the hand flexion task. **C)**. Mean latency of first dorsal interosseous (FDI) at each percentile of the hand flexion task. **D)**. Mean latency of EDC at each percentile of the hand extension task. **E)**. Mean latency of FDS latency at each percentile of the hand extension task. **F)**. Mean latency of FDI at each percentile of the hand extension task. Mean latency of responses in the presence (SCM+) and absence (SCM-) of sternocleidomastoid activity are shown by the dotted lines. Error bars represent standard error of the mean.

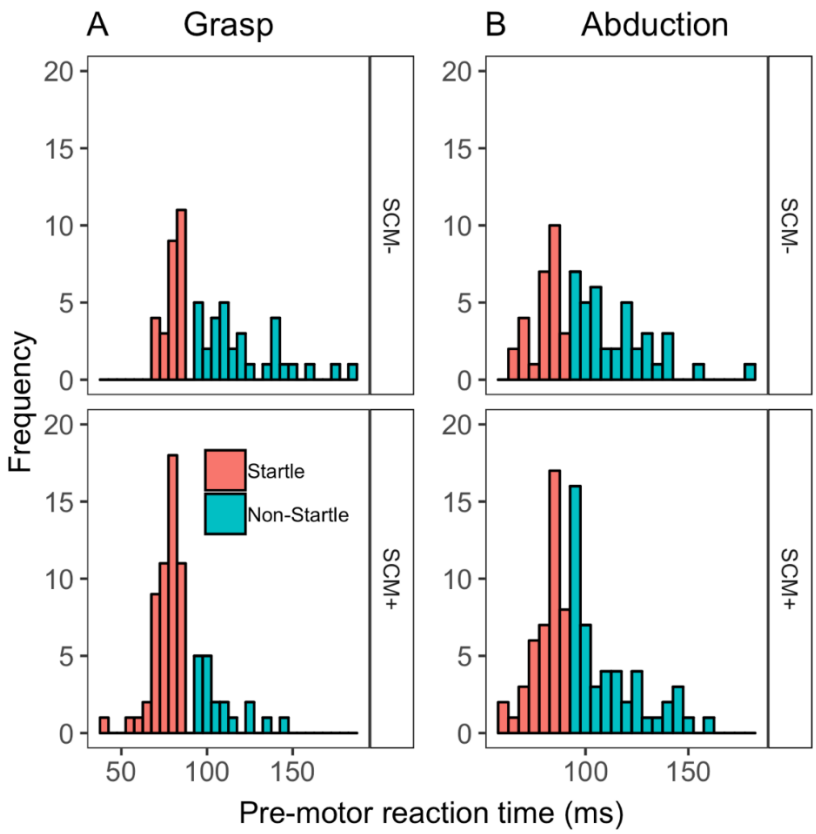


Figure 2.10. Histogram displaying frequency of response times in Honeycutt et al.'s (2013) data. SCM+ and SCM- responses across Startle and Non-Startle percentile categories are displayed. **A)**. First dorsal interosseous (FDI) latency in grasp task. **B)**. FDI latency in finger abduction task.

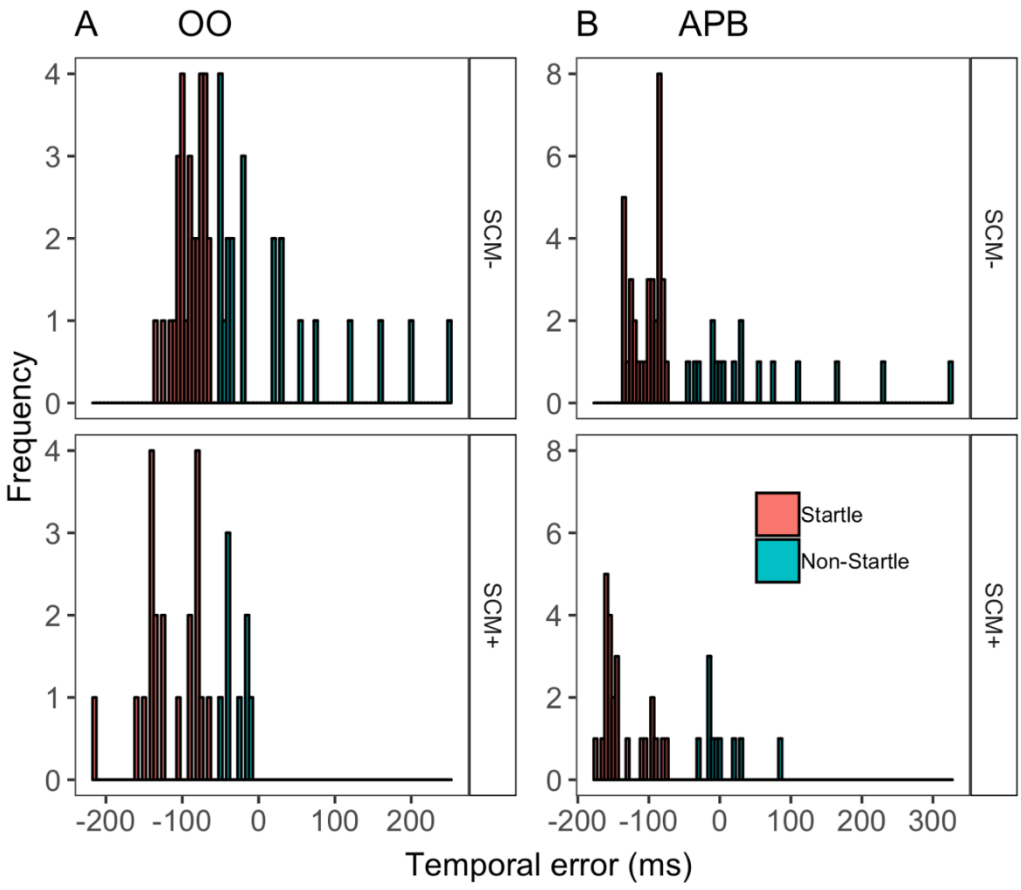


Figure 2.11. Histogram displaying frequency of temporal error in Marinovic et al.'s (2014) data. SCM+ and SCM- responses across Startle and Non-Startle percentile categories are displayed. **A)**. Temporal error of orbicularis oris (OO). **B)**. Temporal error of abductor pollicis brevis (APB).

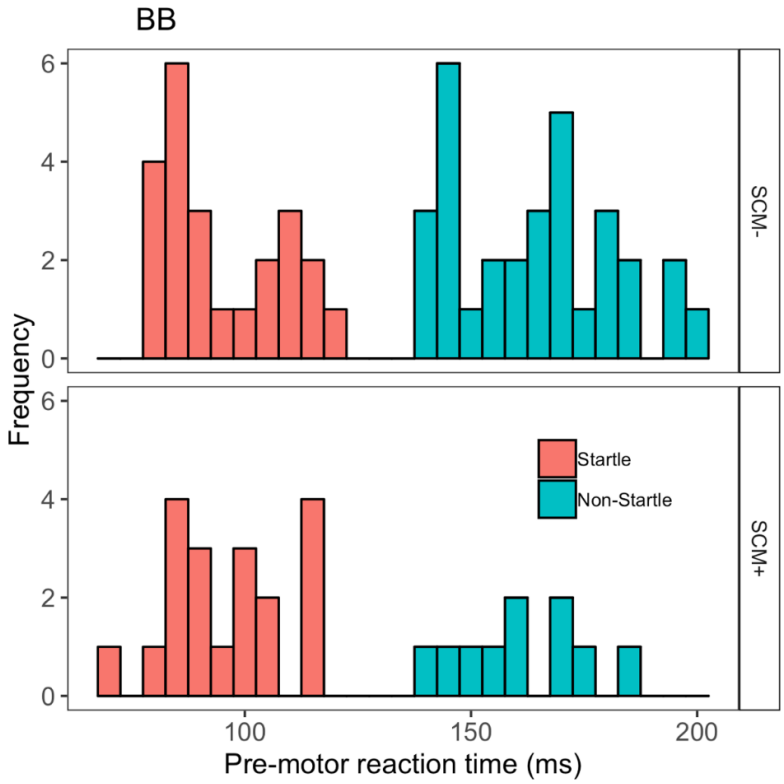


Figure 2.12. Histogram displaying frequency of response times of biceps brachii in Marinovic et al.'s (2015) data. SCM+ and SCM- responses across Startle and Non-Startle percentile categories are displayed. BB = biceps brachii.

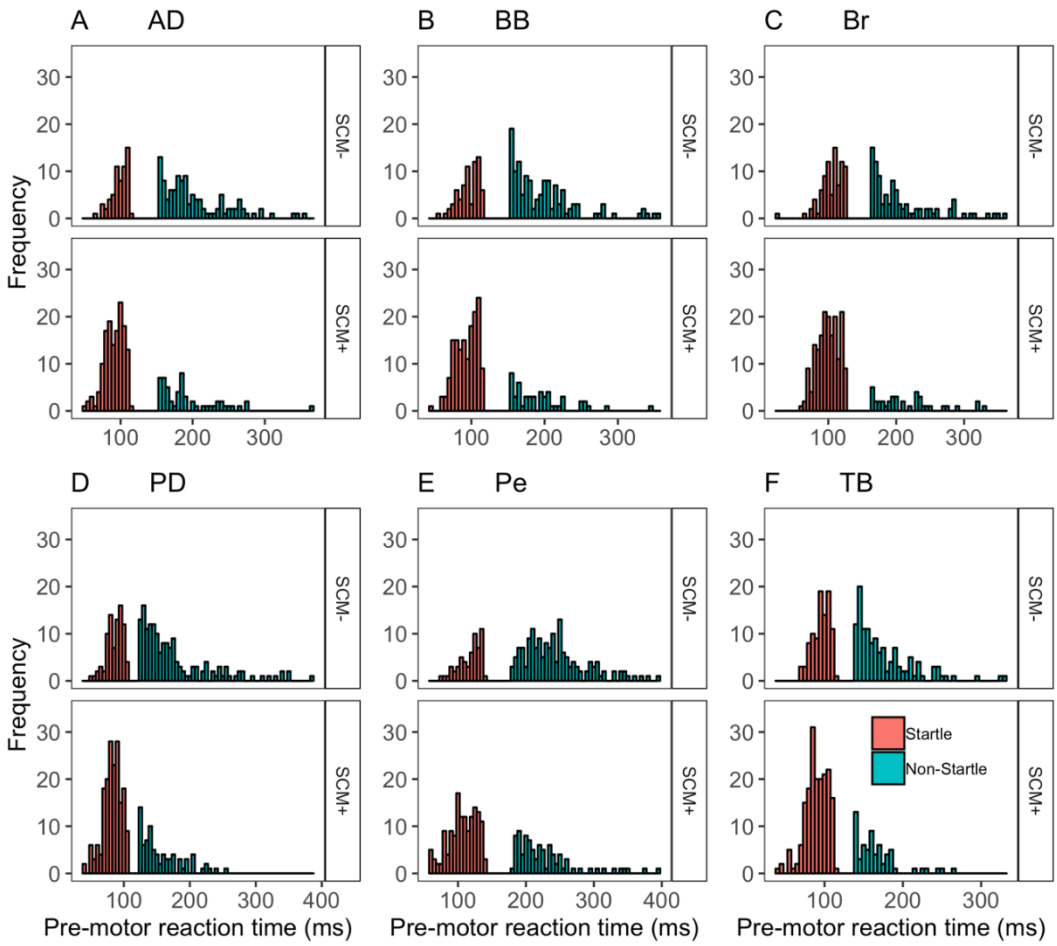


Figure 2.13. Histogram displaying frequency of response times in Ossanna et al.'s (2019) data. SCM+ and SCM- responses across Startle and Non-Startle percentile categories are displayed. **A)**. Anterior deltoid (AD) latency **B)**. Biceps brachii (BB) latency **C)**. Brachioradialis (Br) latency **D)**. Posterior deltoid (PD) latency **E)**. Pectoralis (Pe) latency **F)**. Triceps brachii (TB) latency.

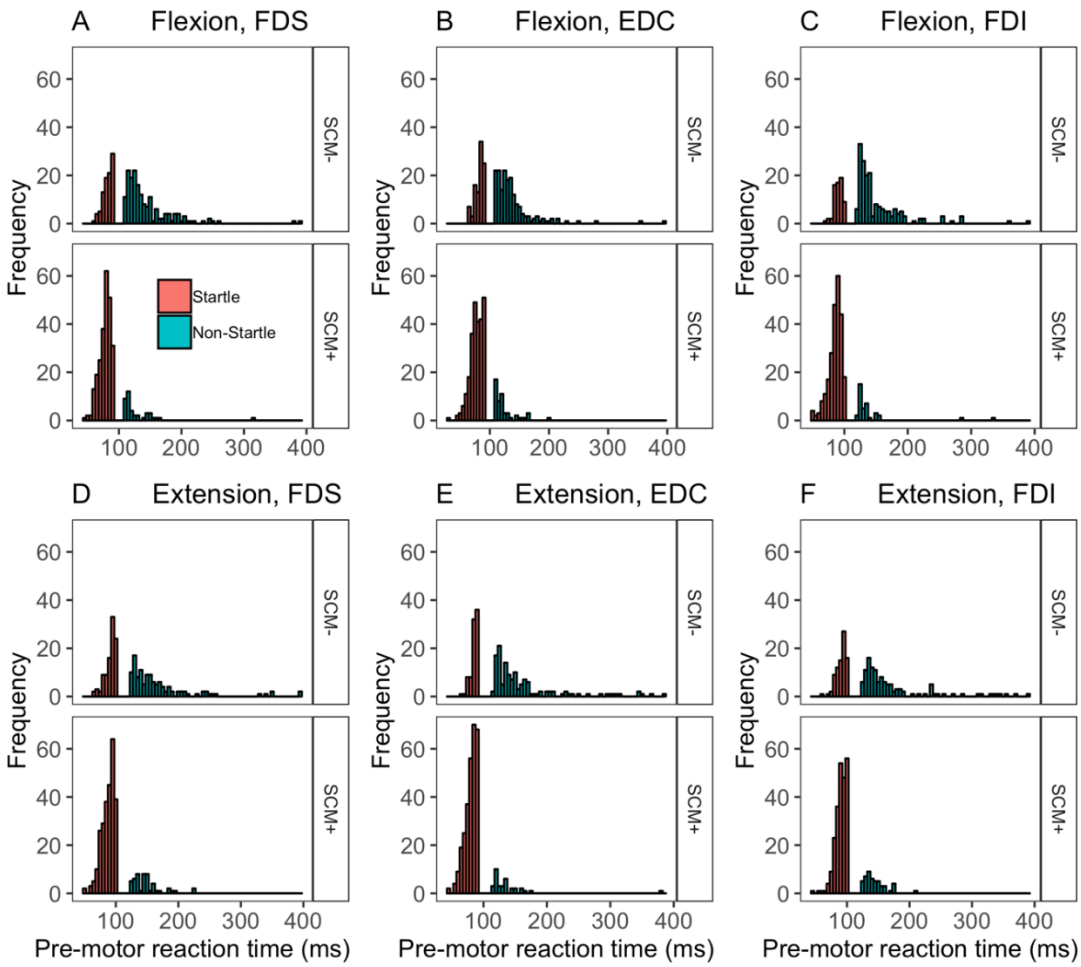


Figure 2.14. Histogram displaying frequency of response times in Honeycutt et al. (2014) and Tresch et al.'s (2014) data. SCM+ and SCM- responses across Startle and Non-Startle percentile categories are displayed. **A)**. Flexion task, flexor digitorum superficialis (FDS) latency. **B)**. Flexion task, extensor digitorum communis (EDC) latency. **C)**. Flexion task, FDI latency. **D)**. Extension task, EDC latency. **E)**. Extension task, FDS latency. **F)**. Extension task, FDI latency.

2.9 Appendix D

Given a large proportion (max = 56.32%) of responses in the Non-Startle categorisation of RT occurred with SCM activity (see **Table 2.2**), we conducted a series of Bayesian tests of association (Albert, 1997) to examine whether the presence of SCM activity depends on our Startle and Non-Startle RT categories. The resulting Bayes Factors (BFs) are reported in **Table 2.5**.

Table 2.5. Bayes factors calculated by Bayesian tests of association for each movement type across all datasets analysed. Bayes Factors (BFs) indicate the degree of evidence to support the dependence of SCM activity and response latency categorisation. BF = 1 indicates no support for the null or alternative hypothesis, BF > 3 indicates substantial evidence, BF > 10 indicates strong evidence, BF > 30 indicates very strong evidence, and BF > 100 indicates decisive evidence for the alternative hypothesis (Jeffreys, 1961).

Authors (year)	*Task*	*Muscle*	*Bayes Factor (BF)*
Castellote & Kofler (2018)	Elbow flexion	BB	2.5×10^{12}
	Finger pinch	FDI	6
	Combine flex-pinch	BB	2×10^{25}
		FDI	8.2×10^{16}
Honeycutt et al. (2013)	Finger abduction	FDI	0.4
	Grasp	FDI	71.2
Marinovic et al. (2014)	Lip press	OO	0.8
	Button press with thumb	APB	0.4
Marinovic et al. (2015)	Arm supination	BB	2.7
Ossanna et al. (2019)	5D Arm reaching task	AD	3.4×10^{12}
		BB	1.2×10^{14}
		Br	5.4×10^{10}
		PD	5.3×10^{12}
		Pe	6.2×10^{11}
		Tr	1.5×10^{9}
Honeycutt et al. (2014); Tresch et al. (2014)	Hand flexion	FDS	1.4×10^{28}
		EDC	1.2×10^{29}
		FDI	9.4×10^{29}
	Hand Extension	FDS	5.2×10^{18}
		EDC	1.4×10^{29}
		FDI	5.5×10^{18}

2.10 Appendix E

Our analyses provided weak evidence to support the hypothesis that the presence of SCM activity is always dependent on percentile categorisation. That is, a significant proportion of SCM+ responses are not only found in the Startle category, but also within the Non-Startle category which approximates SCM- response latencies. Therefore, we examined an alternative approach for investigating triggering mechanisms of responses via intense sensory stimuli: categorisation via percentiles of RT. Response times at the 45th percentile or earlier – the fast onset percentiles - were likely to be indicative of responses which occur more often in the presence of SCM activity and which are likely to be indicative of any distinct neurophysiological mechanism responsible for the StartReact effect that may be present. Similarly, response times at the 55th percentile or later were chosen to represent the slower onset responses which less frequently occur with SCM activity. We conducted a series of linear mixed-effects models on each dataset using these percentile categories (means shown in **Figure 2.15**) to examine any interactions of percentile categorisation with the muscle and task factors to determine whether differing neurophysiological contributions to different movement types alter their benefit received from the intense auditory probe. The resulting statistics are shown in **Table 2.6**.

Table 2.6. Statistical output of our linear mixed effects models.

Authors (year)	*Main Effect/Interaction*	*df*	*F*	*p*
Castellote & Kofler (2018)	Percentile	1, 422	533.7	<.001
	Task	1, 422	32.5	<.001
	Muscle	1, 422	26.6	<.001
	Percentile*Task	1, 422	18.6	<.001
	Percentile*Muscle	1, 422	0.0	.814
Honeycutt et al. (2013)	Percentile	1, 187	158.1	<.001
	Task	1,187	11.1	.001
	Percentile*Task	1, 187	2.7	.099
Marinovic et al. (2014)	Percentile	1, 130	62.2	<.001
	Muscle	1, 130	0.1	.745
	Percentile*Muscle	1, 130	0.0	.905
Marinovic et al. (2015)	Percentile	1, 89	103.7	<.001
Ossanna et al. (2019)	Percentile	1, 579	501.8	<.001
	Muscle	5, 579	29.9	<.001
	Percentile*Muscle	5, 579	5.2	<.001
Honeycutt et al. (2014); Tresch et al. (2014)	Percentile	1, 1944	825.7	<.001
	Task	1, 1944	43.8	<.001
	Muscle	2, 1944	27.5	<.001
	Percentile*Task	1, 1944	2.6	.108
	Percentile*Muscle	2, 1944	0.1	.899

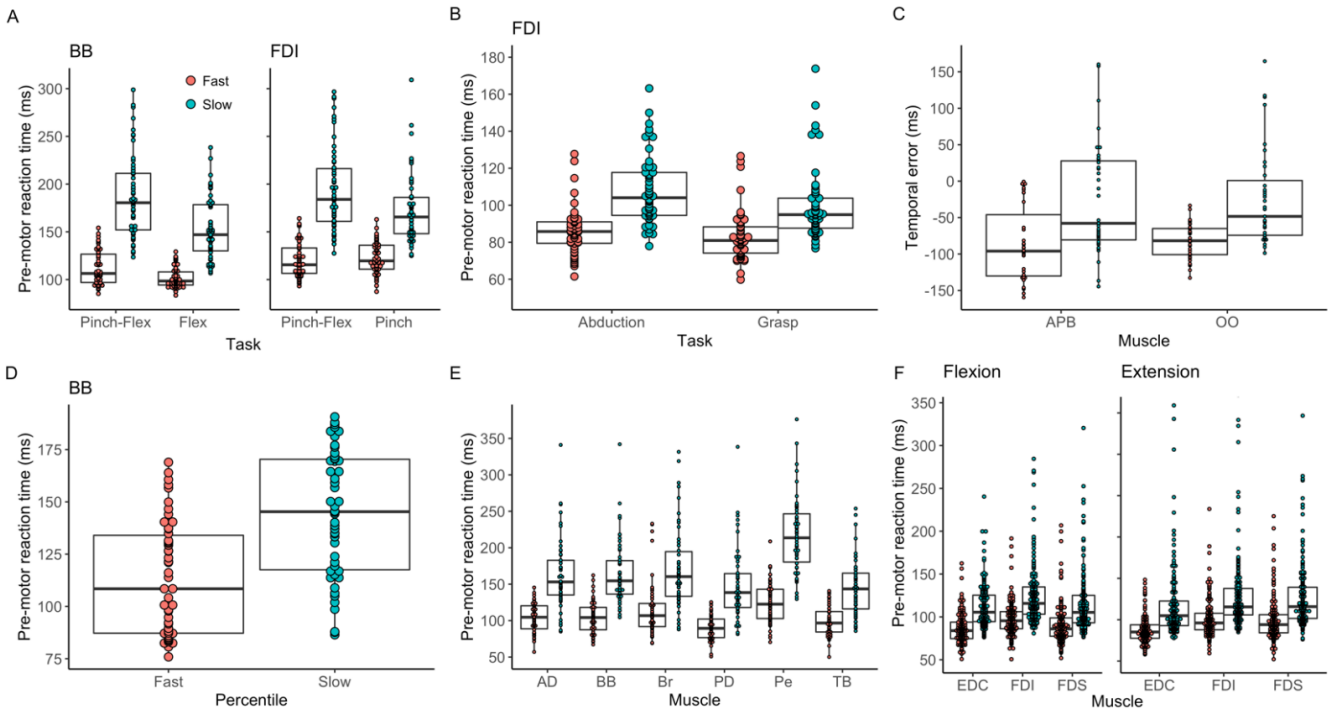


Figure 2.15. Box plots displaying median response times with first and third quartiles. **A).** Response times for Fast and Slow Percentiles across muscles and tasks in Castellotte and Kofler (2018). **B).** Response times for Fast and Slow Percentiles in Honeycutt et al.'s (2013) finger and grip tasks. **C).** Response times for Fast and Slow Percentiles across muscles and tasks in Marinovic et al. (2014). **D)**. Response times for Fast and Slow Percentiles Marinovic et al.'s (2014) arm supination task. **E)**. Response times for Fast and Slow Percentiles across muscles recorded in Ossanna et al. (2019). **F)**. Response times for Fast and Slow Percentiles across muscles and tasks in Honeycutt et al. (2014), Tresch et al. (2014).

2.11 Appendix F

To further highlight the benefit of using CDFs as a method to investigate the StartReact effect, here we present additional analyses that can provide further insight to the triggering of different movements. For example, percentiles may be modelled as a fixed factor in linear mixed-effects analyses. Here, using Castellote and Kofler's (2018) data, we conducted a linear mixed-effects model with percentile (5/15/25/35/45/55/65/75/85/95th), muscle (BB/FDI), and task (single/combined) set as fixed factors, and subjects set as a random factor. In keeping with the finding of a significant interaction of percentile categorisation (Fast/Slow) with task type presented in the results, we found a significant interaction on RT of percentile with task type, $F_{(9, 390)} = 7.91$, $p < .001$. Examination of **Figure 2.16** shows the mean RT at each percentile for each of the movement types. The slopes for the single movements (BB Flex and FDI Pinch) are flatter - with a smaller increase across the earlier percentiles (5 to 45th), indicating a shorter range of RTs for these movements in comparison to the BB and FDI RTs in the Pinch-Flex task. As such, the data suggest there is a narrower distribution of RTs for movements produced in isolation. This narrower distribution indicates these types of movements are less prone to triggering delays.

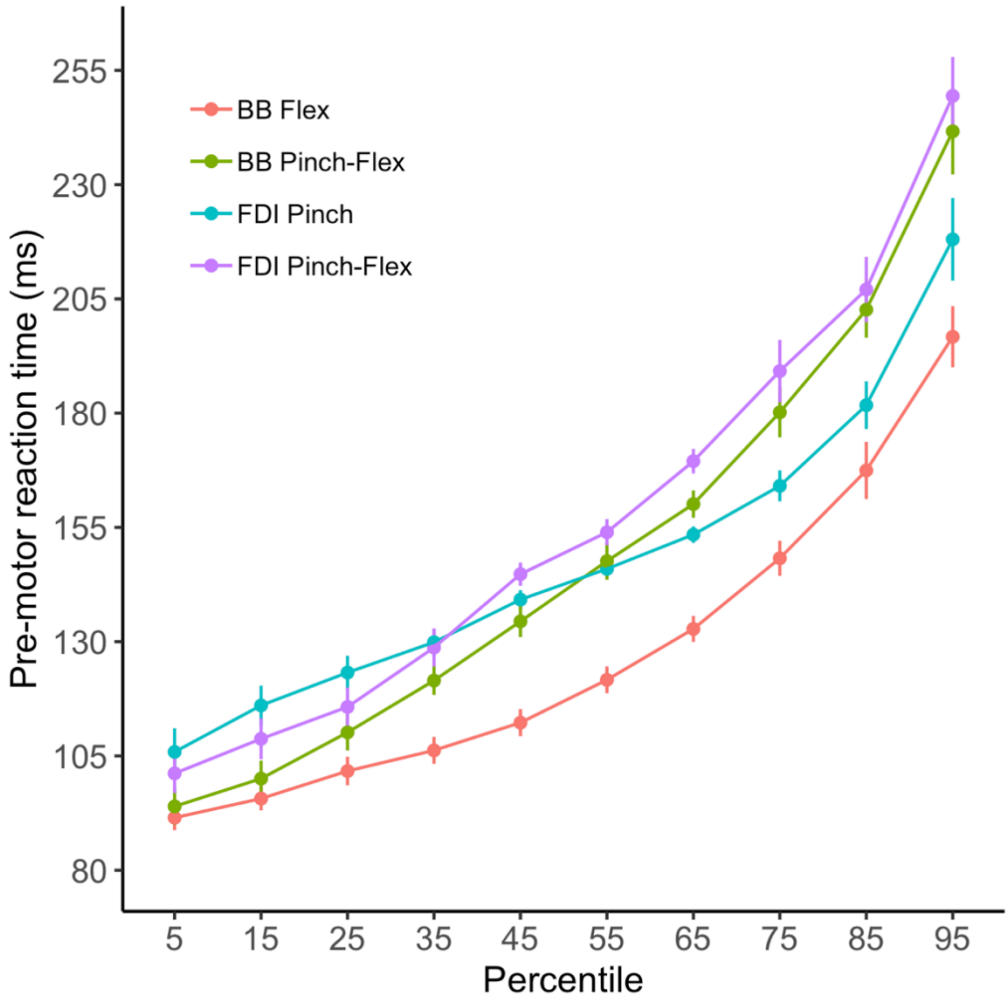


Figure 2.16. Mean reaction times plotted at each percentile for each of the movement types in Castellote and Kofler's (2018) data.

Furthermore, examination of the CDF curve for different movement types together can provide additional information which can guide the analysis method. For example, **Figure 2.2** shows similar SCM+ trial onset latencies for BB in both the flex task and pinch-flex task. However, as shown in our results, after our categorisation of trials on the basis of RT and subsequent analysis of the fast onset data, we found a significant difference between BB onset latency in the flex task and BB onset latency in the pinch-flex task. Examination of these tasks in **Figure 2.16** suggests that RTs in the flex task could not be reduced much further (a floor effect). This is a likely explanation for the similar latencies observed for BB in the flex and pinch-flex tasks at the 5th and 15th percentiles. **Figure 2.16** further shows a divergence after these early percentiles, which was captured by our categorisation of fast onset trials at the 45th percentile or earlier and is evident in our analysis of the

fast onset data. In this case, it appears this method of categorisation of the fast onset trials also has the added benefit in that it avoids this RT floor effect whilst also collating trials at the fastest latencies within the distribution for analysis. This may be a useful consideration for those employing this method in future research whereby differences in triggering across the RT spectrum, along with any potential impacts on data as a result of a RT floor effect, can be explored when taking into account the CDF method.

CHAPTER THREE: NEURAL GAIN INDUCED BY STARTLING ACOUSTIC STIMULI IS ADDITIVE TO PREPARATORY ACTIVATION

This is the peer reviewed version of the following article:

McInnes, A. N., Corti, E. J., Tresilian, J., R., Lipp, O. V., & Marinovic, W. (2020). Neural gain induced by startling acoustic stimuli is additive to preparatory activation. *Psychophysiology, 57*(3), e13493.,

which has been published in final form at https://doi.org/10.1111/psyp.13493. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

3.0 Abstract

Loud acoustic stimuli presented during movement preparation can shorten reaction time and increase response forcefulness. We examined how efferent connectivity of an agonist muscle to reticulospinal and corticospinal pathways, and the level of prepared movement force, affect reaction time and movement execution when the motor response is triggered by an intense acoustic stimulus. In Experiment 1, participants executed ballistic wrist flexion and extension movements of low and high force in response to visual stimuli. A loud acoustic stimulus (105dBA) was presented simultaneously with the visual imperative stimulus in probe trials. In Experiment 2, participants executed ballistic wrist flexion movements ranging from 10-50% of maximum voluntary contraction with a loud acoustic stimulus presented in probe trials. The shortening of response initiation was not affected by movement type (flexion or extension) or prepared movement force. Enhancement of response magnitude, however, was proportionally greater for low force movements and for the flexor muscle. Changes in peak force induced by the intense acoustic stimulus indicated that the neural activity introduced to motor program circuits by acoustic stimulation is additive to the voluntary neural activity that occurs due to movement preparation, rather than multiplicative.

3.1 Introduction

Prepared motor responses can be triggered by the presentation of a loud acoustic stimulus (LAS). These triggered responses typically occur at shorter latencies and tend to be more forceful than would be produced voluntarily (Anzak et al., 2011; Honeycutt & Perreault, 2012; Kumru & Valls-Solé, 2006; Marinovic et al., 2015; Valls-Solé et al., 1999), potentially a product of increased neural activation being introduced to motor program circuits when a LAS is presented (Carlsen et al., 2012; Jaskowski et al., 1995; Marinovic et al., 2013; Marinovic et al., 2014a; Marinovic et al., 2014c; Marinovic et al., 2017; Tresilian & Plooy, 2006; Ulrich et al.,1998). It is unclear how characteristics of the prepared motor response, such as the forcefulness or muscle used to execute the response, may impact how much (or how quickly) LAS-evoked neural activity is injected into motor program circuits. One manner in which LAS-evoked activity may be modulated is via the strength of efferent reticulospinal connections that project to the agonist muscle. For example, as the reticular formation is implicated in both the startle reflex and voluntary motor control pathways, rather than the typical pathways used for voluntary motor control, early triggering of prepared responses may occur through a shorter pathway mediated by the reticular formation (Honeycutt et al., 2015; Rothwell et al., 2002; Yeomans & Frankland, 1995). Therefore, the amount of neural activity introduced to motor program circuits by a LAS and speed at which this activity is evoked may be expected to be greater when the agonist muscle has stronger efferent connectivity to the reticular formation. Differences in the shortening of RT via intense acoustic stimuli have been found between movements that engage muscles which have more reticulospinal projections, such as the biceps brachii, and muscles which have greater corticospinal connectivity, such as the first dorsal interosseous (Carlsen et al., 2009; Honeycutt et al., 2013). However, these differences in RT shortening between muscles may be a product of the functionality of movements that were tested, rather than muscle connectivity (Marinovic & Tresilian, 2016; Castellote & Kofler, 2018). Other studies (Marinovic et al., 2014b) found no effect of muscle connectivity on RT shortening via intense acoustic stimuli, however, a comparison of movements which engage muscles that are functionally and anatomically similar remains to be tested.

While these aforementioned studies have investigated how preparation for movement of different muscles affects how quickly LAS-evoked activity is injected into motor program circuits, they have not examined how muscle connectivity affects the amount of activity that is accumulated after the presentation of a LAS, nor how the amount of force that is prepared for a movement may impact the level or speed of LAS-evoked activation. We hypothesised the level of activation evoked in motor program circuits by a LAS is affected by the amount of force prepared for a response in one of two ways. First, the amount of activation injected into motor program circuits by a LAS will increase as the amount of force prepared for a motor response increases, in which the enhancement of response magnitude scales with the required force of the prepared movement. This enhancement of response magnitude in a multiplicative manner would suggest that as prepared response force increases, a larger network of motor neurons are engaged which can subsequently be more easily recruited by the LAS. Alternatively, the amount of activation injected into motor program circuits by a LAS may be constant regardless of the amount of force prepared for a motor response, so that the enhancement of response magnitude remains constant with increasing prepared response force. Such enhancement of response magnitude in an additive manner would suggest activation introduced via functional connections between the auditory cortex and primary motor cortex (M1) (Marinovic et al., 2014c), or more directly from the brainstem to the spinal cord, converges with the activation associated with the prepared response. Furthermore, in flexors and extensors of the wrist – muscles which differ in their corticospinal and reticulospinal connectivity (Cheney & Fetz, 1980; Clough et al., 1968; de Noordhout et al., 1999; Fetz & Cheney, 1980; Godfrey et al., 2013; Koganemaru et al., 2010; McMillan et al., 2004; Palmer & Ashby, 1992; Park & Li, 2013; Vallence et al., 2012), we investigated how muscle connectivity may impact the level or speed of LAS-evoked neural activation by examining how our proposed multiplicative or additive effects, and/or the shortening of RT, may be modulated by efferent connectivity to the reticular formation. As the reticular formation has been proposed to mediate the shortening of RT via intense sensory stimulation, we predicted a greater enhancement of response magnitude and shortening of RT would be observed in muscles which are more strongly connected to the reticular formation.

3.2 Method

3.2.1 Participants

Twenty-four participants (19 female) volunteered to participate in Experiment 1 (mean age = 22.5, *SD* = 5.41, range = 19 - 45). A second sample of 26 volunteers (19 female) was recruited for Experiment 2 (mean age = 22.85, *SD* = 4.48, range = 18 – 34). Participants in both experiments were self-reportedly right-handed, with normal or corrected-to-normal vision, and no apparent or known auditory impairments, neurological conditions, or injuries which could have affected their performance in the experiments. Informed, written consent was obtained from all subjects prior to participation, in accordance with the Declaration of Helsinki and approved by Curtin University's local human research ethics committee.

3.2.2 Procedures – Experiment One

Participants were seated in front of a 22" Samsung SyncMaster 2233LCD monitor (120 Hz refresh rate, 1680x1050 resolution), at a distance of 0.8m. This monitor was used to present visual stimuli to participants during the experiment. Subjects were asked to respond to visual targets by making ballistic wrist movements in two directions, flexion and extension, with their right (dominant) hand and forearm secured in a custom-made wrist device housing a six degree of freedom force/torque sensor (JR3 45E15A-I63-A 400N60S, Woodland, CA; see de Rugy et al. (2012); pictured in **Figure 3.1**). This device held the hand and forearm in a neutral position throughout the experiment, recorded forces produced by wrist movements, and controlled a cursor which was presented visually on screen. The device was tightened snugly to the hand and forearm of each participant to prevent any time delay between muscle activation and recording of force. The experimental setup is pictured in **Figure 3.1**. All participants completed the same procedures as follows.

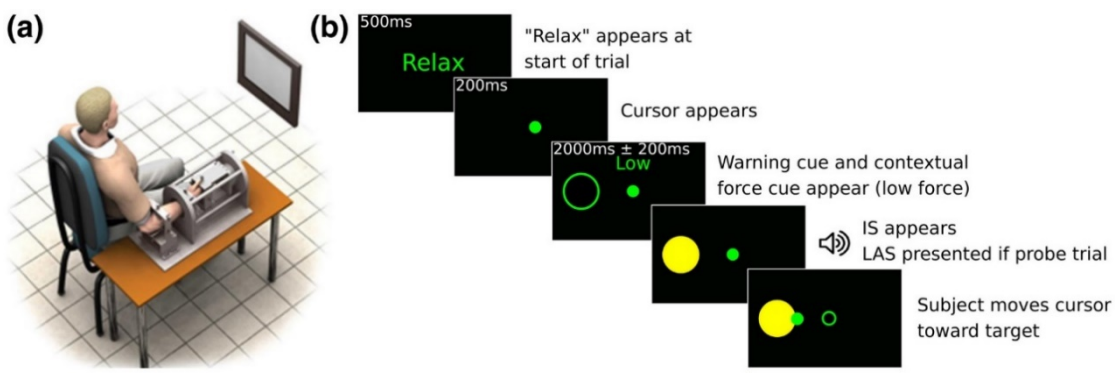


Figure 3.1. A). Diagram of experiment setup. Participant is seated in front of a monitor with arm secured in the wrist device. Adapted from de Rugy et al. (2012). **B).** Sequence of events requiring a low force flexion response.

Due to the more frequent use and subsequent strength of flexor muscles in comparison to extensor muscles (Salonikidis et al., 2011), differences in muscle strength within individuals were controlled in order to allow comparison between these two muscles. Therefore, a maximum voluntary contraction procedure was completed for both flexion and extension movements prior to the start of the experiment (see Selvanayagam et al., 2016). In this procedure, subjects made six (three flexions, three extensions) isometric maximum voluntary contractions of the wrist toward a target for three seconds and the peak force (Newtons, N) was measured. The maximum voluntary contraction was calculated as the average peak force of the three contractions for each type of movement. Subsequently, this data was used to determine the degree of force required to reach low and high force targets during the experiment. These targets were represented as 20% or 60% of maximum voluntary contraction, depending on the force requirement of the relevant trial.

Participants were next trained in the movements that were required in the experiment, until they were able to accurately initiate movements within 250ms after the imperative stimulus (IS). 224 experimental trials were completed, half of which constituted a block of flexion movements and the remaining half required extension movements. The order in which participants completed flexion or extension movements was counterbalanced. As shown in **Figure 3.1**, each trial began with the presentation of the word “relax”, indicating to the participant to keep their hand stationary for the start of the trial. A cursor which could be controlled by moving the hand then appeared in the centre of the screen. During each trial, a circular warning signal (WS) was presented, indicating the impending presentation of the IS. The colour of the WS and text presented at the top of the screen (“low” or “high”) indicated the force requirement of the trial (see **Figure 3.1**). Participants were instructed to prepare to move during this period. After two seconds (±200ms), the IS appeared and participants responded with a ballistic wrist movement, aiming to stop the movement once the target had been reached. The inter-stimulus interval (WS - IS) was randomly determined from a Gaussian distribution. The IS appeared as a

yellow circle in place of the warning cue. The size of the target and distance to reach the target remained consistent regardless of trial type, whereas the position of the target (on the left or right side of the screen) depended on the movement direction of the trial – extension or flexion. 25% of trials were probe trials, in which a LAS was presented as an accessory stimulus pseudo-randomly with the IS, so that two consecutive trials could not occur as probe trials. Visual and auditory stimuli were presented using Cogent 2000 graphics running in Matlab 8.4. Participants were instructed to ignore the LAS when it was presented. At the end of each trial, feedback was presented on screen indicating RT, so as to maintain participant motivation and encourage quick responses. In probe trials, this feedback was not presented so as to prevent subjects becoming aware of the study's aims and modifying their responses.

3.2.3 Loud Acoustic Stimulus

In probe trials, a LAS was presented in brief (50ms with a rise and fall time <1.5ms) white noise bursts. The peak amplitude of the stimulus was measured using a Bruel and Kjaer sound level meter (Type 2205, A weighted; Brüel & Kjaer Sound and Vibration Measurement, Naerum, Denmark). Limits of the neuromuscular system may often complicate interpretations of data in regard to the early release or vigour of responses elicited by acoustic stimuli. That is, RT flooring effects or ceiling effects in the production of force may limit meaningful observations, particularly when comparing muscles in this context. Subsequently, we employed a LAS at two intensities during Experiment 1; a high intensity stimulus at 105dBA, and a lower, albeit still startling stimulus at 90dBA. We chose to incorporate a lower intensity stimulus that would still be capable of eliciting a startle response in the case that the StartReact effect relies on mechanisms that rely on activation of startle circuits (see Carlsen et al., 2007). The LAS was generated by the soundcard of the computer used to run experiments, and presented binaurally through stereophonic active noise cancelling headphones (Bose QC25).

3.2.4 Procedures – Experiment Two

The same procedures from Experiment 1 were used in Experiment 2, with the following exceptions. Only flexion movements were employed in this experiment,

and as such, no extension trials were contained in the maximum voluntary contraction, practice, or experimental trials. In addition, five force levels (10%, 20% 30%, 40%, and 50% of maximum voluntary contraction) were required in the experiment. All participants completed 230 trials, making up five blocks of 46 trials each. Each block was randomised to one of the five required force levels, with the required force of each block indicated by the colour of the warning stimulus, as well as text presented at the start of each trial and block. 20% of trials were probe trials in which a LAS was presented at one intensity, 105dBA.

3.2.5 Data reduction and analysis

Median RT, peak rate of force development, and peak force were subjected to statistical analyses. RT was measured as the time difference between the presentation of the IS and movement onset, expressed in milliseconds (ms). For each trial, the full time series of force data was collected from the force/torque sensor and sampled at 2000Hz using a National Instruments data acquisition device (NI USB-6229). Estimations of the time of movement onset were determined from the tangential speed time series using a two-stage algorithm suggested by Teasdale et al., (1993). The derivative of the torque signal over time was used to measure the peak rate of force development in Newtons per second (N/s). To measure the extent of facilitation that occurred due to the LAS, differences and ratios between probe and control trials were calculated. Differences in RT are reported, calculated as $RT_{LAS} - RT_{Control}$. To examine the extent of change in movement execution that was elicited by the LAS, ratios are reported for rate of force development and peak force. For peak force, this was calculated as ($Peak\ Force_{LAS}$ / $Peak\ Force_{Control}$). The same was calculated for peak rate of force development.

Cases in which subjects responded prematurely before the presentation of the IS (anticipatory response), or responded with a significant delay after the IS (indicating insufficient movement preparation) were identified prior to data analysis and subsequently removed (Whelan, 2008). These cases were identified as a RT <50ms or a RT >1000ms. In Experiment 1, this resulted in the removal of 143 trials in total (2.66% of all trials). 152 trials were removed from Experiment 2 (2.54% of all trials).

A series of linear mixed-effects models were conducted using the lmer function (lmerTest package; version 2.0-36; Kuznetsova et al., 2017) in RStudio (version 1.1.442; RStudio Team, 2015). These were conducted with trial type, muscle type, and required force set as fixed factors and subjects set as a random factor, with Kenward-Roger approximation for degrees of freedom, in order to analyse the effects of trial type (control, low intensity LAS, high intensity LAS), muscle type (extension, flexion), and required force (low, high) on the facilitation of movement initiation and execution in Experiment 1. Along with these main effects, all interactions were tested in each linear mixed-effects model. In Experiment 2, analyses were conducted with required force (10%, 20%, 30%, 40%, and 50% of maximum voluntary contraction) and trial type (control, LAS) as fixed factors and subjects set as a random factor. Examination of residuals for each subject using Q-Q plots indicated no severe violations of the assumption of normality of residuals. Along with F values and p values of analyses, R^2 values, calculated using the r2beta function (r2glmm package; version 0.1.2; Jaeger et al., 2017) are reported to provide an estimate of effect sizes. Post-hoc tests were conducted using the emmeans function (version 1.3) with correction for multiple comparisons using the false-discovery rate procedure (Benjamini & Hochberg, 1995). To complement the traditional frequentist analyses and support inferences based on the null hypothesis, additional Bayesian linear models were conducted using the lmBF function from the BayesFactor package (version 0.9.12; Morey et al., 2018). The resulting Bayes factors (BF_{01}) are reported alongside p values of non-significant results of the main analyses.

As a further consideration for comparing the shortening of RT between muscles, it has been suggested that responses to a LAS fit two different mechanisms (Carlsen et al., 2007). The first may be the usual voluntary pathway for motor control, often without sternocleidomastoid (SCM) activity, and reduced shortening of response latencies – potentially representing a case of simple stimulus intensity and accessory stimulus effects (Marinovic & Tresilian, 2016). If a second mechanism exists, this would represent a distinct StartReact mechanism that produces the shortest response latencies, often – but not always - in the presence of SCM activity (Carlsen et al., 2009; Carlsen et al., 2007; Marinovic & Tresilian, 2016). Therefore, if the StartReact effect does in fact follow a separate, distinct pathway from voluntary motor control pathways, muscles which have stronger

connectivity to this pathway via the reticulospinal tract would be more likely to activate this pathway when a LAS is presented. To test this, a cumulative distribution function (CDF) was used to examine the effects of muscle type, as well as required force, at the 35th and 65th percentiles of RT for probe trials of both intensities. As reported by Leow et al. (2018), 35th and 65th percentiles were chosen for the CDF based on mean RT latencies of trials in the presence (35th percentile) or absence (65th percentile) of SCM activity reported by Honeycutt et al. (2013). While the presence or absence of SCM activity has previously been used in the literature in an attempt to separate responses which may or may not activate neurophysiological mechanisms responsible for the StartReact effect, this is not always reliable (Leow et al., 2018; Marinovic & Tresilian, 2016). For example, short latency responses indicative of the StartReact effect can be observed in the absence of SCM activity (Valls-Solé et al., 2005; Castellote et al., 2017). Furthermore, longer latency responses can occur in the presence of SCM activity (Marinovic & Tresilian, 2016). Therefore, examining trials based on RT percentile latencies, rather than the presence or absence of surface SCM activity, may be a more reliable method of capturing these two potential mechanisms in response to intense acoustic stimuli (Dean & Baker, 2017; Leow et al., 2018; Marinovic & Tresilian, 2016).

To estimate the pattern of neural activation that is accumulated due to the LAS with increasing force, two predicted patterns of the data were proposed. The first, representing a multiplicative effect of the LAS (**Figure 3.2A**) predicts an interaction of trial type and required force. **Figure 3.2B** depicts an additive effect of the LAS and predicts no such interaction. As such an effect relies on observation of the null hypothesis, the data may further be explored with the equations and model presented below.

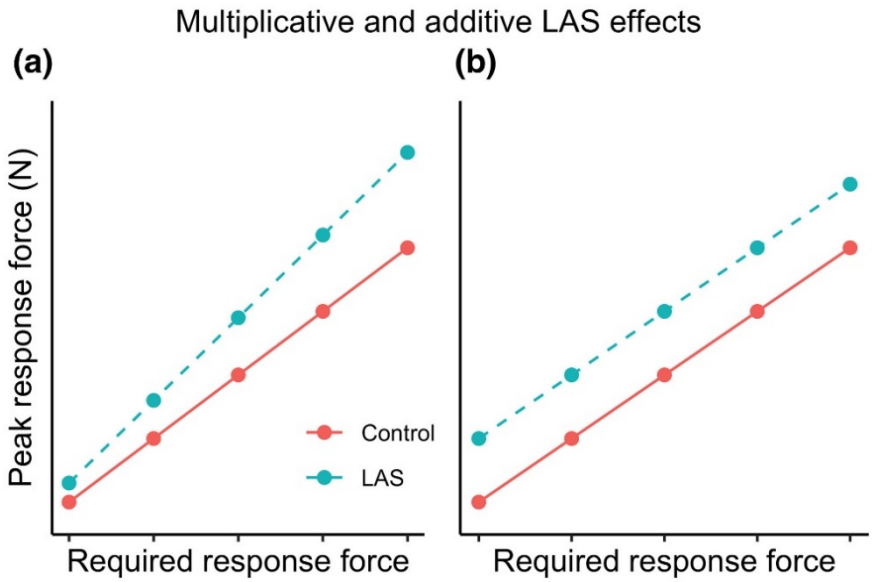


Figure 3.2. A). Multiplicative effect of LAS, where response force induced by the LAS scales with the amount of force prepared for the response. **B).** Additive effect of LAS, where response force induced by the LAS is added at a constant rate to the prepared motor response

The peak force of responses executed in control trials may be described by the following equation, where A is equal to the slope. If responses are biased to present with more or less force than is required by the task, A < 1 or A >1. If there is no bias, then A may be observed as a random variable with a mean = 1.

Equation 1: $(\text{Peak force})_{\text{Control}} = A \times (\text{Required force})$

If the effect of the LAS on response force is multiplicative, and the multiplying factor is denoted as B, then B x A equates to the slope of LAS trials. When B > 1, this results in a slope steeper than control trials, as depicted in **Figure 3.2A**. The equation for LAS trials is therefore described as:

Equation 2: $(\text{Peak force})_{\text{LAS}} = B \times A \times (\text{Required force})$

These equations may then be used to determine how LAS-evoked neural activation is introduced to the system. For example, calculating ratios of LAS to control trials, as denoted by Equation 3, results in the multiplying factor, B. This is depicted in the flat line representing ratios in **Figure 3.3A**.

$$\textbf{Equation 3}: \frac{(\text{Peak force})_{\text{LAS}}}{(\text{Peak force})_{\text{Control}}} = \text{B}$$

Furthermore, differences in response force for the multiplicative predication may be represented by Equation 4. When B > 1, the gradient (A (B – 1)) is positive and corresponds with a slope that linearly increases with required response force, as depicted in the "differences" slope in **Figure 3.3A**.

$$\textbf{Equation 4}: (\text{Peak force})_{\text{LAS}} - (\text{Peak force})_{\text{Control}} = \text{BA(Required force)} - \text{A(Required force)}$$
$$= \text{A(B} - 1)\text{(Required force)}$$

Alternatively, if the effects of the LAS on response force are additive, this may be represented by Equation 5, where F is equal to a constant force added to responses by the LAS, as depicted by a LAS slope parallel to the control slope in **Figure 3.2B**.

$$\textbf{Equation 5}: (\text{Peak force})_{\text{LAS}} = \text{A x (Required force)} + \text{F}$$

Given an additive model, ratios of LAS to control trials are calculated in Equation 6 to determine the neural activation that is introduced to the system by the LAS. This gives a non-linear curve with increasing required force as depicted by the slope for ratios in **Figure 3.3B**.

$$\textbf{Equation 6}: \frac{(\text{Peak force})_{\text{LAS}}}{(\text{Peak force})_{\text{Control}}} = \frac{(\text{Peak force})_{\text{Control}} + \text{F}}{(\text{Peak force})_{\text{Control}}}$$

If differences between LAS and control trials are calculated for the additive model, as in Equation 7, the constant F is derived, equal to the amount of force added to the response by the LAS. This is depicted by the flat line for differences in **Figure 3.3B**.

Equation 7: $(\text{Peak force})_{LAS} - (\text{Peak force})_{Control} = F + (\text{Peak force})_{Control} - (\text{Peak force})_{Control}$

$= F$

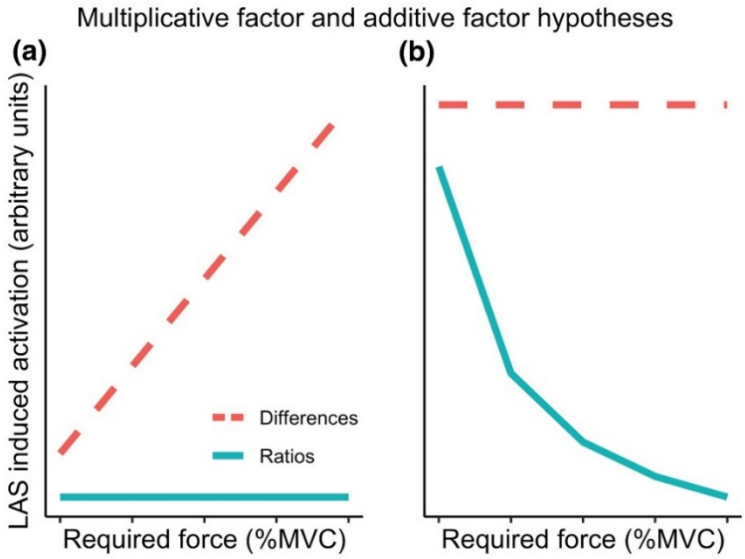


Figure 3.3. A). Multiplying factor hypothesis of LAS-induced neural activation. Differences in peak force between probe and control trials increase, while ratios remain constant. **B).** Adding factor hypothesis of LAS-induced neural activation. Ratios of probe to control trials decrease, while differences remain constant

As the presence of an additive LAS effect as depicted in **Figure 3.2B** cannot be determined by an absence of an interaction of trial type and required force – evidence for the null hypothesis, we compared data from Experiment 2 to our multiplicative and additive models presented in **Figure 3.3**. In this analysis, both ratios of probe to control trials (Peak Force_{LAS} / $\text{Peak Force}_{Control}$) and differences between probe and control trials (Peak Force_{LAS} - $\text{Peak Force}_{Control}$) for peak force data in Experiment 2 were calculated to determine the fit of the data to each model, using polynomial contrasts with the contr.poly function (R Core Team, 2016). The multiplicative hypothesis would be supported by a linear increase in peak force differences and no change in peak force ratios as force increases (see **Figure 3.3A**). Alternatively, an additive hypothesis would be supported by no change in peak force differences and a reciprocal decrease in peak force ratios as required force increases (see **Figure 3.3B**).

3.3 Results

3.3.1 Experiment One

3.3.1.1 Maximum Voluntary Contraction

Our maximum voluntary contraction procedure indicated a statistically significant difference between flexors and extensors in their force production capabilities, $t_{(23)} = 6.12$, $p < .001$, $d = 1.25$, with flexors showing greater maximum voluntary contraction force ($M = 107.15$ N, $SD = 38.31$) compared to extensors ($M = 83.23$ N, $SD = 29.36$).

3.3.1.2 Facilitation of Response Initiation

A statistically significant main effect of trial type indicated RT was shortened by the presentation of the LAS, $F_{(2, 253)} = 203.77$, $p < .001$, $R^2 = .617$. Control trial RT ($M = 194.59$ ms, SD = 20.17) was significantly reduced by both the high intensity ($M = 147.93$ ms, $SD = 20.12$ and low intensity LAS ($M = 150.99$ ms, $SD = 22.25$), with the difference between both probes and control trials resulting in $p < .001$ in post hoc tests. Analysis of the difference between control trials and LAS trials at both intensities indicated no significant main effects or interactions for RT (see **Figure 3.4A**). Subsequent Bayesian analysis of the non-significant effect of muscle type, $F_{(1, 161)} = .98$, $p = .323$, $R^2 = .006$, yielded a Bayes Factor (BF_{01}) of 4.53. This is substantial evidence (Jeffreys, 1961) for the null hypothesis of differences between these muscles in their shortening of RT via a LAS.

Our CDF analysis (**Figure 3.4B**) for all probe trials averaged across both intensities indicated mean RTs at the 35th percentile = 137.07 ms, SD = 15.62. At the 65th percentile mean RT = 162.64 ms, SD = 24.63. A recent meta-analysis comparing RTs based on SCM activity reports a mean difference in RT latency between SCM+ and SCM- trials of -16.9 ms (Leow et al., 2018). Our results indicate a mean difference of -25.57 ms between fast and slow latency responses. Therefore, our analysis based on CDF latencies appears to converge with recent studies adopting the same procedure. No interactions or main effects beyond LAS intensity were statistically significant in analyses of our CDF. Importantly, the main effect of muscle type for the fast percentile did not reach statistical significance, $F_{(1, 69)} = .45$, $p = .506$, $R^2 = .006$. Again, subsequent Bayesian analysis showed substantial support

(Jeffreys, 1961) for this null hypothesis, $BF_{01} = 4.42$. The probability density of RT across conditions is shown in **Figure 3.5**.

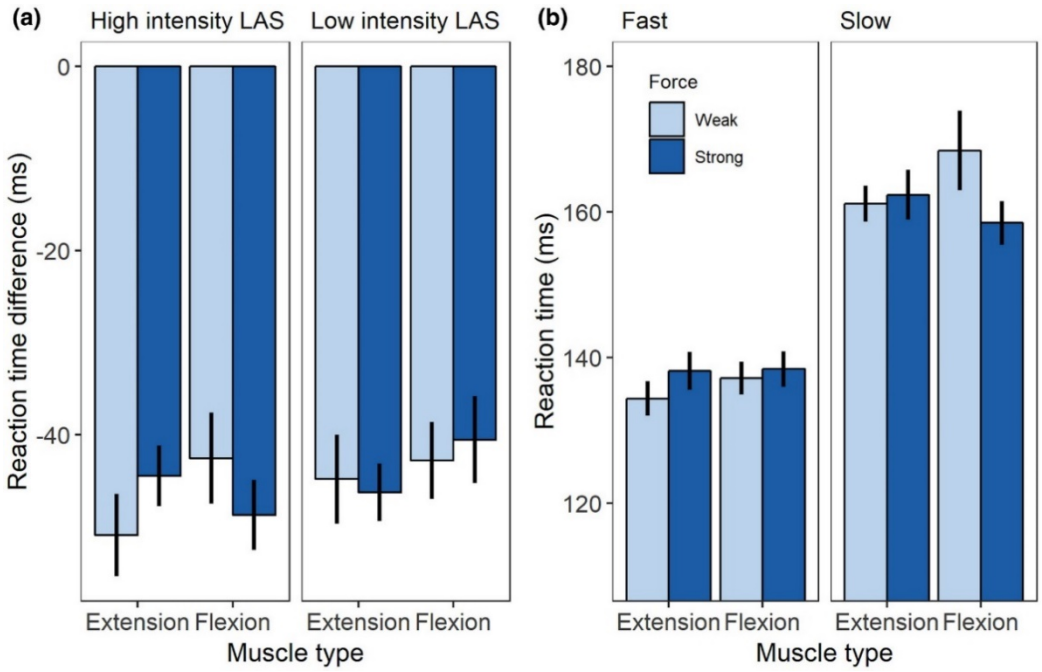


Figure 3.4. A). Difference in RT between both probe conditions and control trials. **B).** RT for fast (35th percentile) and slow (65th percentile) responses over different muscle types and force requirements

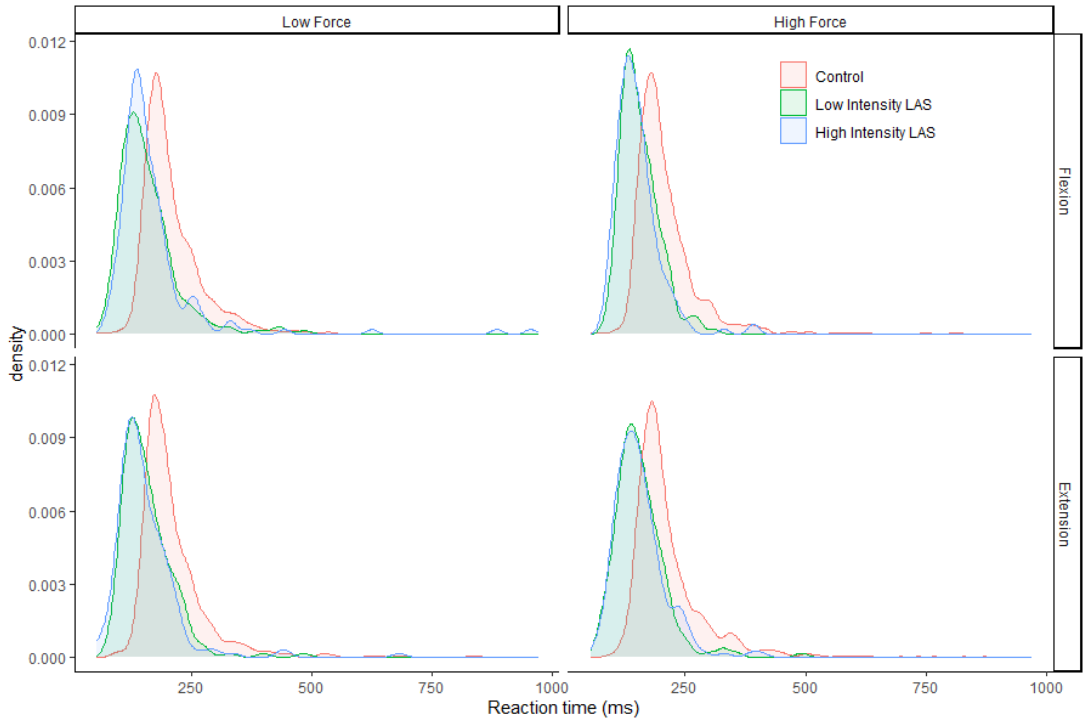


Figure 3.5. Probability density plot of reaction time for each condition.

3.3.1.3 Enhancement of Response Execution

Peak rate of force development showed a main effect of trial type, $F_{(2, 253)} = 18.54$, $p < .001$, $R^2 = .128$, indicating the presentation of the LAS increased response vigour. Rate of force development showed the greatest increase in the high intensity LAS condition ($M = 622.93$ N/s, $SD = 226.08$). The low intensity LAS condition ($M = 606.32$ N/s, $SD = 218.58$) showed some enhancement of rate of force development, with control trials showing the lowest rate of force development ($M = 501.66$ N/s, $SD = 223.35$) (see **Figure 3.6A**). Post hoc tests indicated the difference in peak rate of force development between control trials and probe trials of both intensities was statistically significant, $p < .001$. Similarly, peak force increased from control trials ($M = 37.98$ N, $SD = 20.78$) in both the high intensity ($M = 42.75$ N, $SD = 19.47$), and low intensity LAS conditions ($M = 42.64$ N, $SD = 19.92$), shown by a main effect of trial type on peak force, $F_{(2, 253)} = 3.61$, $p = .028$, $R^2 = .028$ (see **Figure 3.6B**). Post hoc analysis of the difference in peak force between control trials and high intensity probe trials ($p = .033$) and low intensity probe trials ($p = .033$) was statistically significant. The interaction of trial type and required force for peak force was not statistically significant, $F_{(2, 253)} = .17$, $p = .841$, $R^2 = .001$.

Due to the inherent difference between flexors and extensors in their force production capabilities, with flexors showing greater response magnitude regardless of trial type, the raw values of peak rate of force development and peak force were adjusted as a ratio of probe to control trials to examine the proportional facilitatory effects of a LAS on movement execution. Flexors showed larger ratios of peak rate of force development ($M = 1.34$, $SD = .31$) compared to extensors ($M = 1.22$, $SD = .28$) with a statistically significant main effect of muscle type on ratios of peak rate of force development, $F_{(1, 161)} = 14.89$, $p < .001$, $R^2 = .085$. Furthermore, a main effect of required force was found to be significant, $F_{(1, 161)} = 7.90$, $p = .005$, $R^2 = .047$, with larger ratios for low force movements ($M = 1.32$, $SD = .34$) compared to high force movements ($M = 1.24$, $SD = .26$). Ratios for low force movements were larger regardless of the intensity of the acoustic stimulus, with a non-significant main effect of LAS intensity, $F_{(1, 161)} = 1.15$, $p = .285$, $R^2 = .007$. Additionally, the interaction of required force and muscle type was significant, $F_{(1, 161)} = 3.94$, $p = .049$, $R^2 = .024$. The difference between low and high force ratios was statistically significant for flexors, ($p = .002$), but not for extensors ($p = .56$) (see **Figure 3.6C**).

Similar effects were noted for the ratios of peak force. Consistent with the ratios of rate of force development, larger ratios for low force movements (M = 1.22, SD = .26) compared to high force movements (M = 1.14, SD = .20) were observed, with a significant main effect of required force on peak force ratios, $F_{(1, 161)} = 7.93$, p = .005, R^2 = .047. Again, low force movements showed larger ratios regardless of LAS intensity, with a non-significant main effect of trial type for ratios of peak force, $F_{(1, 161)} = .03$, p = .868, R^2 = .000. In addition, the interaction of muscle type and required force was statistically significant for peak force ratios, $F_{(1, 161)} = 4.40$, p = .037, R^2 = .027, with a statistically significant difference between low and high force ratios for flexors, p = .004, but not extensors, p = .735 (see **Figure 3.6B**).

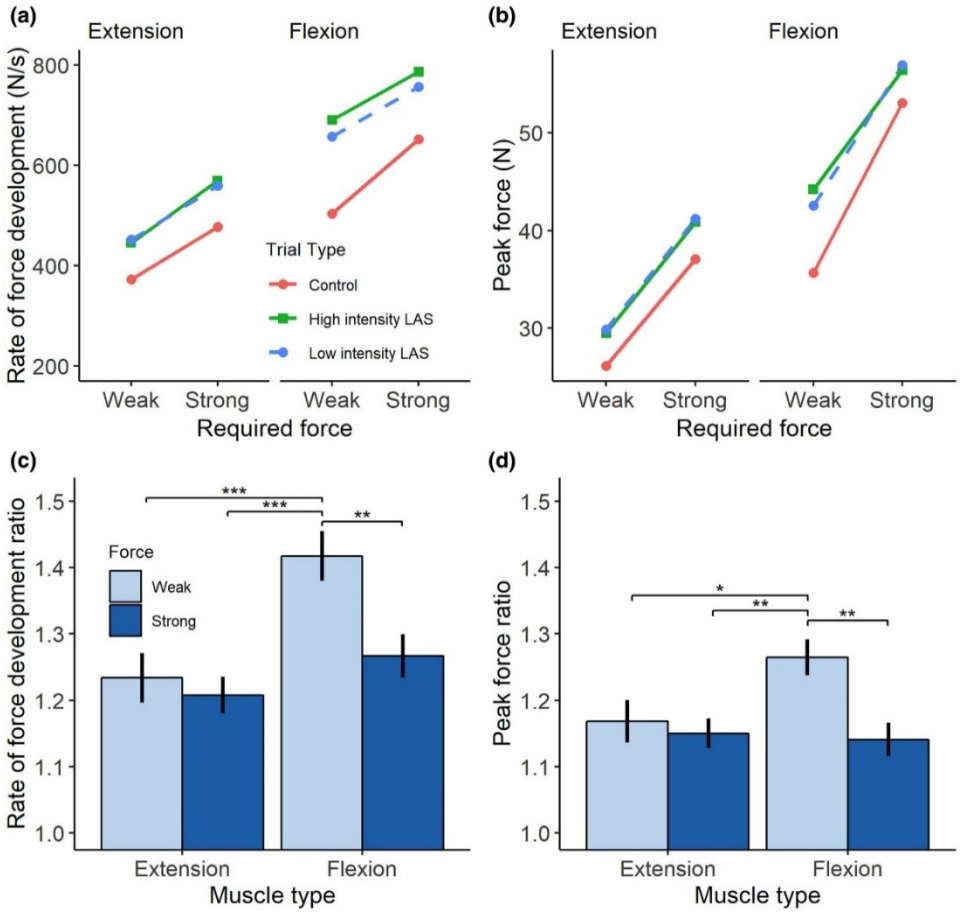


Figure 3.6. A). Peak rate of force development over trial types, muscle types, and force levels. **B).** Peak force over trial types, muscle types, and force levels. **C).** Ratios of probe to control trials for rate of force development. **D).** Ratios of probe to control trials for peak force. ***p < .001; **p < .01; *p < .05

3.3.2 Experiment Two

3.3.2.1 Facilitation of Response Initiation

RT was facilitated by the LAS, with probe trials showing significantly shorter RTs (M = 147.35 ms, SD = 26.18) compared to control trials (M = 194.42 ms, SD = 21.52), as shown by a statistically significant main effect of trial type on RT, $F_{(1, 225)} = 473.16$, $p < .001$, $R^2 = .678$. Analysis of differences between probe and control trials for RT showed no effect of force, $F_{(4, 100)} = 1.56$, $p = .190$, $R^2 = .059$ (see **Figure 3.7**). The probability density of RT across conditions is shown in **Figure 3.8**.

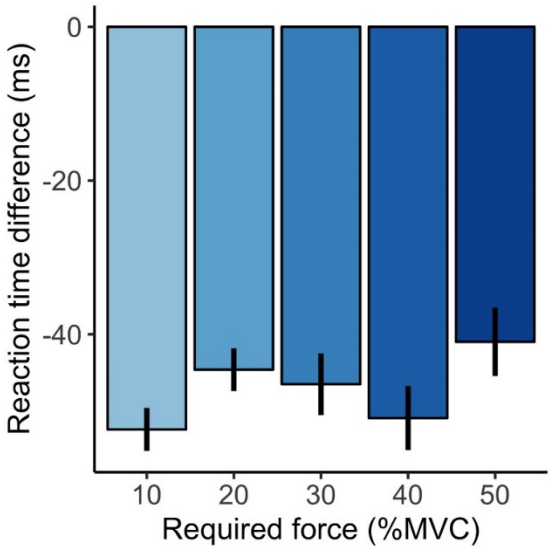


Figure 3.7. RT differences between probe and control trials over increasing force. Required force is represented as percentage of maximum voluntary contraction

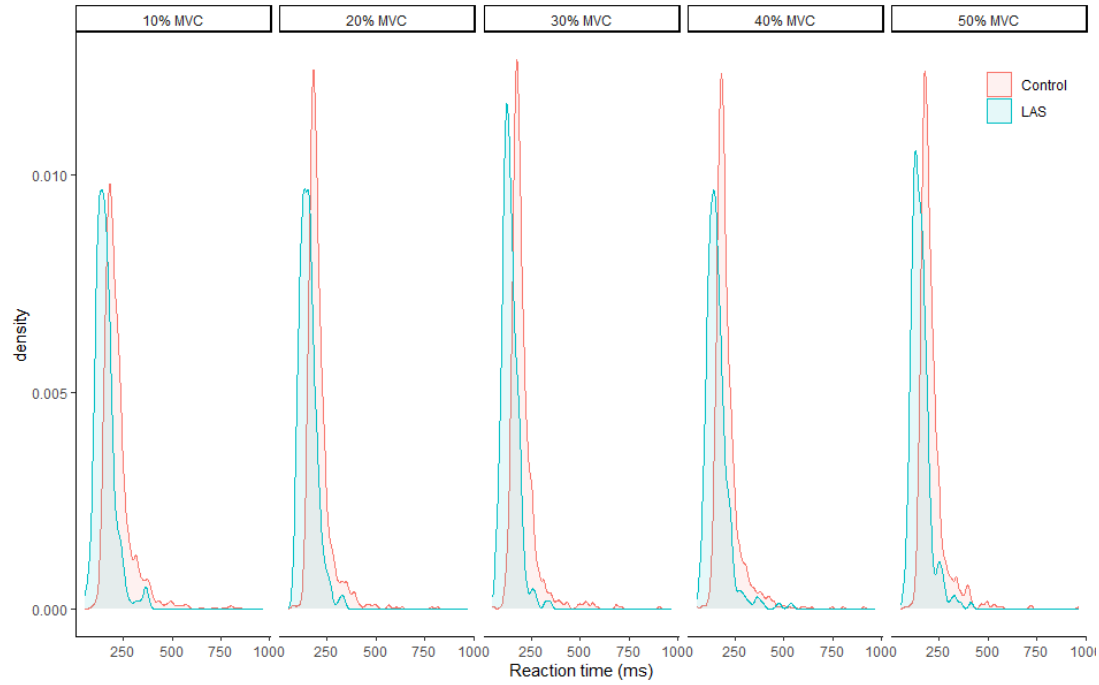


Figure 3.8. Probability density plot of RT for each condition.

3.3.2.2 LAS Induced Activation

Peak force was enhanced at the presentation of the LAS, with a main effect of trial type, $F_{(1, 225)} = 25.10$, $p < .001$, $R^2 = .1$ (see **Figure 3.9**). Similarly to the enhancement of response magnitude by the LAS in Experiment 1, the interaction of trial type and required force was not statistically significant, $F_{(4, 225)} = .168$, $p = .955$, $R^2 = .003$.

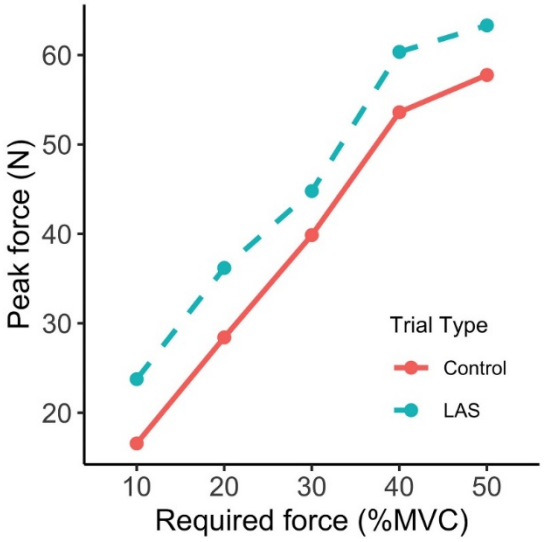


Figure 3.9. Peak force of responses over probe and control trials with increasing required force (percentage of maximum voluntary contraction).

As our analysis failed to indicate a significant interaction of trial type and required force, the data provided direct evidence against our proposed multiplicative LAS effect (**Figure 3.2A**), but not against the additive LAS effect (**Figure 3.2B**). Therefore, we examined the fit of the data to our additive model (**Figure 3.3B**) by calculating ratios (Peak $Force_{LAS}$ / Peak $Force_{Control}$) and differences (Peak $Force_{LAS}$ - Peak $Force_{Control}$) of peak force over trial types. Ratios of peak force (**Figure 3.10A**) showed a significant main effect of required force, $F_{(4, 100)} = 6.35$, $p < .001$, $R^2 = .203$. This effect of force was not significant for peak force differences (**Figure 3.10B**), $F_{(4, 100)} = .846$, $p = .499$, $R^2 = .033$. Polynomial contrasts showed a linear effect of force for peak force ratios, $t_{(100)} = -4.73$, $p < .001$. There was no such linear effect for peak force differences, $t_{(100)} = -1.06$, $p = .289$, indicating a decreasing trend for peak force ratios and a negligible change in the calculated differences of peak force as the required force in the task was increased. These results support our additive model presented in **Figure 3.3B**.

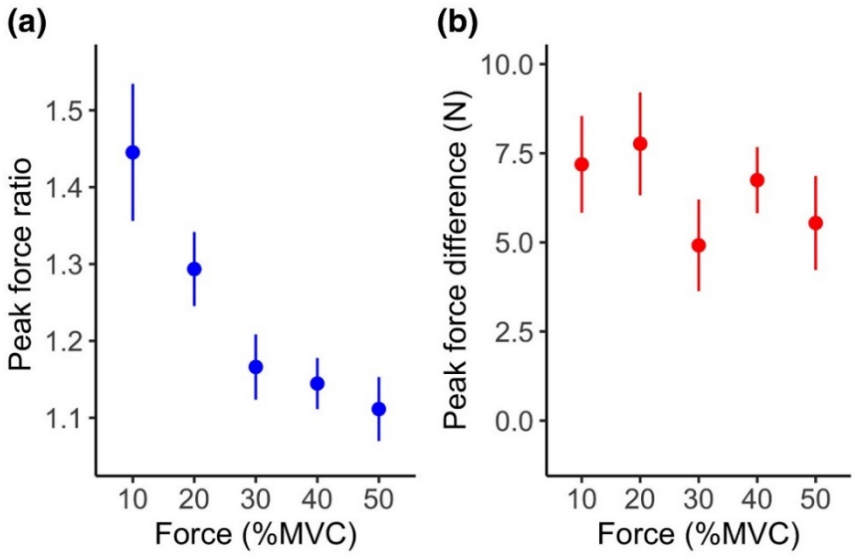


Figure 3.10. A). Ratios of probe to control trials for peak force. **B).** Differences between probe and control trials for peak force.

3.4 Discussion

3.4.1 Reductions of response latency do not differ between muscles

We observed large (≈50 ms) reductions in RT in both low intensity and high intensity LAS conditions in comparison to control trials. In this shortening of RT, no difference between flexion and extension movements emerged. It has been argued that there are two potential mechanisms that underlie responses to a startling stimulus. We conducted a CDF analysis (see Leow et al., 2018) in an effort to capture these two potential mechanisms and determine whether longer latency responses masked our ability to observe differences between muscles in their shortening of RT. Our analysis indicated no difference in response latency between muscle types in the fastest percentile of RT analysed (35%). Moreover, we obtained substantial support for the absence of differences in RT between muscle types from our Bayesian analysis, suggesting connectivity of agonist muscles of the wrist to reticulospinal pathways is not a critical factor in the magnitude of RT shortening via intense acoustic stimulation. Although we tried to avoid a floor effect by using a stimulus with lower intensity than in typical StartReact studies, it is possible that even at 90dBA there was little room for RTs to reduce much further. Future studies may want to consider employing even lower intensities (e.g., 80dBA). However, the magnitude of the shortening of RT observed in our data is arguably less than that observed by others. We have observed a mean difference in RT between probe and control trials of 46.66ms, as compared to a mean difference of 60.1ms reported in Leow et al.'s (2018) mini meta-analysis. In fact, some studies within this meta-analysis (e.g. Tresch et al., 2014) report a mean difference of approximately 90ms. The larger shortening of RT in previous studies may be related to their higher intensity of the acoustic stimulus (128dB in Tresch et al., 2014).

3.4.2 Muscle connectivity affects enhancement of response magnitude

To examine the facilitatory effects of a LAS on response magnitude, in Experiment 1 ratios of probe to control trials were analysed for peak rate of force development and peak force. Ratios for low force movements were larger than high force movements for peak rate of force development and peak force. This difference was observed for both low and high intensity probe conditions, indicating that this effect was not

likely due to high force movements being limited in their facilitation by ceiling effects. Flexors showed a greater rate of force development and peak force enhancement compared to extensors. Interestingly, our maximum voluntary contraction procedure indicated lower force production capabilities for extensors in comparison to flexors. As a function of required force being a percentage of maximum voluntary contraction for each muscle, our task required the execution of weaker extension responses compared to flexion responses. Given low force responses showed greater enhancement of response magnitude in response to the LAS, it may be expected extensors would show a greater increase of response force and vigour. On the contrary, our data indicated a greater enhancement of response magnitude for the stronger flexor muscles. The ratio of force output to firing rate of corticomotoneurons has been found to be larger for extensors in comparison to flexors (Cheney & Fetz, 1980; Clough et al., 1968; de Noordhout et al., 1999; Fetz & Cheney, 1980; Palmer & Ashby, 1992). Given this suggestion that extensors have greater input from corticomotoneurons, flexors being more amenable to the effects of the LAS may suggest increased facilitation of reticulospinal pathways by the LAS; thereby indicating a subcortical mechanism for movement execution that is activated by intense acoustic stimuli and bypasses cortical circuits. However, if this were the case, a similar effect would be expected for our RT data, with shorter RT for muscles that have greater connectivity to reticulospinal pathways. Alternatively, the output to firing rate ratio of corticomotoneuronal cells may not necessarily implicate a greater role of these cells to the innervation of extensor muscles. The lower output to firing rate ratio for flexors may simply allow more precise modulations of force to be made, which is consistent with the fact that flexors are more often used in activities which involve fine motor control, e.g. precision grip (Oliveira et al., 2008; Quinn et al., 2018; Shim et al., 2007). Furthermore,in contrast to earlier reports of greater corticomotoneuronal contributions to extensors, recent neurophysiological reports have indicated greater functional corticospinal excitability for flexors compared to extensors (Godfrey et al., 2013; Koganemaru et al., 2010; McMillan et al., 2004; Park & Li, 2013; Vallence et al., 2012). The greater enhancement of response magnitude via the LAS for flexors which we observed here may thereby be a product of response force being correlated with M1 activity (Ashe, 1997). Subsequently, muscles with stronger corticospinal connectivity may be more sensitive to changes in response force induced by an intense acoustic stimulus. This

is in support of the differences we have observed between muscles in the facilitation of response magnitude but not latency in this task. This further emphasises the utility of considering dynamics of movement such as forcefulness and vigour, rather than just response latency alone. Consistent with this assertion, Vergilino-Perez et al. (2012) have reported a similar pattern of effects in gain and amplitude of saccades (see also Reuter et al., 2019), but not latency. Our maximum voluntary contraction procedure did indicate mechanical differences between the muscles we tested. It is therefore not possible to assert that the altered enhancement of response magnitude between muscles was solely due to innervation. However, our maximum voluntary contraction procedure likely limited the influence of mechanical differences between the muscles by setting the required force for each muscle at an equal proportion relative to the amount of force they were capable of exerting.

3.4.3 Multiplicative and additive models of neural activation accumulation

Examination of the enhancement of response magnitude induced by acoustic stimuli allows estimations of how neural activation may be introduced to motor program circuits. As in **Figure 3.2A**, an interaction of trial type and required force would be expected for peak force if a multiplying effect underlies LAS-induced neural activation. Given no such interaction of trial type and required force was observed, the data in Experiment 1 appeared to suggest an additive effect of the LAS. This was supported by the finding of higher ratios of peak force for low force movements compared to high force movements, for flexion responses. This initial suggestion of an additive effect of the LAS appeared to be impacted by muscle connectivity, with larger change in response magnitude observed for flexors.

In Experiment 2, we aimed to further examine the nature of the neural gain introduced by the LAS with increasing force which we observed in Experiment 1. Peak force was analysed to examine the additional neural activation that is introduced to motor program circuits by the LAS. Again, no interaction of trial type and required force was observed, failing to support the multiplicative model of neural activation. To examine the pattern of this activation, we analysed both ratios of probe to control trials and differences between probe and control trials for peak force. The data (**Figure 3.10**) fit the additive hypothesis (**Figure 3.3B**). As required force increased, peak force differences remained constant, whereas ratios decreased.

This suggests recruitment of motor neurons by the LAS remains constant as prepared force increases, and LAS-induced activation is additive to the signal prepared in motor program circuits. As LAS-evoked activity appears to be added at a constant rate and does not interact with the level of activation of the prepared response, this would suggest neural activity associated with temporal preparation converges with activation introduced to motor program circuits, potentially via functional connections between the auditory cortex and M1, or more directly from the brainstem to the spinal cord (Marinovic et al., 2013; Marinovic & Tresilian, 2016; Marinovic et al., 2014c). While it is important to note that this work represents examination of a theoretical model's fit to behavioural data, a body of work has suggested a linear increase of force output with neuronal firing (see Cheney & Fetz, 1980; Evarts 1968; Russo et al. 2018). It has previously been proposed that LAS induced activation is additive to the voluntary cortical activation that occurs during movement preparation, and that these two processes jointly contribute to the initiation command of the response (Marinovic et al., 2017; Maslovat et al., 2014). Here, similar to the compounded activation that occurs for response initiation, we have shown that activation introduced by the LAS may add to voluntary activation in motor program circuits, and together these can contribute to the amplitude of the accumulated signal that determines the magnitude of response execution. Furthermore, the current study provides a novel report of the additive nature of the activation that is injected into motor program circuits by the LAS.

3.5 Conclusions

In summary, our data indicate that the shortening of RT via intense acoustic stimuli in wrist muscles is not impacted by connections of the muscle to corticospinal or reticulospinal tracts. However, flexor muscles showed a greater enhancement of movement execution by the acoustic stimulus, possibly due to their stronger functional corticospinal connectivity in comparison to extensors. Over increasing force requirements, the benefit of the LAS on peak force did not change, and was therefore proportionally greater for low force movements. These changes in peak force induced by the acoustic stimulus fit our additive activation model and indicate startle-related activation does not interact with the voluntary activation in motor program circuits, but rather adds to this voluntary drive at a constant rate. The

changes in force for the wrist flexor whichsupport M1 involvement in the early triggering of motor actions via intense acoustic stimuli (see Marinovic & Tresilian, 2016), and indicate the forcefulness and vigour of responses elicited by intense sensory stimuli may be enhanced differentially depending on the type of movement prepared. These findings may have implications for potential therapeutic applications of the StartReact effect. For example, prepared motor actions which we have shown to receive more benefit from the acoustic stimulus may be the more promising targets for rehabilitative protocols in conjunction with intense sensory stimuli in order to retrain motor control in neurological conditions in which movement execution is impaired (Honeycutt et al., 2015; Marinovic et al., 2016).

3.6 Appendix A

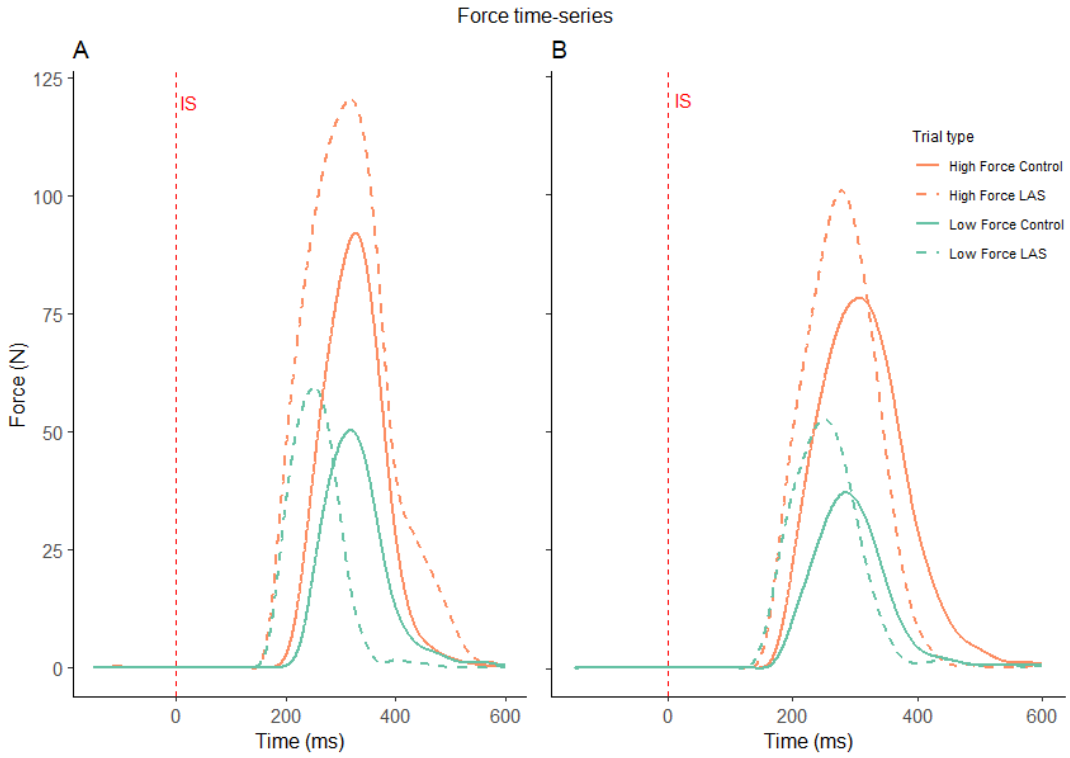


Figure 3.11. Force plotted over the full time-series across Force Level (Low/High) and Trial Type (Control/LAS) for the median trial of each condition for a representative participant in Experiment 1. **A)**. = Flexion trials, **B)**. = Extension trials.

CHAPTER FOUR: MOTOR PATHWAYS IPSILATERAL TO THE MOVING LIMB CAN CONTRIBUTE TO THE FACILITATION OF MOTOR OUTPUT BY LOUD ACOUSTIC STIMULI

4.0 Abstract

When intense sound is presented during light muscle contraction, inhibition of the corticospinal tract is observed. During action preparation, this effect is reversed, with sound resulting in excitation of the corticospinal tract. We investigated how the combined maintenance of a muscle contraction during preparation for a ballistic action impacts the magnitude of the facilitation of motor output by a loud acoustic stimulus (LAS) – a phenomenon known as the StartReact effect. Participants executed ballistic wrist flexion movements and a LAS was presented simultaneously with the imperative signal in a subset of trials. We examined whether the force level or muscle used to maintain a contraction during preparation for the ballistic response impacted reaction time and/or the force of movements triggered by the LAS. These contractions were sustained either ipsilaterally or contralaterally to the ballistic response. The magnitude of facilitation by the LAS was greatest when low force flexion contractions were maintained in the limb contralateral to the ballistic response during preparation. There was little change in facilitation when contractions recruited the contralateral extensor muscle, or when they were sustained in the same limb that executed the ballistic response. We conclude that a larger network of neurons which may be engaged by a contralateral sustained contraction prior to initiation may be recruited by the LAS, further contributing to the motor output of the response. These findings may be particularly applicable in stroke rehabilitation where engagement of the contralesional side may increase the benefits of a LAS to the functional recovery of movement.

4.1 Introduction

The presentation of a loud acoustic stimulus (LAS) during movement preparation can affect the time of movement initiation as well as movement vigour. Actions that are sufficiently prepared at the time a LAS is delivered are involuntarily triggered at much shorter latencies, and are executed with greater force and vigour, than is typically produced voluntarily (Anzak, Tan, Pogosyan, & Brown, 2011; Anzak, Tan, Pogosyan, Djamshidian, et al., 2011; Marinovic et al., 2015; McInnes, Corti, et al., 2020; Valls-Solé et al., 1999). This is referred to as the StartReact effect (Valls-Solé et al., 1999). However, the effects of a LAS on motor circuits are contingent on the state of preparation for action at the time the stimulus is presented. For example, when the task is simply to maintain a light muscle contraction at a stable level, loud acoustic stimuli suppress the excitability of corticospinal pathways (Fisher et al., 2004; Furubayashi et al., 2000; Kuhn et al., 2004). In contrast, during a state of imminent preparation for a discrete action (i.e. the context in which the StartReact effect occurs), corticospinal excitability is increased shortly after the presentation of a LAS, which may provide a neurophysiological means by which motor output can be facilitated in the StartReact effect (Marinovic, Tresilian, et al., 2014). These observations highlight that the effects of a LAS on motor pathways are not fixed, but depend on the state of the motor system. However, the modulation of corticospinal excitability is further nuanced in that inhibition after acoustic stimulation is only observed when there is weak background muscle activity. During maintenance of a slightly stronger contraction, at 10% of maximum voluntary contraction (MVC), suppression of the corticospinal tract is less evident (Chen et al., 2016). This may be due to voluntary activation of the primary motor cortex (M1) suppressing intracortical inhibitory circuits as the amount of contraction force is increased (Roshan et al., 2003).

Furthermore, it is unclear how the maintenance of a muscle contraction during preparation for action may impact the StartReact effect. The potential observations which may be made under these conditions are currently uncertain as the effects of acoustic stimulation on the corticospinal tract during light muscle contraction are opposite (Fisher et al., 2004; Furubayashi et al., 2000; Kuhn et al., 2004) to those observed during action preparation (Marinovic, Tresilian, et al., 2014). Here, we investigated how different types of muscle contractions held during

a preparatory foreperiod may impact the early triggering of motor actions and the enhancement of response vigour when the motor response is triggered by a LAS. If the combined maintenance of a muscle contraction during preparation for a subsequent action results in a decreased StartReact effect (i.e. reduced shortening of RT, reduced enhancement of response force/vigour), this would suggest that the contraction induces a suppressive effect of acoustic stimulation on motor pathways. In accordance with observations that the inhibitory LAS effect depends on the amount of background muscle activity, any putative reduction of the StartReact effect would be expected to be greatest at low contraction force levels. Alternatively, stable background contractions may increase preparatory neural activity prior to the discrete action and subsequently magnify the StartReact effect. During unilateral muscle contraction, excitability of the M1 ipsilateral to the contracting muscle increases as the amount of force is increased (Chen et al., 2019; Perez & Cohen, 2008; Shibuya et al., 2014; Stinear et al., 2001; Uematsu et al., 2010). In addition, regional cerebral blood flow in ipsilateral M1 decreases at light muscle contractions (5% of MVC) and increases in proportion to the strength of the muscle contraction from 10% - 60% of MVC (Dettmers et al., 1996). Therefore, contraction of a muscle during preparation of a contralateral response may result in an enhancement of the StartReact effect that is proportional to the strength of the contraction maintained during preparation. The StartReact effect has also been proposed as a tool to aid in rehabilitation in neurological conditions such as stroke, with startling sensory stimuli capable of reducing movement initiation-related deficits (Choudhury et al., 2019; Coppens et al., 2018; Honeycutt et al., 2015; Honeycutt & Perreault, 2012; Jankelowitz & Colebatch, 2004; Marinovic et al., 2016; Rahimi & Honeycutt, 2020b). Given this, and the fact that stroke survivors typically experience exaggerated movement impairment on one side of the body (Zemke et al., 2003), maintenance of a contraction contralateral to the impaired side may be particularly beneficial for people with stroke if it enhances the benefits derived from intense sensory stimuli. Therefore, we examined how the type of isometric contraction maintained during preparation for a ballistic response impacts the facilitation of movement initiation and execution by a LAS, in both bilateral and unilateral tasks.

4.2 Method – Outline

In experiment one we investigated how the magnitude of the StartReact effect may be impacted by the level force maintained in a sustained contraction contralaterally to the limb engaged in preparation (bilateral task). In experiment two, we examined whether this effect can be observed when the contraction is maintained in the same limb that is engaged in preparation (unilateral task). In the unilateral task we also investigated whether the muscle used to maintain the contraction impacts the magnitude of facilitation observed. In experiment three, we examined whether engagement of different muscles during the sustained contraction impacts the magnitude of facilitation observed in the bilateral task.

4.3 Method – Experiment One

4.3.1 Participants

Thirty participants were recruited for experiment one (20 female, mean age = 20.33, SD = 2.25). Participants in all experiments were self-reportedly right-handed, with normal or corrected-to-normal vision, and no apparent or known auditory impairments, neurological conditions, or injuries which may have affected their performance in the experiment. The study was approved by Curtin University's local human research ethics committee and all participants provided informed, written consent.

4.3.2 Procedures

Participants were seated on a height-adjustable chair with each hand and forearm secured in custom-made manipulanda, each housing a six degree of freedom force/torque sensor (JR3 45E15A-I63-A 400N60S, Woodland, CA; see de Rugy et al. (2012). The forearm was secured in a semi-supinated position with the palms facing inward, and elbows flexed at an approximately 90° angle. Both hands and forearms of each participant were secured snugly in the device to prevent time delays between muscle activation and the recording of force. The manipulanda are pictured in **Figure 4.1**. Participants were seated at a distance of approximately 0.8 m in front of a 24.5 inch monitor (Asus ROG Swift PG258Q, 120Hz refresh rate, 1920x1080 resolution) which presented visual stimuli during the task. Both visual

and auditory stimuli were presented using Psychtoolbox (v3.0.11) running in Matlab 2015b.

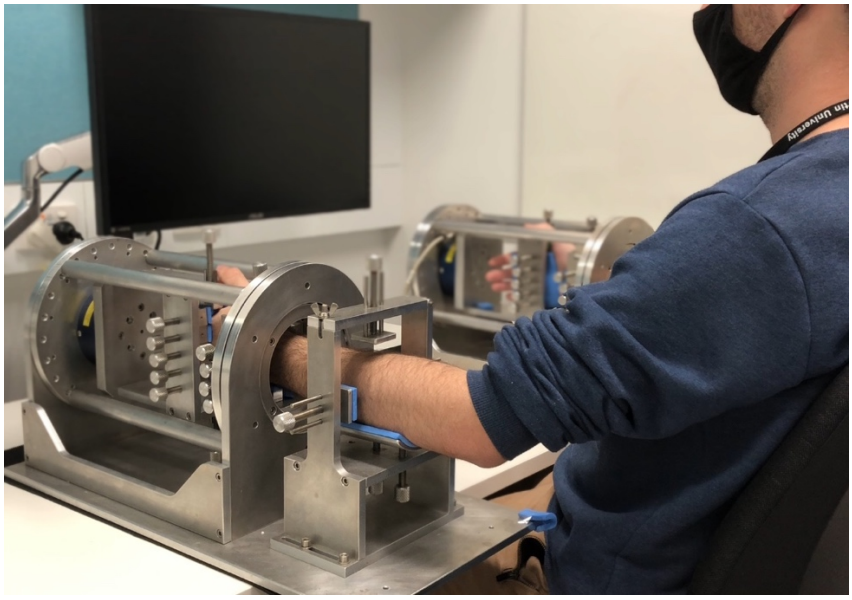


Figure 4.1. Participant with hands and forearms secured in the manipulanda.

Prior to the experimental trials, each participant completed a MVC procedure of wrist flexion in both the left and right hand (see Selvanayagam et al., 2016). In this procedure, force feedback was provided to subjects via a cursor in two-dimensional space (x = flexion/extension, y = abduction/adduction) such that 10 Newtons (N) was required to move the cursor 32 pixels on the computer monitor. In experiment one, subjects produced three isometric MVCs for three seconds toward a target in each direction, and the peak force (Newtons, N) was measured. The mean peak force of the three contractions for each hand was recorded as the MVC for the relevant hand. These data were used to determine the level of force required to reach targets during the experiment.

The experimental task of experiment one required participants to perform a discrete ballistic wrist flexion movement of the right hand in response to an imperative cue. There were four contraction conditions during the experiment and these were each randomised across participants to one of four blocks of trials during the experiment. The contraction condition of the block determined the amount of force which was required to be sustained with the left hand during preparation of the right hand response. These force levels were 0%, 5%, 10%, and 20% of the

participant's left wrist flexor's MVC. In one block, referred to as the "no contraction" condition, participants kept their left hand relaxed while they prepared and executed a ballistic flexion movement of the right hand, aiming to produce a brief force pulse of 20% of the right wrist flexor's MVC. The 20% of MVC flexion ballistic response was chosen as we have previously shown that this muscle and force level is particularly prone to the beneficial effects of a LAS on motor output (McInnes et al., 2020). In the three remaining contraction conditions, the left hand maintained an isometric flexion contraction at either 5%, 10%, or 20% of the left wrist flexor's MVC, during preparation for the ballistic right-hand response. See **Figure 4.2** for the sequence of events during the experiment. Prior to experimental trials, participants completed a block of 12 practice trials, which consisted of three trials of each condition in the experiment. Participants were given verbal feedback regarding their performance, and practice trials were repeated until participants were able to accurately initiate movements within 250 ms after the presentation of the imperative stimulus (IS). One-hundred and sixty-four experimental trials were then completed, split into four blocks of 41 trials each.

Each trial began with the word "relax" presented on-screen, indicating for the participant to keep their hands relaxed and stationary for the start of the trial. Next, a cursor that responded to forces with the left hand, and a contraction target, consisting of two arcs on the right side of the screen were presented. Participants moved their left hand so that the cursor was positioned within the contraction target, and held their hand in this position for the duration of the trial. The amount of force required to reach the contraction target changed each block, depending on the contraction condition (0%, 5%, 10%, or 20% of MVC contraction level). Trials would not proceed until the participant had maintained a contraction of the appropriate force level within a tolerance of ±7.5% of the target, to accommodate minor deviations from the contraction force. Once the participant had maintained this contraction for two seconds, a cursor which could be controlled by the right hand and a warning cue appeared, indicating the impending presentation of the IS. This warning cue appeared as a red circle on the left side of the screen. Participants were instructed to prepare to respond with the right hand during this period. After 500 ms, the contraction target and cursor indicating the position of the left hand was removed from the screen, so that participants would be encouraged to direct their attention to

the warning cue and impending IS and prepare responses appropriately. If the participant unknowingly moved their left hand outside of the contraction target during this preparatory foreperiod, the left hand cursor and contraction target would reappear on screen, requiring the participant to return their left hand within the contraction target before the trial would proceed. The warning cue was presented for two seconds (± 200 ms jitter), after which the IS, a yellow circle in place of the warning cue, was presented. Twenty percent of trials occurred as probe trials in which a LAS was presented as an accessory stimulus simultaneously with the IS. The order of trials was pseudo-randomised so that the LAS would not be presented in two consecutive trials. When the IS was presented, participants reacted by moving their right hand in a ballistic wrist flexion. They were instructed to aim to touch the target and stop the cursor movement once the target had been reached. To encourage participants to respond with the appropriate amount of force required to reach the target, the yellow IS target flashed green when intersected with the cursor. At the end of the trial, feedback regarding RT was presented to encourage quick responses throughout the experiment. In probe trials, this feedback was not presented.

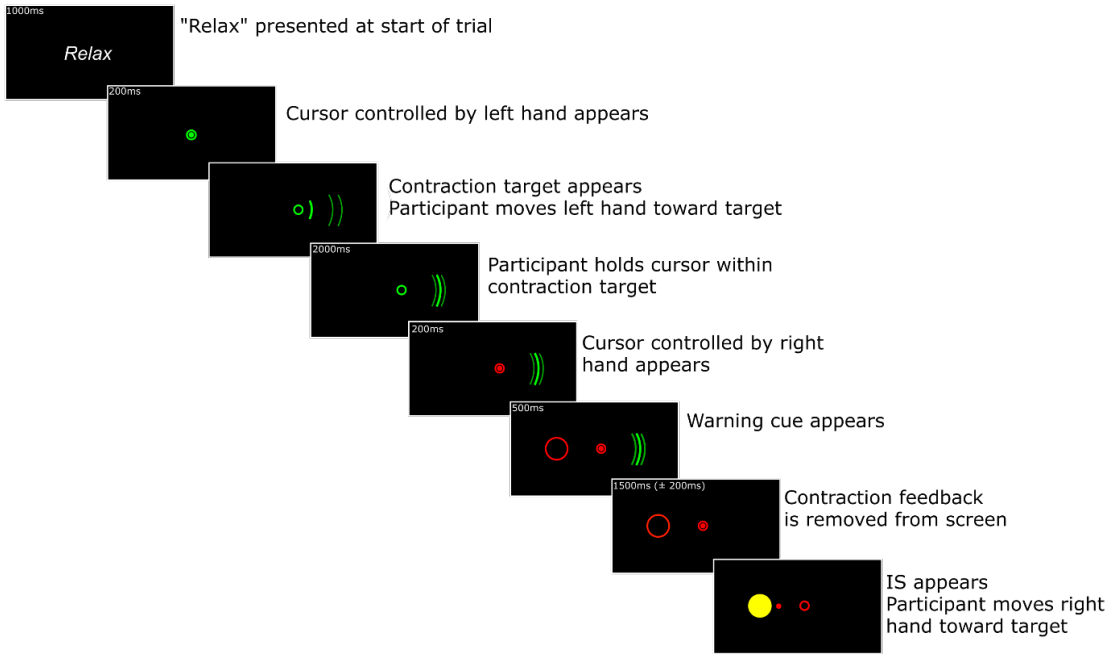


Figure 4.2. Sequence of events during the experiment requiring a left-hand contraction during preparation for a ballistic right-hand response to the IS.

4.3.3 Loud Acoustic Stimulus

In probe trials, a LAS generated by the onboard audio of the computer used to run experiments was presented through high-fidelity stereophonic active noise cancelling headphones (Bose QC25). The peak amplitude of the stimulus was measured at 105dBA using a Bruel and Kjaer sound level meter (Type 2205, A weighted; Brüel & Kjaer Sound and Vibration Measurement, Naerum, Denmark). The LAS was presented for a duration of 50ms and with a rise and fall time of less than 1.5ms.

4.3.4 Statistical analyses

For each trial, the time series of force data were collected from the load cell with a sampling rate of 2 kHz using a National Instruments data acquisition device (NI USB-6229). Movement onset was estimated from the force time series data using Teasdale et al.'s (1993) algorithm, and RT was determined by subtracting the time of IS presentation from the time of movement onset, expressed in milliseconds (ms). The vigour of the ballistic response was determined through the measurement of the derivative of the torque data with respect to time, referred to as the rate of force development (Newtons per second; N/s). Peak rate of force development and peak force were determined as the maximum value of peak rate of force development and peak force over the course of a trial. Statistical analyses were run using R software (*v3.6.0*; R Core Team, 2019).

Prior to analysis, trials with a RT < 60 ms or > 1000 ms were removed on the basis that these were error responses made as a result of premature response initiation due to anticipation of the IS, or delayed responses due to insufficient movement preparation (Whelan, 2008). This resulted in the exclusion of 100 trials (2.03% of all trials) in experiment one. We further used cumulative distribution functions (CDFs) to analyse mean RTs at each percentile of the entire RT distribution to assess whether preparatory contraction conditions resulted in movements being more or less prone to triggering delays. Examination of entire RT distributions between conditions allows an examination of how different conditions are prone to effects such as triggering delays, facilitation, or RT floor effects. We have outlined the method of analysing data using CDFs in a StartReact context in more detail previously (McInnes et al., 2021).

We used the *lmer* function from the *lmerTest* package (*v3.1*; Kuznetsova, Brockhoff, & Christensen, 2017) to conduct a series of linear mixed-effects models. All trials were fed into the linear models with participants set as a random factor. In experiment one, trial type (control, LAS) and contraction level (no contraction, 5%, 10%, 20% contractions) were fixed factors in the model. To determine the extent of facilitation that occurred for RT, peak force, and peak rate of force development that occurred as a result of the LAS, we calculated differences of RT and ratios of peak force and peak rate of force development. For RT differences, the median RT of control trials was calculated for each contraction condition, and each LAS trial was subtracted from the median of control trials to determine a RT difference for each probe trial. A similar procedure was conducted with peak force and peak rate of force development by dividing probe trials by the median of control trials. For all models, degrees of freedom were approximated using the Kenward-Roger procedure. R^2 values, calculated using the *r2beta* function (r2glmm package, v0.1.2) are also reported to estimate effect sizes of all main effects and interactions tested using the linear mixed models. Post-hoc tests were conducted for significant main effects and interactions of the linear mixed models using the *emmeans* function (emmeans package, v1.3.0) with the correction of multiple comparisons using the false discovery rate method (Benjamini & Hochberg, 1995).

4.4 Results – Experiment One

4.4.1 Shortening of response initiation

RT was significantly shortened in probe trials (M = 158.69 ms, SD = 85.37) in comparison to control trials (M = 221.74 ms, SD = 99.86), with a statistically significant main effect of trial type for RT, $F_{(1, 4783.1)} = 639.84$, $p < .001$, $R^2 = .118$. The main effect of contraction level was also statistically significant for RT, $F_{(3, 4783.2)} = 9.50$, $p < .001$, $R^2 = .006$. The interaction of trial type with contraction level was not statistically significant, $F_{(3, 4783.1)} = 0.22$, $p = .886$, $R^2 = .000$. Analysis of the difference in RT between probe trials and the median of control trials for each condition did not indicate a statistically significant main effect of contraction level, $F_{(3, 887.49)} = 0.65$, $p = .583$, $R^2 = .002$. Mean RTs across each condition are shown in **Figure 4.3**.

We further examined each participant's mean RT at each percentile, across the four contraction level conditions. A linear mixed-effects model indicated a significant main effect of contraction level, $F_{(3, 1131)} = 24.10$, $p < .001$, $R^2 = .060$. In comparison to the no contraction condition ($M = 206.21$ ms, $SD = 70.40$), the 10% contraction condition showed significantly shorter RTs across the CDF curve ($M = 198.76$ ms, $SD = 57.25$; $p = .003$), while the 20% contraction condition had significantly longer RTs ($M = 217.18$ ms, $SD = 73.51$; $p < .001$). RTs across the CDF curve for the 5% contraction condition ($M = 201.87$ ms, $SD = 65.48$) were not significantly different from the no contraction condition ($p = .083$), nor the 10% contraction condition ($p = .18$). The interaction of percentile with contraction level was not statistically significant, $F_{(27, 1131)} = 1.28$, $p = .153$, $R^2 = .03$.

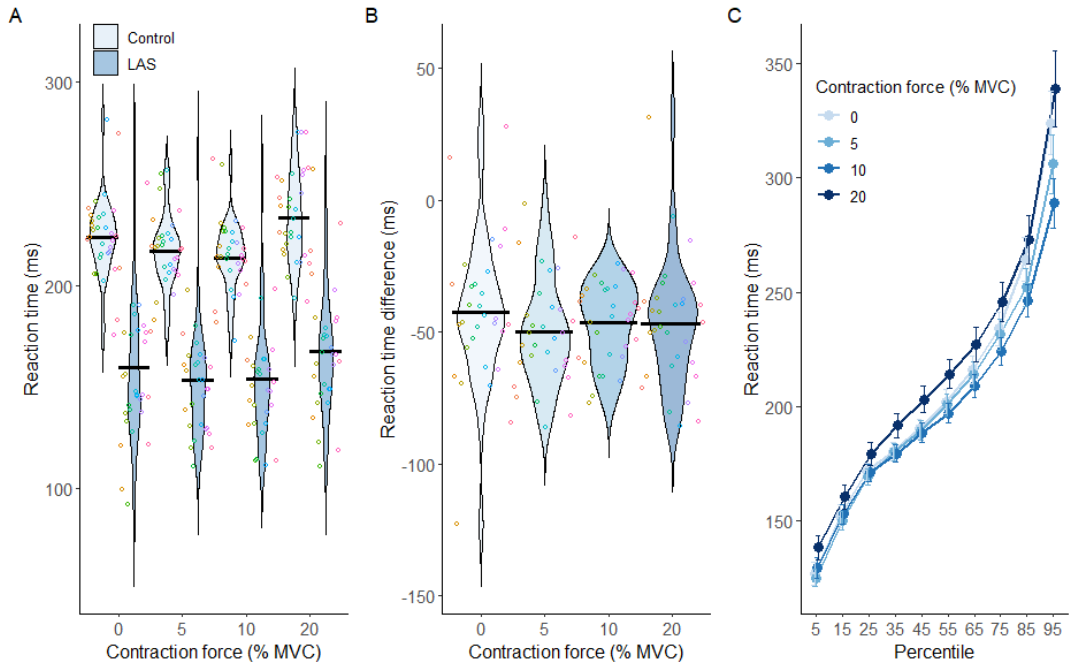


Figure 4.3. **A)**. Mean reaction time over control and probe trials for each contraction level. **B)**. Mean of the difference in RT between all probe trials and the median of control trials for each condition. **C)**. Mean RT across each participant for each percentile of RT. Coloured points represent subject averages.

4.4.2 Enhancement of peak force and vigour

Peak force showed an enhancement in probe trials (M = 36 N, SD = 16.60) compared to control trials (M = 31.95 N, SD = 13.31), as shown by the main effect of trial type which was statistically significant, $F_{(1, 4783)} = 127.09$, $p < .001$, $R^2 = .026$. The main effect of contraction level was also statistically significant, $F_{(3, 4783)} = 10.59$, $p < .001$, $R^2 = .007$. Furthermore, the interaction of trial type and contraction level was statistically significant for peak force, $F_{(3, 4783)} = 6.83$, $p < .001$, $R^2 = .004$. Analysis of the ratios of peak force showed a statistically significant main effect of contraction level, $F_{(3, 908.26)} = 6.35$, $p < .001$, $R^2 = .021$. Post hoc tests indicated that in comparison to the no contraction condition (M = 1.11, SD = .39), ratios of peak force between control trials and probe trials were significantly greater in the 10% contraction condition (M = 1.19, SD = .33; p = .016), but not in the 5% (M = 1.17, SD = .40; p = .059) or 20% contraction conditions (M = 1.07, SD = .36; p = .417).

Similarly to peak force, our analysis showed a statistically significant main effect of trial type for peak rate of force development, $F_{(1, 4783)} = 252.01$, $p < .001$, $R^2 = .050$, with greater peak rate of force development observed on average for probe trials (M = 492.13 N/s, SD = 251.70) in comparison to control trials (M = 410.50 N/s, SD = 185.05). The main effect of contraction level, $F_{(3, 4783)} = 4.56$, $p = .003$, $R^2 = .003$, and the interaction of trial type with contraction level, $F_{(3, 4783)} = 5.43$, $p = .001$, $R^2 = .003$, were statistically significant. The main effect of contraction level for ratios of peak rate of force development was also statistically significant, $F_{(3, 908.25)} = 5.46$, $p = .001$, $R^2 = .018$. In comparison to the no contraction condition (M = 1.17, SD = .52), post hoc tests indicated ratios of peak rate of force development were significantly greater for the 5% (M = 1.25, SD = .44; p = .042) and 10% (M = 1.29, SD = .42; p = .006) contraction conditions but not for the 20% contraction condition (M = 1.16, SD = .45; p = .817). The mean peak force and vigour for each experimental condition are presented in **Figure 4.4**.

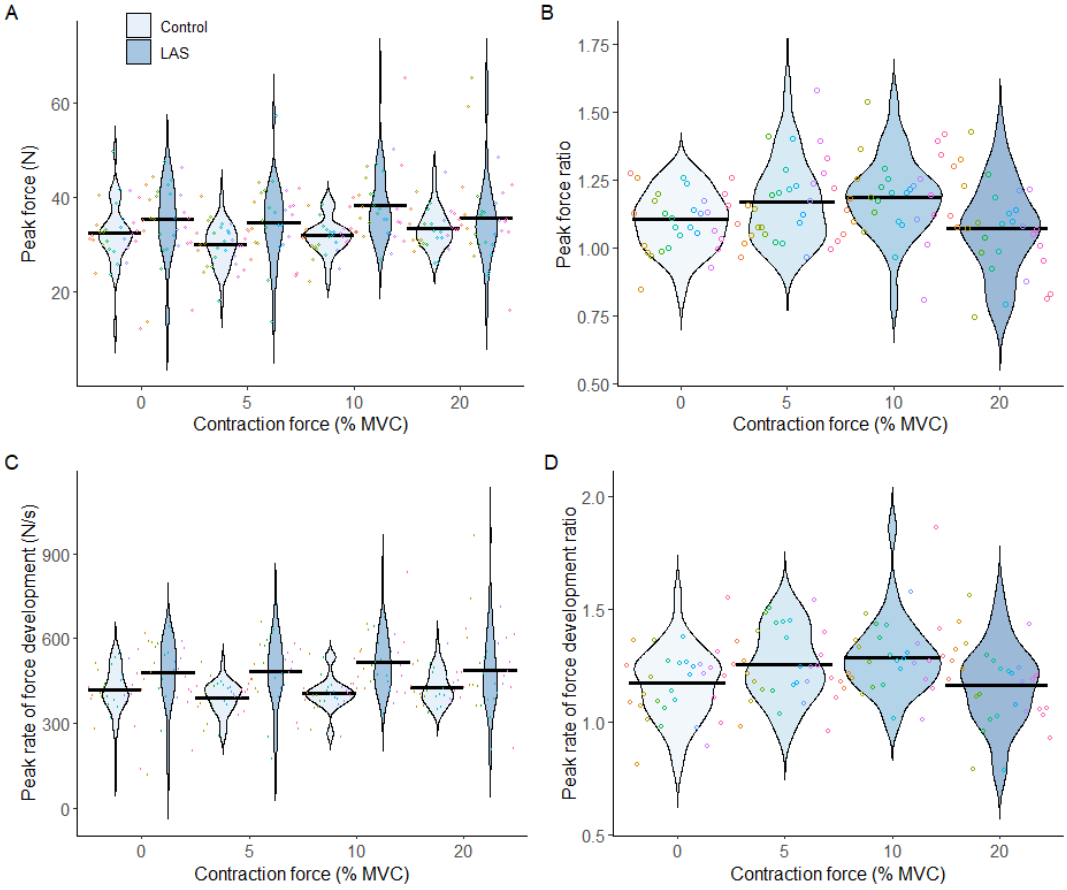


Figure 4.4. **A)**. Mean peak force for control and probe trials over each contraction level. **B)**. Mean peak force ratios over each contraction level. **C)**. Mean peak rate of force development over control and probe trials at each contraction level. **D)**. Mean peak rate of force development ratios for each contraction level. Coloured points represent subject averages.

4.5 Method – Experiment Two

4.5.1 Participants

A second sample of 25 participants (16 female; mean age = 20.28, SD = 1.65) was recruited for experiment two. The same recruitment criteria as experiment one was used for Experiment two.

4.5.2 Procedures

In experiment two, we used a forewarned RT task similar to experiment one, in a unilateral task. In this task, isometric contractions were maintained during preparation for the response to the IS with the same (right) hand. Responses to the IS were again ballistic flexion movements of the right hand at 20% of MVC of the right wrist flexor. The right (responding) hand either remained relaxed during preparation for the response to the IS, or maintained a contraction in either flexion or extension, at 10% of the relevant muscle's MVC. Both flexion and extension contractions were examined as these muscles have been suggested to differ in the strength of their efferent contributions from the corticospinal and reticulospinal tracts (Cheney & Fetz, 1980; Clough et al., 1968; de Noordhout et al., 1999; Fetz & Cheney, 1980; Godfrey et al., 2013; Koganemaru et al., 2010; McInnes et al., 2020; McMillan et al., 2004; Palmer & Ashby, 1992; Park & Li, 2013; Quinn et al., 2018; Vallence et al., 2012). As such, this would demonstrate whether the effects observed are muscle-specific. Contractions were maintained during preparation at 10% of the muscle's MVC as this force level appeared to provide the most benefit in experiment one. The ballistic response always required responsive activation of the flexor muscle at 20% of MVC. For example, during the isometric flexion contraction, the target was set so that from the 10% of MVC position, an additional force of 20% of MVC would be required to meet the target (i.e. the final position of the ballistic response was 30% of flexion MVC). During the isometric extension contraction, the ballistic flexion response of 20% of MVC was required, measured from the point at which the extensor muscle was at rest (i.e. the final position required movement away from 10% extension MVC and toward 20% flexion MVC). We determined this to be the most feasibly equivalent between the flexion and extension contraction conditions of the unilateral task in terms of the amount of activation which would be required to be produced by motor circuits in order to drive the response. Trials were again excluded from analysis on the basis of 60 ms < RT < 1000 ms. This resulted in the exclusion of 98 trials from experiment two in total (1.96% of all trials).

4.6 Results - Experiment Two

4.6.1 Shortening of response initiation

RTs were significantly shorter in probe trials (M = 159.98 ms, SD = 101.61) in comparison to control trials (M = 238.25 ms, SD = 140.42), with a statistically

significant main effect of trial type for RT, $F_{(1, 3648)} = 406.13$, $p < .001$, $R^2 = .100$. The main effect of contraction type was also statistically significant for RT, $F_{(2, 3648.1)} = 10.97$, $p < .001$, $R^2 = .006$. Responses on average showed shorter RTs without the preparatory contraction ($M = 209.65$ ms, $SD = 107.1$) in comparison to when contractions were maintained in both flexion ($M = 223.97$ ms, $SD = 123.41$; $p = .012$) and extension ($M = 233.58$ ms, $SD = 111.75$; $p < .001$) during preparation. Post-hoc tests also indicated the extension contraction condition showed significantly longer RTs than the flexion contraction condition ($p = .043$). The interaction of trial type with contraction type was not statistically significant, $F_{(2, 3648)} = 0.21$, $p = .811$, $R^2 = .002$. Analysis of the difference in RT between probe trials and the median of control trials for each contraction condition did not indicate a statistically significant main effect of contraction type, $F_{(2, 711.16)} = 1.23$, $p = .293$, $R^2 = .003$.

Each participant's mean RT at each percentile contraction conditions was also analysed using a linear mixed-effects model. Analysis indicated a significant main effect of contraction type, $F_{(2, 696)} = 25.38$, $p < .001$, $R^2 = .068$. In comparison to the no contraction condition ($M = 204.08$ ms, $SD = 89.27$), the flexion contraction condition showed significantly longer RTs across the CDF curve ($M = 219.82$ ms, $SD = 113.3$; $p < .001$), as did the extension contraction condition ($M = 230.83$ ms, $SD = 107.94$; $p < .001$). RTs across the CDF curve were significantly longer for the extension contraction condition in comparison to the flexion contraction condition ($p = .003$). The interaction of percentile with contraction type was not statistically significant, $F_{(18, 696)} = 1.17$, $p = .282$, $R^2 = .029$. **Figure 4.5** shows the mean RTs for each condition along with mean RTs at each percentile within the CDF.

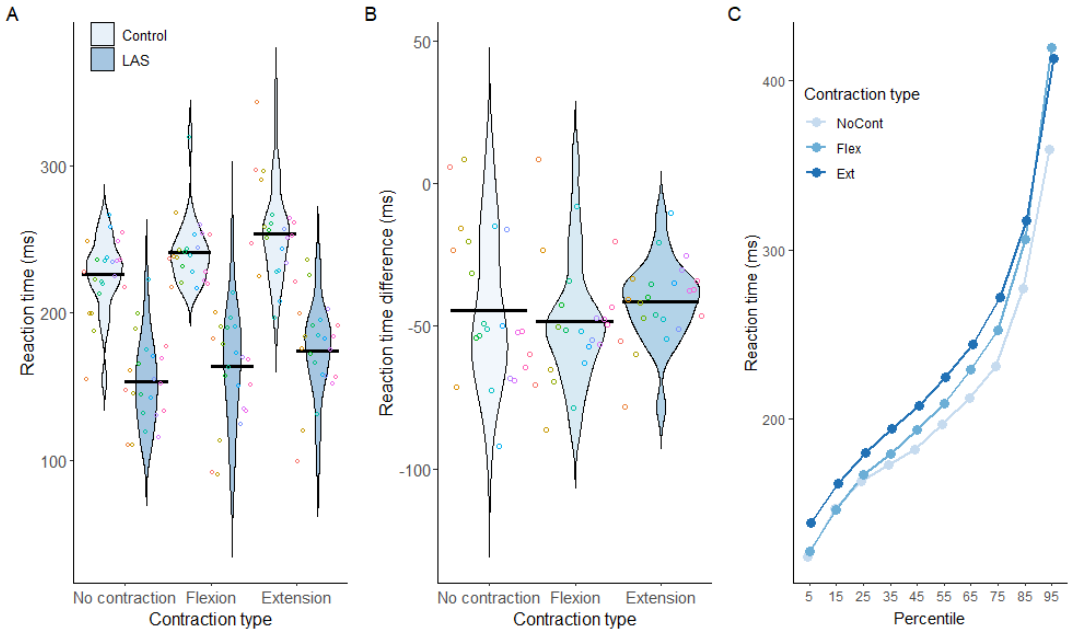


Figure 4.5. **A)**. Mean reaction time over control and probe trials for each contraction type. **B)**. Mean of the difference in RT between all probe trials and the median of control trials for each condition. **C)**. Mean RT across each participant for each percentile of RT. Coloured points represent subject averages.

4.6.2 Enhancement of response force and vigour

Peak force showed an enhancement in probe trials (M = 44.34 N, SD = 18.33) compared to control trials (M = 39.24 N, SD = 16.52), as shown by a statistically significant main effect of trial type, $F_{(1, 3648)} = 111.54$, $p < .001$, $R^2 = .030$. The main effect of contraction type was also statistically significant, $F_{(2, 3648)} = 66.22$, $p < .001$, $R^2 = .035$. The flexion (M = 37.46 N, SD = 12.67) contraction condition showed significantly lower peak force on average in comparison to both the no contraction (M = 37.46 N, SD = 12.69; p = < .001) and extension (M = 43.84 N, SD = 15.38; p = < .001) contraction conditions. Average peak force in the extension contraction condition was also significantly greater than the no contraction condition (p = < .001). The interaction of trial type with contraction type was not statistically significant, $F_{(2, 3648)} = 0.23$, $p = .793$, $R^2 = .000$. The benefit of the acoustic stimulus on peak force did not appear to differ as a function of contraction type, as analysis of the ratios of peak force indicated the main effect of contraction type was not statistically significant, $F_{(2, 711.14)} = 1.02$, $p = .361$, $R^2 = .003$.

Peak rate of force development was also increased by the LAS (M = 567.93 N/s, SD = 288.57) in comparison to control trials (M = 448.40 N/s, SD = 218.79), as indicated by a main effect of trial type, $F_{(1, 3648)} = 313.27$, $p < .001$, $R^2 = .079$. The main effect of contraction type was also statistically significant, $F_{(2, 3648)} = 39.12$, $p < .001$, $R^2 = .021$, however, the interaction of trial type with contraction type, $F_{(2, 3648)} = 1.30$, $p = .273$, $R^2 = .001$, was not significant. The main effect of contraction type for ratios of peak rate of force development was statistically significant, $F_{(2, 711.15)} = 5.83$, $p = .003$, $R^2 = .016$. Post hoc tests indicated peak rate of force development ratios in the flexion (M = 1.40, SD = 0.54; $p = .002$), but not the extension (M = 1.33, SD = 0.54; $p = .088$) contraction condition, were significantly greater than the no contraction condition (M = 1.26, SD = 0.49). The flexion contraction condition also showed significantly greater peak rate of force development ratios in comparison to the extension contraction condition ($p = .088$). Ratios and means of peak force and peak rate of force development are presented in **Figure 4.6**.

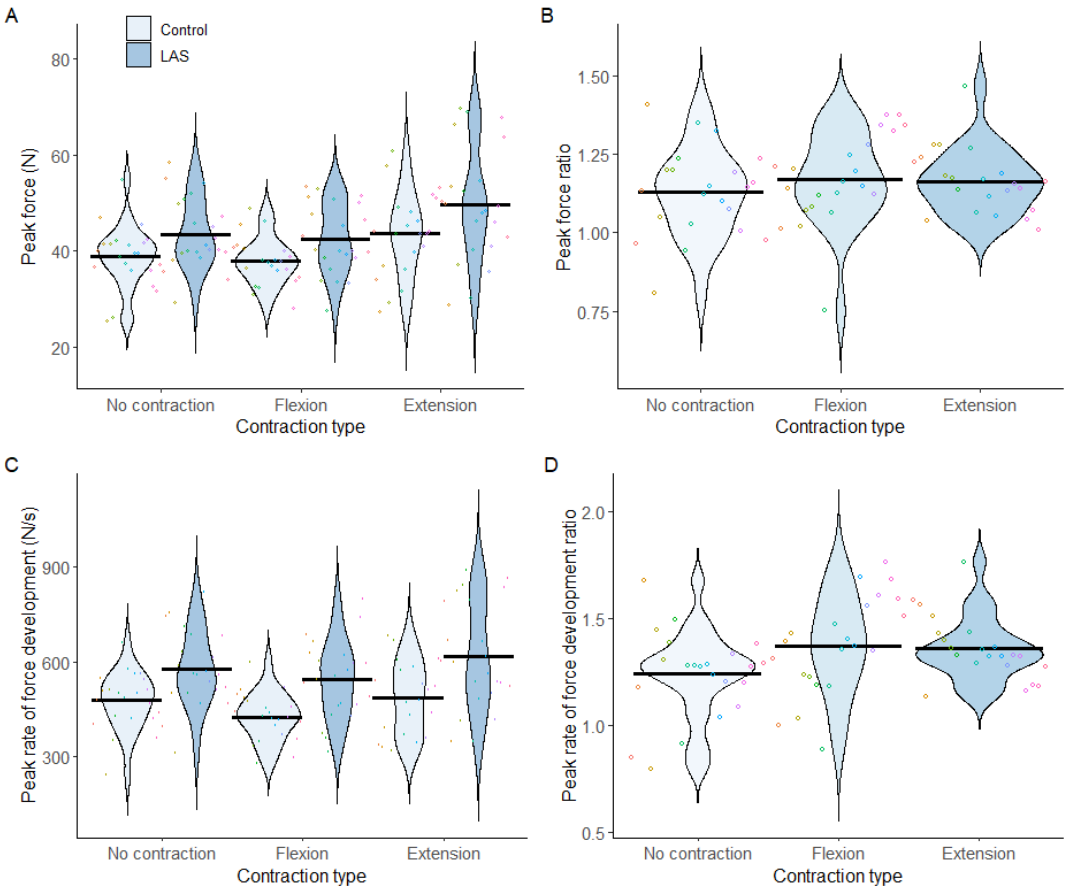


Figure 4.6. A). Mean peak force for control and probe trials for each contraction type. **B)**. Mean peak force ratios for each contraction type. **C)**. Mean peak rate of force development over control and probe trials at each contraction type. **D)**. Mean peak rate of force development ratios for each contraction type. Coloured points represent subject averages.

4.7 Method – Experiment Three

4.7.1 Participants

In Experiment three, a sample of 29 volunteers (different from those recruited in experiment one and two) were recruited (23 female, mean age = 20.72, SD = 3.18). Participants were again required to be right-handed and free from any auditory impairments, neurological conditions, or injuries which may have impacted their performance in the experiment.

4.7.2 Procedures

Experiment three followed similar procedures to those of the bilateral task of experiment one, except contractions were made with the left hand in both directions of flexion and extension during preparation in an anticipatory timing task requiring a response from the right hand. The use of both flexion and extension contractions during preparation in the bimanual task again allowed us to examine whether the effects observed in experiment one were muscle specific. Alternatively, effects may be movement specific. For example, modulations of corticospinal excitability in M1 during ipsilateral movement have been suggested to be more strongly associated with whether the direction of movement is toward or away from the midline of the body, rather than the specific agonist muscle used in the movement (Duque et al., 2005). An anticipatory timing task was used as the effects of the LAS on peak force and vigour were larger than those observed for the latency of movement onset in experiment one. Therefore, an anticipatory timing protocol would allow us to examine whether the effects of the LAS on movement execution become more or less pronounced when the stimulation is delivered temporally more closely to movement onset. We presented contraction feedback as an outer ring of a circle, with the contraction target at the 12 o'clock position of the circle. Rather than the

presentation of a WS and IS, as in the previous experiments, the centre of the contraction feedback would fill in a clockwise motion, and participants were instructed to initiate their movement in synchrony with the time the circle was completely filled and the clock hand intersected at the 12 o'clock position. Contractions during preparation were set at one required force level – 10% of MVC, as the facilitation of motor output was greatest at this contraction force level in experiment one. As in the previous experiments, responses were made with the right hand at 20% of MVC. The LAS was presented in synchrony with the expected time of movement onset and, therefore, we did not anticipate a main effect of LAS on the temporal error of movement onset. In experiment three, responses to the IS with temporal error < -150 ms or > 150 ms were excluded from analysis. This resulted in the exclusion of 519 trials in experiment three (12.14% of all trials).

4.8 Results - Experiment Three

4.8.1 Temporal error of movement onset

Mean temporal error of movement onset was earliest in the no contraction condition (M = -24.83 ms, SD = 51.13), followed by that of the extension contraction (M = -16.68 ms, SD = 50.50) and flexion contraction (M = -10.77 ms, SD = 50.18) conditions. Our analysis of temporal error of movement onset data in experiment three indicated a statistically significant main effect of contraction type (no contraction/flexion/extension), $F_{(2, 3733.4)} = 22.76$, $p < .001$, $R^2 = .012$. The time of movement initiation in the no contraction condition was significantly earlier than both the flexion ($p < .001$) and extension ($p < .001$) contraction conditions. The difference in temporal error between the flexion and extension contraction conditions was also statistically significant ($p = .006$). As expected given the timing of LAS presentation, the main effect of trial type (LAS/control) was not statistically significant, $F_{(1, 3732.5)} = 2.59$, $p = .107$, $R^2 = .001$, nor was the interaction of trial type with contraction type, $F_{(2, 3732.4)} = .02$, $p = .977$, $R^2 = .002$. Mean temporal error for each condition is shown in **Figure 4.7**.

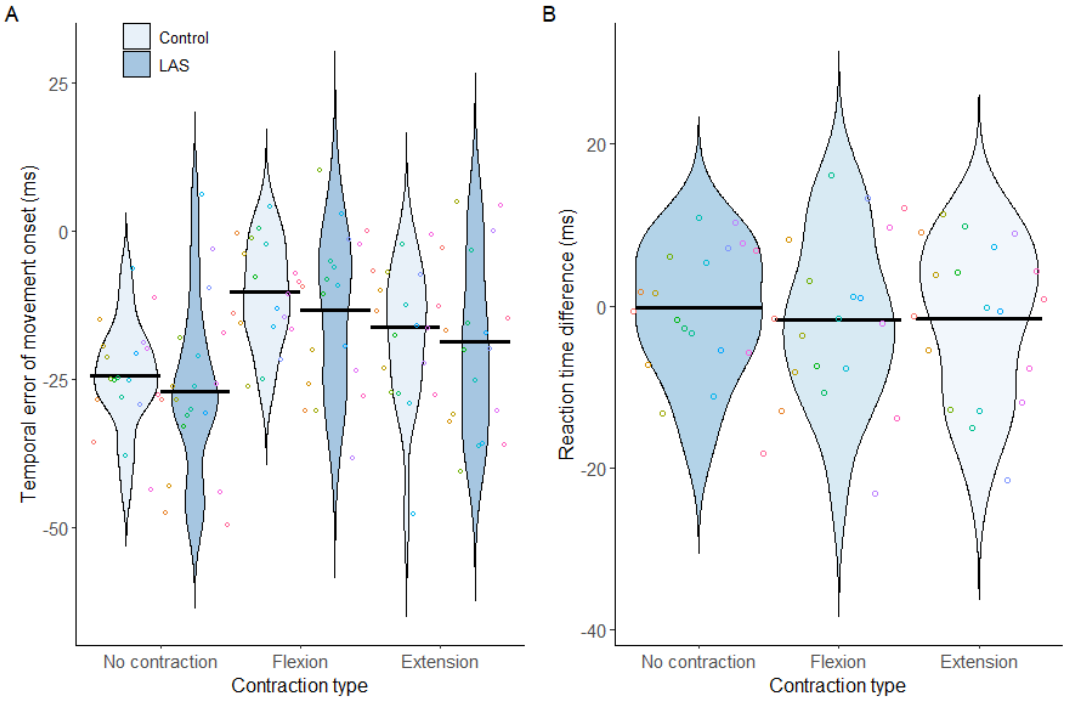


Figure 4.7. Mean temporal error of movement onset for control and probe trials across contraction conditions. Coloured points represent subject averages.

4.8.2 Enhancement of response force and vigour

Peak force was enhanced by the LAS (M = 27.9 N, SD = 14.03) in comparison to control trials (M = 23.54 N, SD = 9.64). The main effect of trial type for peak force was statistically significant, $F_{(1, 3732)} = 197.77$, $p < .001$, $R^2 = .050$. The main effect of contraction type was not statistically significant, $F_{(2, 3732)} = 2.38$, $p = .092$, $R^2 = .001$. Furthermore, the interaction of trial type with contraction type failed to reach statistical significance, $F_{(2, 3732)} = 1.84$, $p = .159$, $R^2 = .001$. Ratios of peak force were largest in the flexion contraction condition (M = 1.26, SD = .51), with smaller ratios of peak force being found for the no contraction (M = 1.19, SD = .42) and extension contraction conditions (M = 1.18, SD = .47). A linear mixed model of peak force ratios indicated a significant main effect of contraction type, $F_{(2, 725.52)} = 3.34$, $p = .036$, $R^2 = .009$. Post hoc tests indicated a significant difference in peak force ratios between the flexion contraction condition and the no contraction condition ($p = .049$), between the flexion contraction condition and the extension contraction condition ($p = .049$), but not between the no contraction and extension contraction conditions ($p = .872$).

On average, peak rate of force development was also increased by the LAS (M = 347.98 N/s, SD = 225.50) in comparison to control trials (M = 258.41 N/s, SD = 121.88). Linear mixed-effects models of peak rate of force development indicated a significant main effect of trial type, $F_{(1, 3732)} = 435.27$, $p < .001$, $R^2 = .104$. The main effect of contraction type, $F_{(2, 3732)} = 4.44$, $p = .012$, $R^2 = .002$, as well as the interaction of trial type with contraction type, $F_{(2, 3732)} = 7.90$, $p < .001$, $R^2 = .004$, were statistically significant. Analysis of the ratios of probe trials over control trials for peak rate of force development indicated a significant main effect of contraction type, $F_{(2, 725.39)} = 7.22$, $p < .001$, $R^2 = .020$, with larger ratios of peak rate of force development occurring in the flexion contraction (M = 1.49, SD = .80, $p < .001$) and extension contraction (M =1.41, SD = .72, $p = .032$) conditions in comparison to the no contraction condition (M = 1.29, SD = .63). The difference between the flexion contraction and extension contraction conditions was not statistically significant (p = .150). The means and ratios of peak force and peak rate of force development are shown in **Figure 4.8**.

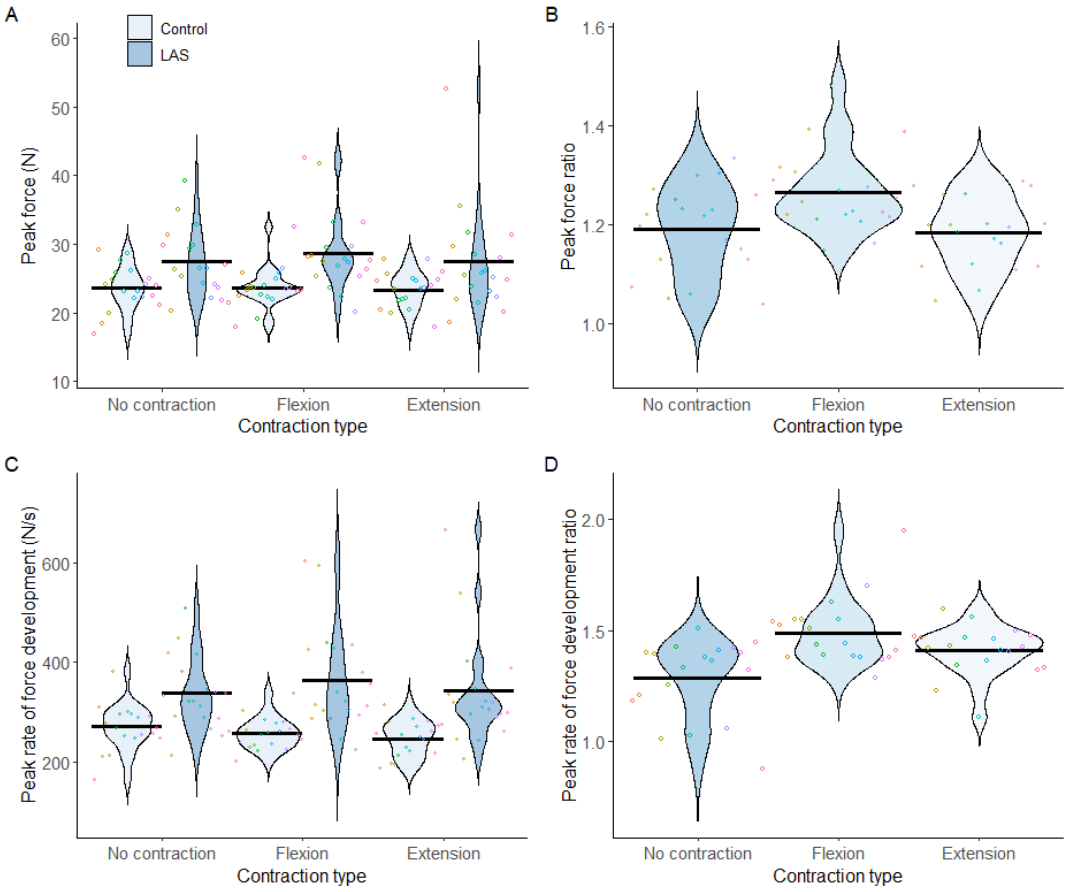


Figure 4.8. A). Mean peak force for control and probe trials for each contraction type. **B)**. Mean peak force ratios for each contraction type. **C)**. Mean peak rate of force development over control and probe trials at each contraction type. **D)**. Mean peak rate of force development ratios for each contraction type. Coloured points represent subject averages.

4.9 Discussion

Intense sounds have paradoxical effects on the motor system, depending on the evolving state of the central nervous system during preparation. During maintenance of a stable low-force muscle contraction, a LAS has inhibitory effects on the corticospinal tract (Fisher et al., 2004; Furubayashi et al., 2000; Kuhn et al., 2004). In contrast, during preparation for a discrete movement, a LAS has an excitatory effect on the corticospinal pathway (Marinovic, Tresilian, et al., 2014). Facilitation of movement via intense sound (the StartReact effect) is observed when a LAS is delivered whilst the central nervous system is in a high state of preparation (close to movement initiation time ~ 200 ms). As such, this excitatory effect of sound during preparation may provide a neurophysiological means by which motor performance can be enhanced in the StartReact effect (Marinovic, Tresilian, et al., 2014; Marinovic & Tresilian, 2016). Observations of paradoxical effects of sound on corticospinal excitability which are contingent on the motor system's state of preparation raise the question of whether combined muscle contraction and motor preparation enhance, or diminish, the StartReact effect. Therefore, here we investigated how the combined maintenance of a muscle contraction during preparation for action impacts the facilitation of motor output induced by a LAS. Raw RTs of movements executed in the bilateral task of experiment one provided no evidence that preparatory contractions of different force levels impact the degree to which a LAS can shorten RT. However, analysis of the entire RT distribution using CDFs indicated some overall benefit of a 10% MVC preparatory contraction on RT, and an overall delay of RT when a 20% of MVC contraction was maintained during preparation. Consideration should also be given to a potential RT floor effect which may have resulted in RTs being already close to the limits of the central nervous

system and therefore limiting the facilitation of actions by the LAS in terms of their initiation.

In experiment two, our CDF analysis indicated that sustained flexion contractions at 10% of MVC, which produced the most benefit on RT in the bilateral task of experiment one, resulted in a delay of movement initiation across the RT spectrum when performed in the unilateral task prior to initiation of the ballistic movement. A similar delay was also produced by the sustained extension contraction during the unilateral task. Attention has previously been shown to modulate intracortical inhibitory circuits of M1 (Bell et al., 2018; Binkofski et al., 2002; Kuhn et al., 2017), with external focus of attention, as opposed to an internal one, increasing short-interval intracortical inhibition (SICI) during sustained contraction (Kuhn et al., 2017). The authors suggest that this serves to regulate the amount of M1 outflow and subsequently increase the time taken for muscle fatigue to occur. This may be applicable to our data, as participants were provided with visual feedback regarding their hand position so that the correct amount of force would be exerted during the sustained contraction. However, we removed this visual feedback prior to LAS presentation and movement onset. Therefore, preparation of the ballistic response may have produced a similar modulation of SICI – either through a shift of attention toward the impending IS presentation, or by the process of motor preparation itself. As such, these attentional-dependent effects may have been induced within the cortical hemisphere that was engaged for the prepared response in the unilateral task but not the bilateral one. This increase of SICI within intracortical circuits might explain why this delay of RT for both contraction directions was observed during the unilateral task but not during the bilateral task.

Similar to the apparent benefit on RT that was produced by the 10% MVC contraction in the bilateral task (experiment one), the LAS provided a larger facilitatory effect on peak force and vigour when a contraction 10% of MVC was maintained contralateral to the hand engaged in preparation. Interestingly, the contralateral sustained flexion contraction during preparation in experiment three replicated this magnification of the LAS effect on peak force, however, the contralateral extension contraction did not. Rather, the LAS effects on peak force of the ballistic response were no more beneficial than the simple unilateral response when an extension contraction was maintained contralaterally. Given the flexion

sustained contraction enhanced the StartReact effect but the extension one did not, this may suggest that the magnification of the StartReact by such sustained contractions can be muscle (or directionally) specific. During bilateral movements, interhemispheric inhibition has been found to be greater during isometric contraction of homologous muscles (i.e. flexion-flexion and extension-extension), whereas this inhibition is decreased during contraction of non-homologous muscles (i.e flexion-extension) (Perez et al., 2014). This decrement of interhemispheric inhibition only during asymmetrical movement appears to be incompatible with the findings we present here of an increased StartReact effect during the bilateral task when the limbs are moving congruently, but not when they are moving incongruently. However, it is difficult to directly compare these findings, given the multitude of evidence which suggests that the modulation of M1 excitability is particularly sensitive to the background state of motor circuits and the dynamics of the movements which are being executed (Carson, 1995; Chen et al., 2016; Cheney & Fetz, 1980; Dettmers et al., 1996; Marinovic et al., 2014). As such, given Perez et al. (2014) employed bilateral isometric contractions whereas we used a task engaging isometric contraction of one limb during active preparation for a ballistic response of the contralateral limb, this may contribute to the incompatibility of our findings.

These data demonstrate that the inhibitory effects on motor pathways that are induced by acoustic stimulation during the maintenance of a muscle contraction can be reversed if motor preparation coincides with certain types of contractions. The engagement of a contralateral muscle contraction may engage a wider and more distributed neural network during preparation which can subsequently be more easily recruited by the LAS and add to the accumulation of preparatory neural activity which summates to produce the final magnitude of motor output (McInnes et al., 2020). Similar suggestions have been made to describe previous observations that the facilitation of movement triggering via the StartReact effect can vary between different movement types of the same muscle, depending on the task functionality of the movement employed (e.g. Honeycutt et al.'s (2013) finger pinch versus grip task, see Marinovic, de Rugy, et al., 2014). The finding that at least in terms of peak force, a contralateral flexion contraction increases the benefit of the LAS could be a result of the efferent connectivity of the flexor muscle. For example, it has been suggested that flexor muscles receive greater functional contributions from the corticospinal

tract in comparison to extensors (Godfrey et al., 2013; Koganemaru et al., 2010; McInnes et al., 2020; McMillan et al., 2004; Park & Li, 2013; Vallence et al., 2012), which may allow a greater facilitation of force due to the correspondence of force generation with primary motor cortex (M1) activity (Ashe, 1997). Alternatively, these effects may be due to the congruency of the sustained contraction with the ballistic response.

We also observed a greater benefit of the LAS on force and vigour of the ballistic response for sustained contractions at lower force levels – particularly at 10% of MVC – than for the higher force contraction (20% of MVC). The direction of this effect is opposite to our prediction of an increase of the StartReact effect which is proportional to the strength of the contraction maintained during preparation. The use of positron emission tomography has identified that at lower force levels, there is a rapid increase of M1 activity as the amount of force produced is increased, but that the rate of this rise diminishes at higher force levels, producing a logarithmic relationship between force production and M1 activity (Dettmers et al., 1996). Single cell recordings have also suggested weak forces are primarily produced by corticomotoneurons (Maier et al., 1993), a finding which may reflect the use of weak forces in fine motor control such as precision grip (Oliveira et al., 2008; Quinn et al., 2018; Shim et al., 2007; Yu et al., 2010). Furthermore, it has been argued that the reticulospinal tract becomes increasingly important for the production of higher levels of force (Baker, 2011), given ipsilateral motor evoked potentials, which are likely mediated by the reticulospinal tract (Ziemann et al., 1999), can be more easily elicited during strong background muscle activity (Alagona et al., 2001). Therefore, the heightened use of the corticospinal tract at lower forces may have led to these force producing neurons to be more readily recruited by the LAS when engaged in a light muscle contraction during preparation, adding to the final motor output. However, this was only observed when the sustained contraction was performed contralaterally to the ballistic response, and not when it was performed ipsilaterally. Therefore, any potential interaction of both facilitatory and inhibitory effects which act during ipsilateral contraction and preparation should be considered — such as a potential modulation of SICI induced in the hemisphere that is engaged in preparation, as discussed earlier.

Finally, the sustained contractions appeared to be more beneficial to motor output when they were maintained during preparation of the contralateral limb, rather than the ipsilateral one. There are a number of neurophysiological mechanisms which may underpin this finding. For example, tonic contraction of one limb can increase activity in ipsilateral M1 (Carson et al., 2004; Kawashima et al., 1998; Liepert et al., 2001; Muellbacher et al., 2000), which may be mediated by interhemispheric modulations of excitability via the corpus callosum (Carson et al., 2004; Di Lazzaro et al., 1999; Perez et al., 2014). This increased activity in M1 may then be recruited by the LAS when triggering a movement that is prepared in those related circuits, which subsequently adds to the final output of the response. Alternatively, engagement of the motor pathways contralateral to the side that is engaging in preparation may allow activation of ipsilateral descending pathways by the LAS which may contribute to the motor output. One such descending pathway is the cortico-reticulo-propriospinal pathway, a descending tract which has been suggested to be important in functional recovery after stroke (Bradnam et al., 2013). The ipsilateral hemisphere may also contribute to motor output through the small number of corticofugal fibres which project to ipsilateral spinal motoneurons, rather than crossing at the pyramidal decussation (Phillips & Porter, 1964). These explanations assume that the input provided by the LAS is of cortical origin. A cortical origin of the descending LAS-induced activity is supported by the fact that the descending pathways which innervate primarily contralateral muscles (i.e. the corticospinal and rubrospinal tracts) receive significant projections from the cortex (Lemon, 2008). The subcortical dorsolateral pathways, in contrast, project bilaterally (Lemon, 2008) and transmission via these pathways would likely be evident regardless of whether the task was unimanual or bimanual. Alternatively, facilitation of a bilaterally projecting pathway in the bilateral task may explain why we observed facilitatory effects induced by the LAS in the bilateral task but not the unilateral one. However, we believe this explanation seems less likely based on the multiple lines of evidence we have already discussed, suggesting a potential role of the cortex in the facilitation provided by a LAS. For example, if facilitation of a bilateral pathway (i.e. the reticulospinal tract) underlies the effects we observed, then magnification of the StartReact effect would be expected to be greatest at higher force levels due to a potentially greater involvement of reticulospinal circuits at higher force levels

(Baker, 2011). It is also possible that the engagement of a muscle contraction during preparation may simply raise the level of preparatory activity to a higher state and thereby enhance the magnitude of the StartReact effect. However, we deem this to be unlikely, given the modulation of gains introduced to the ballistic response by the LAS were dependent on the force and muscle of the sustained contraction, and the effect was far more pronounced in the bilateral tasks rather than in the unilateral one.

Regardless of the specific mechanisms underpinning the greater facilitation of motor output provided by the LAS in the presence of a sustained contralateral muscle contraction during preparation, this finding may have important practical implications for using the StartReact effect as a rehabilitative tool. Engagement of the contralesional side can be used to increase the benefits of acoustic stimulation and further aid in the functional recovery of movement after neurological conditions such as stroke. This is particularly promising given the ipsilateral cortex has been suggested to be capable of compensating for contralateral cortex deficits after stroke (Serrien et al., 2004; Strens et al., 2003). While the StartReact effect has been shown to facilitate the initiation and execution of movement in a range of neurological disorders (for review see Carlsen et al., 2012; Nonnekes et al., 2015), it is yet to be determined whether these improvements in motor function transfer to situations where the intense sensory stimulus is no longer present. It has been demonstrated that the presentation of a LAS during a learning period of sensorimotor adaptation can boost adaptation of reaching movements to rotated movement feedback (Leow et al., 2021). This enhanced adaption was observed to remain present 24 hours after the initial learning period. Therefore, there is reason to believe that modulation of central nervous system excitability via a LAS can produce effects which last on a timescale beyond the period in which the LAS was presented. However, further investigation is needed to determine how movement initiation and execution may be affected by a LAS on a longer timescale. It should be noted that the use of the StartReact effect in combination with traditional rehabilitation protocols presents potential benefits in promoting neuroplasticity and preventing muscular atrophy (Kiper et al., 2016; Metoki et al., 2003). Such application of intense sensory stimulation is therefore a promising method of improving the functional control of movement after neurological conditions which impair motor control. Finally, given contralateral muscle activity during preparation was shown to modulate the

StartReact effect at even moderately low force levels, it may be an important consideration for researchers studying the StartReact effect to observe participants during experimental sessions to ensure they are not unknowingly activating task-irrelevant muscles.

CHAPTER FIVE: PREMOVEMENT INHIBITION PROTECTS MOTOR ACTIONS FROM INTERFERENCE

5.0 Abstract

Shortly before movement initiation, the corticospinal system undergoes a transient suppression. This phenomenon has been observed across a range of motor tasks, suggesting that it may be an obligatory component of movement preparation. We probed whether this was also the case when the urgency to perform a motor action was high, in a situation where little time was available to engage in preparatory processes. We controlled the urgency of an impending motor action by increasing or decreasing the foreperiod duration in an anticipatory timing task. Transcranial magnetic stimulation (TMS; experiment one) or a loud acoustic stimulus (LAS; experiment two) were used to examine how corticospinal and subcortical excitability were modulated during motor preparation. Preparatory inhibition of the corticospinal tract was absent when movement urgency was high, though motor actions were initiated on time. In contrast, subcortical circuits were progressively inhibited as the time to prepare increased. Interestingly, movement force and vigour were reduced by both TMS and the LAS when movement urgency was high and enhanced when movement urgency was low. Our findings indicate that preparatory inhibition may not be an obligatory component of motor preparation. The behavioural effects we observed in the absence of preparatory inhibition were induced by both TMS and the LAS, suggesting that accessory sensory stimulation may disrupt motor output when such stimulation is presented in the absence of preparatory inhibition. We conclude that preparatory inhibition may be an adaptive strategy which can serve to protect the prepared motor action from external interference.

5.1 Introduction

During preparation for a motor action, the corticospinal (CS) tract goes through systematic changes of excitability. Measurement of CS excitability via transcranial magnetic stimulation (TMS) of the primary motor cortex (M1) approximately 100 ms before electromyogram (EMG) onset in the effector muscle indicates an increase in excitation of CS neurons (Chen et al., 1998; Leocani et al., 2000; Starr et al., 1988). However, prior to this ramp-up of excitability, there is a period of CS inhibition, indicated by a gradual suppression of motor evoked potentials (MEPs) induced by TMS, up until the point excitation occurs (Hasbroucq et al., 1997a; Hasbroucq et al., 1997b; Hasbroucq et al., 1999; Ibáñez et al., 2020; Marinovic et al., 2011). There are three main explanations which have been put forward regarding the role of this suppression of motor circuits before movement onset. First, the competition resolution account suggests that CS inhibition may be necessary to suppress the initiation of competing response selections (Burle et al., 2004). Second, the impulse control hypothesis proposes that inhibition may be necessary to prevent a prepared movement from being triggered prematurely (Duque et al., 2010, 2017; Duque & Ivry, 2009). Finally, the spotlight hypothesis suggests premovement inhibition allows the speeded initiation of movement by increasing the signal to noise ratio in motor circuits (Greenhouse et al., 2015).

The available data, however, are not completely consistent with these three explanations. In choice RT time tasks, CS inhibition has been found to increase in the selected effector, rather than the non-selected effector after an action is specified (Duque & Ivry, 2009). This finding is not consistent with the competition-resolution hypothesis. Furthermore, the impulse control explanation implies that any facilitatory input to the CS tract should be suppressed. This is incompatible with findings that there is specificity in the suppression of volleys evoked by TMS (Hannah et al., 2018). In addition, shorter RTs are associated with greater levels of preparatory inhibition (Hannah et al., 2018), and CS suppression is observed in self-timed actions which do not rely on initiation taking place at a particular time (Ibáñez et al., 2020). These findings are incompatible with preparatory inhibition acting to prevent premature initiation. Finally, the spotlight account is incompatible with the observation of CS suppression in non-selected muscles – it could be argued that this would be maladaptive in that it may lead to an increased risk of unintentionally

triggering task-irrelevant actions. As such, it is unclear whether preparatory inhibition serves an entirely different role, or whether the phenomenon involves the combination of some or all of the above explanations (Duque et al., 2014). For example, a more global inhibition may initially take place at a short-time scale, acting on non-selected effectors to prevent their unintentional triggering. This may eventually unfold into a more specific inhibition acting in selected effectors consistent with the impulse control and/or spotlight hypotheses.

Preparatory inhibition has been observed using different neurophysiological techniques besides TMS. These include the Hoffman's reflex (Derosiere, 2018; Hannah et al., 2018) and the eye-blink reflex, which, similarly to the CS tract, has been shown to undergo a facilitation close to movement onset (Lipp et al., 2001; Marinovic et al., 2013) and suppression earlier in preparation (Anthony & Putnam, 1985; Nguyen et al., 2020). While the Hoffman's reflex can be exploited to derive information regarding the excitability of separate populations of corticospinal neurons, the startle-blink reflex can be used as a measure of global motor-related subcortical excitability (Kumru & Valls-Solé, 2006). One advantage of using startling stimuli to probe the excitability of subcortical circuits is that it also has a well-known effect on response initiation and execution: the StartReact effect (Anzak et al., 2011; Marinovic et al., 2016; McInnes et al., 2020; Valls-Solé et al., 1999). More specifically, by employing startling stimuli, one is able to test both the excitability of subcortical circuits as well as the effects of the startle eliciting sounds on motor output (Kumru & Valls-Solé, 2006; Marinovic et al., 2013; Nguyen et al., 2020).

We hypothesised that time constraints imposed on preparation would limit the ability of motor circuits to undergo suppression during preparation. As such, we modified the urgency of an anticipatory action by shortening or lengthening the duration of time between the point at which an action should be prepared and when it should be initiated. Changes in CS and startle circuit excitability that occur shortly prior to action initiation were probed using TMS and a startling stimulus in experiments one and two, respectively. We predicted reduced CS inhibition when urgency is high and, due to the lack of sufficient inhibition, movements that are triggered by an intense sensory stimulus would occur earlier and with greater vigour than movements for which ample time was allowed for preparation. Furthermore, if

a generalised suppression of motor circuitry precedes a more specific one, then time-related constraints on preparation should act to a lesser extent on the suppression of subcortical circuits (potentially representing global inhibition) than on the more focal inhibition which would be observed in the CS tract. In contrast, when sufficient time for motor preparation is allowed, we expected greater CS and subcortical inhibition and smaller behavioural effects induced by accessory stimulation — smaller increase in vigour and force — than in the high urgency condition.

5.2 Method – Experiment One

5.2.1 Participants

Eighteen participants were recruited for experiment one (10 female; mean age = 25.33, *SD* = 7.71). All participants had normal or corrected vision and no apparent or known auditory impairments, neurological conditions, or injuries which may have impaired their ability to complete the task. Sixteen of the participants were self reportedly right handed and two participants reported being ambidextrous. Participants were screened for potential contraindications to TMS in accordance with the guidelines proposed by Rossi et al. (Rossi et al., 2009).

5.2.2 Procedures

Participants were seated comfortably ~70 cm in front of a 24.5” monitor (ASUS ROG PG258Q; 240Hz refresh rate, 1920 x 1080 resolution). The experiment routines were run using custom scripts run in MATLAB 2015b. Timing and presentation of visual and electromagnetic stimuli were controlled using Psychtoolbox (v3.0.11) and MAGIC (v0.2; Habibollahi Saatlou et al., 2018) toolboxes. During the task, participants applied pressure to a force sensor (SingleTact 10 N calibrated sensor), which was held in a custom-made housing, with their right index finger in synchrony with the sweeping of a clock hand which was presented on the monitor. The sensor and housing are pictured in **Figure 5.1**. Participants were instructed to apply ballistic force with the index finger in abduction of the first dorsal interosseous (FDI) and to initiate the response in time with the clock hand reaching the 12 o’clock position. The first 10 trials in each experimental block were control trials in which no TMS was delivered. These initial

10 trials were excluded from analysis and were included in the experimental session only to ensure participants were familiar with the timing of that particular block before TMS was delivered. Participants completed two experimental blocks of 100 trials each (200 experimental trials total). In each trial, prior to the clock sweeping, the clock remained stationary on screen for a duration randomised from a uniform distribution of 3 – 5 s, in order to prevent anticipatory motor preparation. The sweeping of the clock hand was counterbalanced to one of two speeds (high/low urgency) in each experimental block. The time from the start of the sweep to the intersection of the hand at the 12 o'clock position was either 350ms (high urgency), or 1400ms (low urgency). As the clock moved toward the 12 o'clock position, the centre remained white up until -25 ms prior to the intersection at 12 o'clock, and remained green for a duration of 50 ms so that the centre flashed green ± 25 ms around the expected time of movement onset. In 40% of trials, TMS was presented either two seconds after the beginning of the resting period during which the clock was stationary (baseline TMS), or 250 ms prior to the expected time of movement onset (probe TMS). Ibanez et al. (2020) demonstrated preparatory inhibition of CS excitability begins at some point between 500 ms to 200 ms prior to the expected time of movement onset in an anticipatory timing task. Furthermore, Marinovic (2011) found preparatory suppression of CS excitability was greatest during the time window 250 ms – 201 ms prior to the expected time of movement onset in an anticipatory timing task. However, suppression of OOc responses to a LAS during preparation for anticipatory finger movement has been observed as early as 392 ms prior to the expected time of movement onset (Nguyen et al., 2021). We therefore opted to deliver the probe TMS (experiment one) and LAS (experiment two) at 250 ms prior to the expected time of movement onset as this stimulation time was most likely to capture both CS and subcortical suppression, given the early subcortical suppression of OOc and maximal CS suppression between 250 – 201 ms prior to the expected time of movement onset. Feedback regarding the temporal error of movement onset was provided at the end of each trial, with the exception of probe trials. Feedback was not provided in probe trials to avoid participants changing their responses due to the probe stimulus interfering with their ability to initiate their movements on time. For movements initiated within ± 25 ms of the clock hand intersection, a "Good timing!" message was presented in green text. For movements

initiated < -25 ms or > 25 ms, a message of “Too early” or “Too late”, respectively, was presented in red text. Feedback regarding the temporal error of movement onset was also presented visually in a horizontal bar which depicted temporal error = 0, temporal error = 25, and temporal error = -25, along with temporal error of the current trial (see **Figure 5.2**). This feedback was presented for two seconds. Along with temporal error feedback, points were awarded to participants for each “good timing” response to encourage participants to initiate actions as close to the expected time of movement onset as possible. The ongoing score was presented along with temporal error feedback at the end of each trial. Prior to commencing experimental trials, participants completed 12 practice trials (six trials for each foreperiod length) and before beginning the task 20 TMS pulses were presented in order to measure MEPs at rest. Participants remained with their hands at rest in the same position they would hold the force sensor during the task and looked at a fixation cross presented on screen while the resting TMS pulses were presented.

Figure 5.1. Force sensor in custom-made housing.

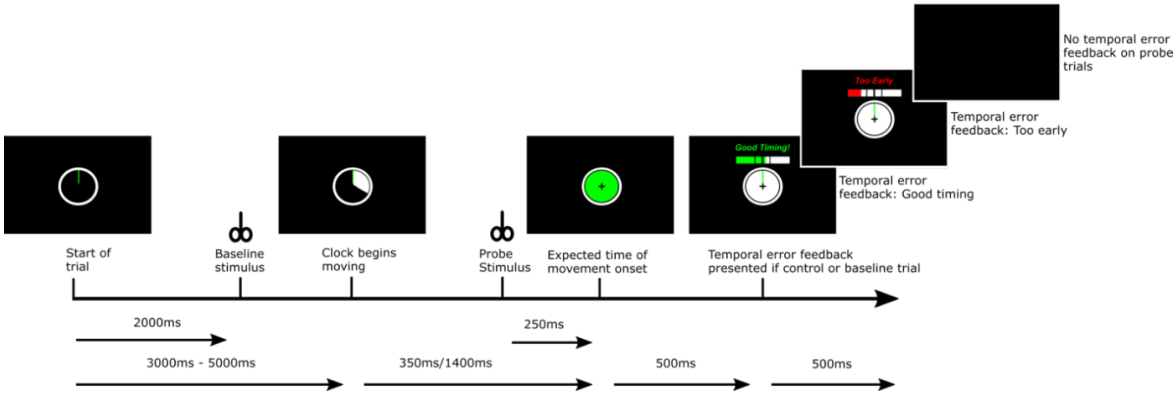


Figure 5.2. Sequence of events during experiment one. Note baseline and probe stimuli were presented on separate trials.

5.2.3 Transcranial magnetic stimulation

TMS was delivered using a Magstim BiStim^2 magnetic stimulator using a 70mm Magstim D70^2 figure-of-eight coil. The coil was held tangentially to the participants' scalp over the primary motor cortex (M1) and with the handle facing the rear of the head, placed at a 45° angle to the sagittal plane. Before commencing the experimental trials, the hotspot for the FDI (the primary agonist muscle) was located and the resting motor threshold was determined by using the Rossini-Rothwell procedure (Rossini et al., 1994) to find the lowest stimulus intensity to the nearest 1% of maximum stimulator output that could elicit a motor evoked potential with a peak to peak amplitude greater than 50 μV in five out of 10 test trials. Test pulses during the experiment were delivered at 120% of resting motor threshold. The mean resting motor threshold was 38.33% of maximum stimulator output (range = 32% – 49%). TMS was presented in 40% of trials (20% baseline timing, 20% probe timing) and trials were pseudorandomised so that no two consecutive trials could occur as a TMS trial.

5.2.4 Data acquisition, reduction, and analysis

5.2.4.1 Acquisition of force and electromyogram data

Data were acquired using a National Instruments USB-6229 data acquisition device and sampled continuously at 2 kHz each trial. The data acquired from the force sensor were detrended and multiplied by a factor of 10 to convert the voltage output

of the Singletact sensor to Newtons (N). These data were then used to determine temporal error of movement onset (difference in ms between the intersection of the clock hand at 12 o'clock and the time of movement onset, calculated using the algorithm suggested by Teasdale et al. (1993)), peak force (maximum force applied to the sensor over the course of a trial), and peak rate of force development (maximum derivative of the force signal over time occurring over the course of a trial; N/s). EMGs were recorded from the right FDI using bipolar 24 mm electrodes with a reference electrode placed over the styloid process of the right ulna. The EMG signal was amplified with a gain of x1000 using a pre-amplifier (Digitimer NeuroLog NL844) and amplifier (Digitimer NeuroLog NL820A) and the signal was band-pass filtered using a low-pass filter at 500 Hz (Digitimer Neurolog NL135) and high-pass filter at 20 Hz (Digitimer Neurolog NL144).

5.2.4.2 Processing of electromyogram data

All analyses were conducted using R software (v3.5.1). EMG data from FDI were down sampled to 1 kHz and EMG peak to peak amplitudes between 20 ms – 80 ms after TMS presentation were automatically calculated. All trials were visually inspected and peak to peak amplitudes were manually marked if they were incorrectly marked by the algorithm. Trials were excluded from analysis if visual inspection indicated significant noise, artifacts, or voluntary contraction, which obscured the detection of peak to peak amplitude, were present in the EMG record. Visual inspection was conducted blindly with respect to experimental condition. The manual rejection of trials resulted in the removal of 135 (participant median = 5, range = 0 – 21) trials. In addition, after manual trial rejection we calculated the root mean square of FDI EMG activity 200 ms prior to TMS presentation for all trials. Median root mean square of FDI EMG activity 200 ms prior to TMS presentation was calculated for each participant and if for a single participant any trial exceeded that median value by a factor of 1.4, the trial was excluded from the analysis of FDI EMGs. This resulted in the removal of a further 106 trials (participant median = 2, range = 0 – 25; n baseline timing = 58, n probe timing = 48). In total, 241 trials out of 1440 probe trials (16.74%) were excluded from the analysis of FDI EMGs. The findings observed from our analyses using these trial exclusion criteria were consistent with those obtained from analyses for which all trials were retained. In

addition, we ran linear mixed-effects models to examine whether voluntary muscle contraction, as indicated by FDI EMG root mean square values 200 ms prior to TMS presentation, systematically differed as a function of foreperiod length, trial type, or as an interaction between the two.

5.2.4.3 Analysis of behavioural and electromyogram data

The behavioural data (temporal error of movement onset, peak force, peak rate of force development), and EMG data (MEP peak to peak amplitude) were subject to statistical analyses. In the analysis of behavioural data, control trials were excluded from analysis if their temporal error of movement onset was < -150 ms or > 150 ms. This resulted in the removal of 98 trials (participant median = 4, range = 0 – 29, 4.54% of all control trials) from the analysis of behavioural data. A series of linear mixed-effect models were conducted using the *lmer* function (lmerTest package, v3.0). Models were conducted with foreperiod length (350 ms, 1400 ms) and trial type (control, baseline, probe) as fixed factors and participant IDs as random factors. All valid trials were run in the models and all main effects and interactions were tested. The same models were conducted with MEP peak to peak amplitude as the dependent variable. In addition, EMG amplitudes of probe trials were calculated as a percentage of EMG amplitude in baseline trials to determine the magnitude of change in amplitude that occurs from baseline to probe responses. This involved calculating the median value of baseline trials for each foreperiod length and dividing each probe trial value by the median baseline value for its corresponding foreperiod length, and multiplying the result by a factor of 100 (i.e. $\left(\frac{EMG\ Amplitude\ Probe\ (Foreperiod\ x)}{Median(EMG\ Amplitude\ Baseline\ (Foreperiod\ x))}\right) \times 100$). We also examined MEPs to the pre-experimental resting TMS and compared these to MEPs to the experimental baseline TMS to examine whether CS excitability, as measured by MEPs in experimental baseline trials, was modulated by our manipulation of foreperiod length. Similarly to our calculation of amplitudes of EMG in probe trials as a percentage of amplitudes in baseline trials, we calculated MEP amplitude of experimental baseline trials as a percentage of median EMG amplitude of pre-experimental MEPs. The resulting *F* values, with Kenward-Rogers approximation for degrees of freedom, and *p* values from all linear mixed models are reported along with R^2 values which were calculated using the *r2beta* function (r2glmm package,

v0.1.2) in order to provide an estimate of effect size. Post-hoc tests were conducted using the *emmeans* function (emmeans package, v1.3.0) with the false discovery rate correction method for multiple comparisons (Benjamini & Hochberg, 1995). In addition to our linear mixed-effects analyses, we complemented our frequentist methods with Bayesian analyses using the *BayesFactor* package (v0.9.12) in order to provide the degree of support for the null hypothesis when such evidence would be relevant to the study aims. As such, BF_{01} values are reported to indicate the level of evidence to support the null hypothesis, and BF_{10} values are reported to indicate the level of evidence to support the alternative hypothesis. Furthermore, the *ttestBF* function was used to derive a Bayes factor (BF) indicating the degree of support the data provide for a facilitation of probe MEPs (> 100% of baseline), as well as a BF indicating the degree of support for an inhibition of probe MEPs (< 100% of baseline). The resulting BFs of a facilitatory effect were then divided by BFs indicating the probability of an inhibitory effect ([probability of data if excitatory effect / probability of data if null effect] / [probability of data if inhibitory effect / probability of data if null effect]). In this analysis, BF_{10} values are reported to indicate the level of support for a facilitatory effect and BF_{01} values are provided to indicate the level of support for an inhibitory effect.

5.3 Results – Experiment One

5.3.1 Urgency effects on temporal error

Average temporal error did not significantly differ between foreperiod lengths, as indicated by a non-significant main effect of foreperiod duration in the linear mixed-effects model of temporal error of movement onset, $F_{(1,\ 3446.3)} = 1.16$, $p = .282$, $R^2 = .000$. The main effect of trial type was statistically significant, $F_{(2,\ 3446.3)} = 11.76$, $p < .001$, $R^2 = .007$. Post-hoc tests indicated temporal error of movement onset was earliest for the probe TMS condition ($M = -14.71$ ms, $SD = 96.99$), with later temporal error of movement onset occurring for the control condition ($M = -7.83$ ms, $SD = 51.4$; $p = .003$), and baseline TMS condition ($M = -1.11$ ms, $SD = 56.8$; $p < .001$). The interaction of trial type with foreperiod length was not statistically significant, $F_{(2,\ 3446.2)} = 2.06$, $p = .128$, $R^2 = .001$. **Figure 5.3A** shows mean temporal error of movement onset for each trial type across both of the foreperiod durations.

5.3.2 Urgency effects on peak force and vigour

Analysis of peak force indicated that on average, responses in the 350 ms foreperiod duration (M = 3.35 N, SD = 2.07) were executed with greater force than those in the 1400 ms foreperiod duration (M = 3.15 N, SD = 1.73), as indicated by a significant main effect of foreperiod duration in the linear mixed model of peak force data, $F_{(1, 3479)} = 7.94$, $p = .004$, $R^2 = .002$. The main effect of trial type was also statistically significant, $F_{(2, 3479)} = 4.97$, $p = .006$, $R^2 = .003$. Average peak force was significantly reduced in probe TMS trials (M = 3.11 N, SD = 1.88), from control trials (M = 3.29 N, SD = 1.59; $p = .005$). Average peak force in baseline TMS trials (M = 3.26 N, SD = 1.61) was not significantly different from control trials ($p = .583$), nor probe TMS trials ($p = .054$). The linear mixed-effects model also showed a significant interaction of foreperiod length with trial type, $F_{(2, 3479)} = 7.38$, $p < .001$, $R^2 = .004$. Post-hoc tests indicated a significant difference in peak force between control trials and probe TMS trials for the 350 ms foreperiod duration ($p < .001$), but not for the 1400 ms foreperiod duration ($p = .459$; see **Figure 5.3B**).

Analysis of peak rate of force development indicated that on average, responses in the 350 ms foreperiod duration condition (M = 51.71 N/s, SD = 37.11) were executed with greater peak rate of force development than those in the 1400 ms foreperiod duration condition (M = 46.17 N/s, SD = 28.24), as indicated by a significant main effect of foreperiod duration in the linear mixed model, $F_{(1, 3479)} = 23.51$, $p < .001$, $R^2 = .007$. The main effect of trial type was not statistically significant, $F_{(2, 3479)} = 0.28$, $p = .754$, $R^2 = .000$. The linear mixed-effects model also showed a significant interaction of foreperiod duration with trial type, $F_{(2, 3479)} = 13.22$, $p < .001$, $R^2 = .008$. Post-hoc tests indicated a significant decrease in peak rate of force development from control trials to probe TMS trials for the 350 ms foreperiod length ($p = .001$), and in contrast, peak rate of force development showed a significant increase from control trials to probe trials during the 1400 ms foreperiod duration ($p = .012$; see **Figure 5.3C**).

5.3.3 Suppression of motor evoked potentials

Background EMG activity was assessed by analysing the root mean square of EMG 200 ms prior to the presentation of TMS. A linear mixed-effects model indicated a significant main effect of foreperiod length, $F_{(1, 1176.5)} = 16.93$, $p < .001$, $R^2 = .014$

and indicated EMG background activity was greater in the 350 ms foreperiod length (M = 4.87 μV, SD = 1.63 × 10) than in the 1400 ms foreperiod length (M = 4.6 μV, SD = 1.61 × 10^{-3}). However, this difference was small, with the difference between mean EMG root mean square in the 350 ms and 1400 ms conditions = 0.027 μV. Importantly, both the main effect of trial type, $F_{(1, 1176)} = 0.79$, $p = .374$, $R^2 = .001$, and the interaction of foreperiod length with trial type, $F_{(1, 1176)} = 0.33$, $p = .568$, $R^2 = .000$, were not statistically significant. A Bayesian linear model of the interaction of trial type with foreperiod length indicated $BF_{01} = 346.12$, providing decisive evidence for the null hypothesis (Jeffreys, 1961). Furthermore, we calculated the baseline MEP amplitude for each foreperiod duration condition as a percentage of median MEP amplitude when participants were at rest prior to the commencement of experimental trials. A linear model indicated a statistically significant main effect of the foreperiod duration condition of the block which the baseline probe was contained in, $F_{(1, 581.41)} = 4.9$, $p = .027$, $R^2 = .008$. These percentages were larger for the 350 ms condition (M = 208.76%, SD = 233.51) than for the 1400 ms condition (M = 180.92%, SD = 196.32), suggesting there may have been some ongoing enhancement of corticospinal excitability prior to the start of the clock sweep for the 350 ms condition.

MEPs showed a modulation of amplitude with the manipulations of foreperiod length and TMS timing, with the linear mixed model of MEP amplitude showing statistically significant main effects of foreperiod length, $F_{(1, 1177.4)} = 40.48$, $p < .001$, $R^2 = .033$, and trial type, $F_{(1, 1176.1)} = 29.91$, $p < .001$, $R^2 = .025$. The interaction of foreperiod length and trial type was also statistically significant, $F_{(1, 1176.1)} = 32.59$, $p < .001$, $R^2 = .027$. Post-hoc tests indicated that baseline MEP amplitudes did not differ between the foreperiod duration conditions ($p = .717$). Furthermore, for the 1400 ms foreperiod length, MEP amplitudes were significantly reduced relative to baseline TMS (M = 1.58 mV, SD = 1.16) in probe TMS trials (M = 0.94 mV, SD = 1.01; $p < .001$). This reduction in MEP amplitude from baseline (M = 1.61, SD = 1.23) in probe (M = 1.63, SD = 1.13) TMS trials was not present during the 350 ms foreperiod length ($p = .864$). A Bayesian paired-samples t-test provided no support ($BF_{01} = 1.92 \times 10^{-3}$) for the null hypothesis of differences in participant mean MEP amplitudes between baseline and probe trials for the 1400 ms condition, and provided decisive evidence (Jeffreys, 1961) for the alternative hypothesis, BF_{10}

= 521.48. Conversely, for the 350 ms foreperiod length, a Bayesian paired-samples t-test provided substantial evidence for the null hypothesis of differences in MEP amplitudes between baseline and probe TMS trials, $BF_{01} = 3.84$ (Jeffreys, 1961). **Figure 5.3D** shows mean MEP amplitudes across probe and baseline TMS trials for each of the foreperiod lengths. MEP amplitudes in probe TMS trials were also calculated as a percentage of the median amplitude of MEPs in baseline TMS trials for each foreperiod length. A linear mixed model indicated a significant main effect of foreperiod length for these percentages, $F_{(1, 590.33)} = 84.97$, $p < .001$, $R^2 = .126$. Probe MEP amplitudes as a percentage of baseline MEP amplitudes trended toward facilitation for the 350 ms foreperiod length, with mean percentage being > 100% ($M = 106.6\%$, $SD = 83.35$). However, a one-sample t-test of the mean of these percentages against a mean of 100% was not statistically significant ($p = .127$). Probe MEP amplitudes showed a clear inhibition during the 1400 ms foreperiod length ($M = 63.12\%$, $SD = 78.7$), and a one-sample t-test indicated these percentages were significantly reduced from 100% ($p < .001$). We calculated BFs which indicate the magnitude of evidence to suggest observation of a facilitatory effect in probe MEP amplitudes over baseline amplitudes, for each foreperiod duration. Trials in the 350 ms foreperiod duration indicated $BF_{10} = 14.49$, providing strong evidence (Jeffreys, 1961) to suggest facilitation of probe MEPs. In contrast, analysis returned a $BF_{10} = 2.61 \times 10^{-22}$ for the 1400 ms condition, providing no support for facilitation in this condition, but rather decisive evidence for an inhibitory effect, $BF_{01} = 3.83 \times 10^{21}$. **Figure 5.3E** shows mean probe MEPs as a percentage of median baseline MEPs for each foreperiod duration.

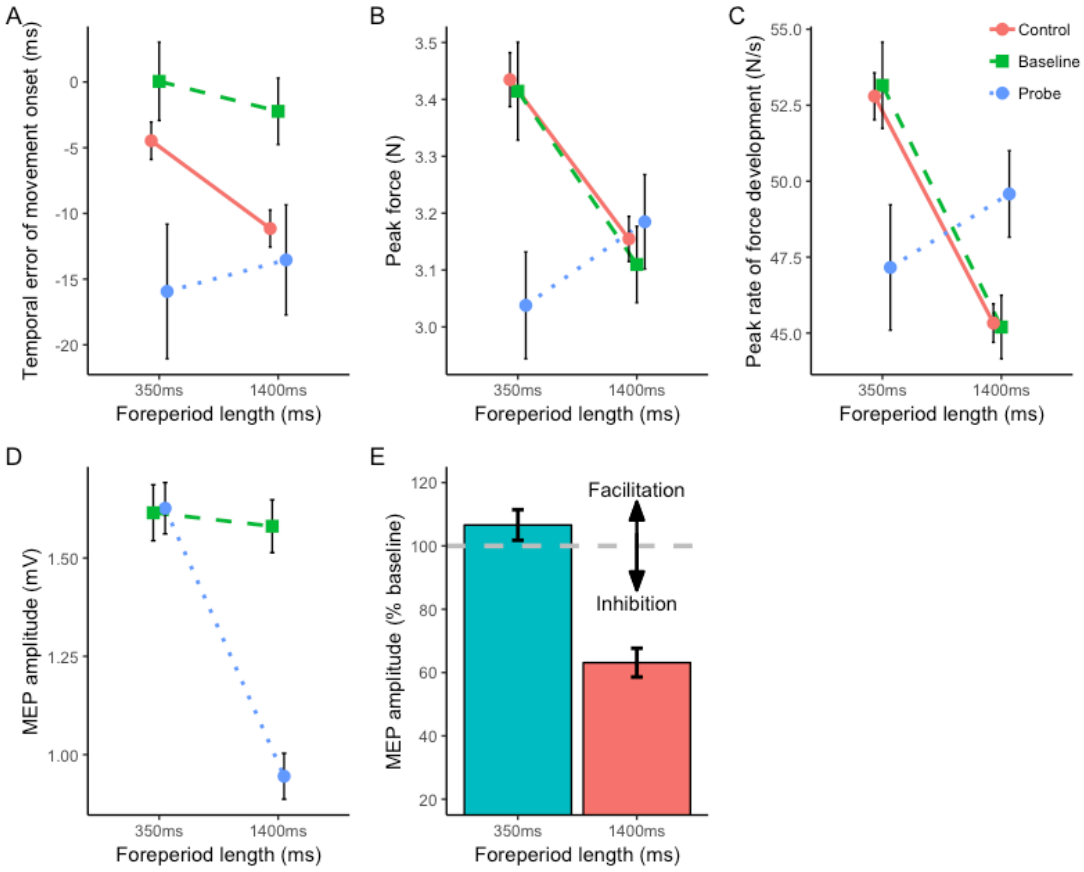


Figure 5.3. **A)**. Temporal error of movement onset for control, baseline and probe trials over foreperiod lengths. **B)**. Mean peak force of movements executed in control, baseline, and probe trials over foreperiod lengths. **C)**. Mean peak rate of force development of movements executed in control, baseline, and probe trials over foreperiod lengths. **D)**. Mean motor evoked potential (MEP) amplitudes for baseline and probe trials across each foreperiod length. **E)**. MEP amplitudes in probe trials as a percentage of baseline amplitude over foreperiod lengths. Error bars represent standard error of the mean.

5.4 Method – Experiment Two

5.4.1 Participants

Twenty-three healthy participants were recruited for experiment two (12 female; mean age = 23.61, *SD* = 5.84). Twenty-two participants were self-reportedly right-handed, and one participant reported being ambidextrous. All participants had normal or corrected vision and no apparent or known auditory impairments,

neurological conditions, or injuries which may have impaired their ability to complete the task.

5.4.2 Procedures

A similar procedure was employed in experiment two, with the following exceptions. Three foreperiod lengths were employed, high urgency (350 ms), medium urgency (700 ms), and low urgency (1400 ms), in order to reference a time point at which preparatory inhibition emerges. Each of the foreperiod durations was randomised to one of three blocks which consisted of 100 trials each (300 trials total). Furthermore, rather than single pulse TMS, a loud acoustic stimulus (LAS) was presented at the same timings as the TMS in experiment one. A LAS was used in this experiment as it has recently been shown that preparatory inhibition can be observed in the modulation of the startle eyeblink response during preparation (Nguyen et al., 2020), and it would allow us to discern whether the behavioural effects we observed in experiment one were due to activation of the corticospinal tract or whether this was due to accessory effects of the TMS coil's discharge. Prior to commencing experimental trials, participants completed 12 practice trials (4 trials for each foreperiod length). The LAS was also presented four times to participants before beginning the task in order to measure their OOc EMG response at rest.

5.4.3 Data acquisition, reduction and analysis

EMG from the right OOc was recorded with surface bipolar 8 mm Ag/AgCl sintered electrodes with a 24 mm reference electrode placed over the right mastoid process. Onset latency and amplitude of OOc EMG in experiment two were measured using several steps. EMG data from OOc were downsampled to 1 kHz, rectified using the *rectification* function (biosignalEMG package, v2.1.0), and the *rollapply* function (zoo package v1.8) was used to smooth data using a five-point moving average. The latency of OOc EMG onset was detected using the Bonato (1998) method with the *onoff_Bonato* function (biosignalEMG package, v2.1.0; sigma n = 2 times the standard deviation of activity within 0 – 200 ms prior to the LAS). Multiple passes of the Bonato method were run until onset of EMG could be detected. If no onset of OOc EMG could be detected, the threshold was increased by an increment of 0.2x(Baseline variability) for a maximum of 10 passes, after which the threshold was

decreased from 1 by increments of 0.2x(baseline variability) for a maximum of two passes, until an onset of EMG could be detected between 20 – 80 ms with respect to LAS onset. Amplitude of the EMG was automatically calculated as the difference between the maximum of the rectified EMG signal after blink onset and baseline amplitude of the rectified signal. All trials were visually inspected, and adjustments were made to the latency of EMG onset or amplitude if necessary. Trials were excluded from analysis of OOc EMG if their onset was < 20 ms or > 80 ms (Blumenthal et al., 2005), if no EMG response occurred, or if excessive noise, artifacts, or voluntary activation within 20 ms of LAS onset were present in the EMG record. Seven participants were excluded from analysis of EMG data in experiment two due to excessive noise or artifacts in EMG recordings, or insufficient OOc EMG response to the LAS, and as such, EMG data are reported for the remaining 16 participants. After removal of these participants, our trial exclusion criteria resulted in the exclusion of 128 trials (9.27% of all LAS trials). Finally, t-scores were calculated for OOc EMG amplitude using the *rescale* function and setting $M = 50$ and $SD = 10$. Exclusion of control trials of behavioural data for which temporal error of movement onset was < -150 ms or > 150 ms resulted in the exclusion of 117 trials in experiment two (2.83% of all control trials).

5.4.4 Loud acoustic stimulus

A LAS was presented in 40% of trials, 20% at baseline timing and 20% at probe timing. Trials were pseudorandomised to either control, baseline LAS, or probe LAS trials so that no two consecutive trials could occur as a LAS trial. The onboard audio of the computer used to run the experiment generated the LAS as brief bursts of white noise (50 ms burst duration with a rise and fall time < 1.5 ms). The LAS was presented binaurally through stereophonic active noise cancelling headphones (Bose QC25). At a distance of 2 cm from the speaker cone, the peak amplitude of the LAS was measured at 105 dBA.

5.5 Results - Experiment Two

5.5.1 Urgency effects on temporal error of movement onset

On average, movement onset was shortest for the 700 ms foreperiod length (M = -15.64 ms, SD = 62.91), and the 1400 ms condition (M = -12.88 ms, SD = 58.59), with the 350 ms foreperiod length resulting in the longest movement onsets (M = 3.53, SD = 60.24). The linear mixed-effects model of temporal error of movement onset data indicated a statistically significant main effect of foreperiod duration, $F_{(2, 6750)} = 50.7$, $p < .001$, $R^2 = .015$. Post-hoc tests indicated in comparison to the 350 ms condition, both the 700 ms ($p < .001$) and 1400 ms ($p < .001$) foreperiod durations resulted in significantly earlier movement onsets. The main effect of trial type was also statistically significant, $F_{(2, 6750.1)} = 42.02$, $p < .001$, $R^2 = .012$, with movements on probe trials (M = -24.49 ms, SD = 88.05) being initiated earlier on average than on baseline (M = -4.5 ms, SD = 67.32, $p < .001$) and on control trials (M = -7.08 ms, SD = 57.18, $p < .001$), but with no significant difference between control and baseline trials ($p = .267$). The interaction of foreperiod length with trial type was also statistically significant, $F_{(4, 6750)} = 2.76$, $p = .026$, $R^2 = .002$. However, analysis of the difference in temporal error between probe trials and control trials indicated the main effect of foreperiod duration was not statistically significant, $F_{(2, 665)} = 0.18$, $p = .832$, $R^2 = .001$. **Figure 5.4A** shows mean temporal error of movement onset for control, baseline, and probe trials for each foreperiod duration.

5.5.2 Urgency effects on peak force and rate of force development

Mean peak force was greatest for the 350 ms foreperiod duration (M = 6.43 N, SD = 2.43), with lower forces executed in the 700 ms (M = 6.11 N, SD = 2.01) and 1400 ms (M = 5.94 N, SD = 1.92) conditions. The main effect of foreperiod duration was statistically significant for peak force, $F_{(2, 6752)} = 13.27$, $p < .001$, $R^2 = .008$. Post-hoc tests indicated that in comparison to the 350 ms condition, average peak force was significantly lower in both the 700 ms ($p = .003$) and 1400 ms ($p < .001$) foreperiod durations. The main effect of trial type was not statistically significant, $F_{(2, 6752)} = 1.77$, $p = .17$, $R^2 = .001$, however, the interaction of foreperiod length with trial type on peak force was significant, $F_{(4, 6752)} = 2.52$, $p = .039$, $R^2 = .004$. Post-hoc tests indicated a significant difference in peak force between control and probe trials for the 1400 ms foreperiod duration ($p = .031$), but not for the 700 ms ($p = .071$), or 350 ms foreperiods ($p = .177$). **Figure 5.4B** shows mean peak force executed during control, baseline, and probe trials for each foreperiod duration.

Mean peak rate of force development was also greatest in the 350 ms foreperiod length, (M = 85.7 N/s, SD = 37.05), with movements showing decreased peak rate of force development in the 700 ms (M = 78.82 N/s, SD = 29.13) and 1400 ms (M = 79.09 N/s, SD = 27.84) foreperiods. This was indicated by a significant main effect of foreperiod duration, $F_{(2, 6752)} = 17.75$, $p < .001$, $R^2 = .005$. The main effect of trial type was also statistically significant for peak rate of force development, $F_{(2, 6752)} = 4.13$, $p = .016$, $R^2 = .001$, with mean peak rate of force development being greatest in probe LAS trials (M = 83.88 N/s, SD = 34.77), with lower peak rate of force development executed in control trials (M = 80.9 N/s, SD = 31.5) and baseline LAS trials (M = 80.92 N/s, SD = 31.28). The interaction of trial type with foreperiod length was not statistically significant, $F_{(4, 6752)} = 1.38$, $p = .237$, $R^2 = .001$. **Figure 5.4C** shows mean peak rate of force development executed during control, baseline, and probe trials for each foreperiod duration.

5.5.3 Orbicularis oculi electromyogram onset latency and amplitude

The amplitudes of OOc EMG responses to the LAS at baseline timing for each foreperiod duration were calculated as a percentage of EMG amplitudes to the LAS presented at rest prior to experimental trials. These percentages (M_{350} = 107.86%, SD = 23.86; M_{700} = 106.48%, SD = 24.75; M_{1400} = 106.85%, SD = 23.45) were not significantly different between foreperiod durations, $F_{(2, 393.5)} = .152$, $p = .859$, $R^2 = .001$. This suggests motor preparation at the time the baseline LAS was presented was not significantly different between foreperiod durations. A Bayesian linear model was run to examine the degree of support for the null hypothesis and returned $BF_{01} = 34.19$, indicating very strong evidence (Jeffreys, 1961) for the null hypothesis of differences between foreperiod durations. We also examined OOc EMG amplitude in baseline trials and a Bayesian linear model provided very strong evidence to suggest EMG amplitude did not differ as a function of foreperiod duration, $BF_{01} = 33.92$. Similarly, a Bayesian linear model of OOc onset latency after the baseline LAS indicated $BF_{01} = 14.50$, suggesting strong evidence to support the null hypothesis of OOc latency being modulated by the foreperiod duration of experimental trials (Jeffreys, 1961).

Statistically significant main effects of foreperiod duration, $F_{(2, 870.07)} = 21.92$, $p < .001$, $R^2 = .048$, and trial type, $F_{(1, 870.25)} = 12.01$, $p < .001$, $R^2 = .014$ were

observed for the linear mixed-effects models of OOc EMG onset latency. Furthermore, the interaction of foreperiod length with trial type was statistically significant, $F_{(2, 870.1)} = 7.27$, $p < .001$, $R^2 = .016$. Post-hoc tests indicated onsets of EMG in probe trials ($M = 38.06$ ms, $SD = 6.35$) were not significantly different from baseline trials ($M = 38.61$ ms, $SD = 5.55$) for the 350 ms foreperiod length ($p = .419$). However, EMG onset latencies for probe trials were significantly delayed in the 700 ms ($M = 41.52$ ms, $SD = 7.57$) and the 1400 ms ($M = 42.97$ ms, $SD = 6.74$) when compared to the respective onset latencies for baseline trials ($M_{700} = 39.93$ ms, $SD = 6.76$, $p = .038$; $M_{1400} = 39.83$ ms, $SD = 6.08$, $p < .001$). **Figure 5.4D** shows mean OOc EMG latency in baseline and probe trials for each foreperiod length.

The amplitude of OOc EMG was reduced from baseline ($M_{t\text{-}score} = 53.02$, $SD = 14.22$) in probe trials ($M_{t\text{-}score} = 46.72$, $SD = 12.22$), as indicated by a significant main effect of trial type, $F_{(1, 879.39)} = 101.66$, $p < .001$, $R^2 = .104$. However, the interaction of foreperiod duration with trial type was not statistically significant, $F_{(2, 879.69)} = 2.25$, $p = .106$, $R^2 = .005$. **Figure 5.4E** shows mean OOc EMG amplitude in baseline and probe trials for each foreperiod duration. Examination of OOc EMG amplitude t-scores as a percentage of baseline across foreperiod durations indicated a significant main effect of foreperiod duration, $F_{(2, 410.88)} = 6.54$, $p = .002$, $R^2 = .031$. Pairwise comparisons indicated the 1400 ms foreperiod length resulted in a significantly greater reduction of OOc EMG amplitudes from baseline to probe in comparison to the 350 ms foreperiod length ($p = .001$) and the 700 ms foreperiod length ($p = .041$; see **Figure 5.4F**). Finally, we calculated BFs to evaluate the degree of evidence to suggest the OOc response amplitudes after the probe stimulus showed an inhibitory effect. In contrast to corresponding analysis of MEP amplitude in experiment one, OOc responses showed evidence of inhibition for all foreperiod duration conditions. Evidence for an inhibitory effect increased with increasing preparation time, $BF_{10} = 24134.85$, 5465834, and 223496324928 for the 350 ms, 700 ms, and 1400 ms conditions, respectively.

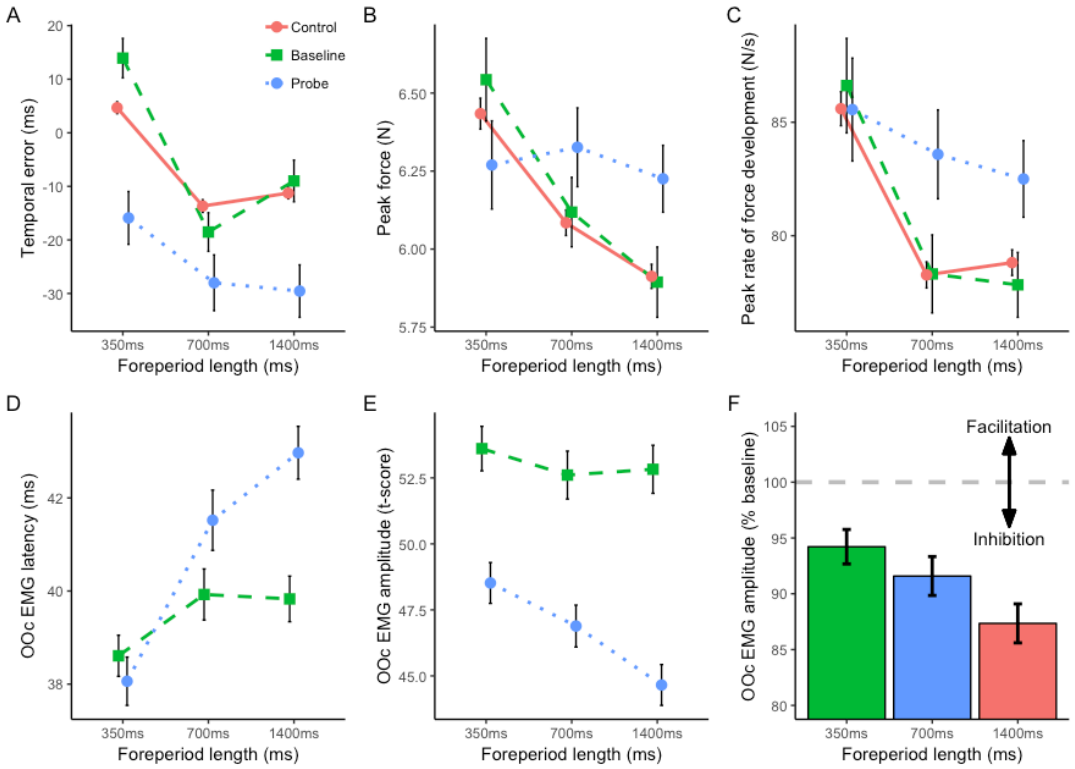


Figure 5.4. **A)**. Mean temporal error of movement onset across control, baseline, and probe trials for each foreperiod length. **B)**. Mean peak force of movements across control, baseline, and probe trials for each foreperiod length. **C)**. Mean peak rate of force development of movements across control, baseline, and probe trials for each foreperiod length. **D)**. Mean latency of orbicularis occuli electromyogram onset for baseline and probe trials across each foreperiod length. **E)**. Mean t-scores of Orbicularis oculi electromyogram amplitude in baseline and probe trials over foreperiod lengths. **F)**. Mean orbicularis oculi electromyogram amplitude after the probe stimulus as a percentage of median electromyogram amplitude after the baseline stimulus. Error bars represent standard error of the mean.

5.6 Discussion

In this work, we examined whether changes in the magnitude of preparatory suppression can be observed when urgency to prepare a motor response is manipulated, impacting the time available for motor circuits to engage in preparatory inhibition. These manipulations of urgency were achieved by modifying the duration of time that occurs between the start of the movement of a clock hand and the time a

prepared motor action should be initiated. We predicted time constraints on preparatory processes would reduce CS suppression and subsequently, in experiment one, examined whether inhibition of the CS tract is modulated by the urgency of preparation. We supplemented this in experiment two by using a LAS to examine whether inhibition of subcortical startle circuits was similarly modulated by the time available for preparation. Intense sensory stimuli are known to trigger prepared actions early and increase the magnitude of movement execution. Therefore, we analysed motor output after the LAS and examined how the effects of a LAS on motor output may also be modulated depending on the urgency of preparation.

5.6.1 Preparatory inhibition during low-urgency preparation

When urgency to perform a movement was low, in experiment one we observed evidence of the inhibition of CS pathways, as indicated by a reduction of the amplitude of MEPs elicited during motor preparation in comparison to MEPs elicited at a baseline period before the clock sweep began. Interestingly, in experiment two we also observed evidence of an inhibition of subcortical circuits related to the startle reflex, as measured by the latency and amplitude of startle related OOc responses. This is consistent with a recent report of a delay and reduction of magnitude of OOc responses to a LAS presented during preparation and prior to movement onset (Nguyen et al., 2020). Given preparatory suppression was clearly observed for low urgency movements as indexed by both MEPs and startle related OOc EMGs, it may be assumed that during low-urgency preparation, inhibitory effects can affect both cortical and subcortical motor-related circuits. However, there may be multiple inhibitory processes acting on the central nervous system over the course of action preparation. These separate processes may be difficult to discern from one another when there are little time constraints on preparation and the system is able to freely engage in preparation. As such, the examination of inhibition in both the CS tract and subcortical circuits when there is limited time available for preparation can be particularly useful in differentiating these potential processes, as discussed next.

5.6.2 Preparatory inhibition during time-constrained preparation

There was no evidence of CS suppression when preparation urgency was high in experiment one. In contrast, OOc amplitude after the probe LAS in experiment two was reduced for all foreperiod durations, although the magnitude of suppression was progressively increased as the amount of preparation time was increased. As such, there may be separate processes of premovement suppression which can be observed at different levels of the central nervous system. Therefore, a distinction should be made between preparatory inhibition of the CS tract, and a potentially more global inhibition which can be observed in subcortical circuits. Furthermore, the phenomenon of preparatory inhibition does in fact appear to be modified by the urgency of an impending motor action. In simple and choice RT tasks, preparatory inhibition is known to be sensitive to foreperiod duration. The magnitude of preparatory inhibition has previously been shown to be reduced at longer foreperiod durations (e.g. > 2000 ms) in simple and choice RT tasks (Davranche et al., 2007; Lebon et al., 2016; Touge et al., 1998; Van Elswijk et al., 2007). This is potentially due to impaired temporal estimation of the imperative signal as the foreperiod duration is increased (Jaskowski & Verleger, 1993), limiting the capability of the system to effectively engage preparatory processes. Here we used an anticipatory timing task which maintains high temporal predictability. We have shown an impairment of CS inhibition which appears to occur due to time constraints on preparatory processes. In this context, actions could be initiated in time which suggests CS suppression may not be an obligatory component of preparation.

5.6.3 Effects of sensory stimulation during time-constrained preparation

5.6.3.1 Sensory stimuli disrupt motor output in the absence of corticospinal suppression

A striking finding was that response vigour was enhanced in probe trials in comparison to control trials when movement urgency was low, but was reduced when movement urgency was high. This indicates that preparatory inhibition may modulate the effects of sensory stimulation on the execution of motor actions. The enhancement of force and vigour observed in the low urgency condition is consistent with previous reports in the StartReact literature, in which a LAS may add neural activity to motor program circuits which results in a greater magnitude of movement execution (Anzak et al., 2011; Marinovic et al., 2015; McInnes et al., 2020; Tresilian

& Plooy, 2006; Ulrich et al., 1998). The similar effects on peak force and vigour that occurred in probe trials regardless of whether the probe was a LAS or TMS pulse may be attributable, in experiment one, to accessory stimulation induced by the TMS coil discharge, rather than the stimulation provided by TMS itself (Hershenson, 1962). For example, when discharging, the TMS coil produces an auditory click and produces some tactile sensation on the head. Consequently, the TMS coil may have provided bimodal stimulation which contrasts to the unimodal LAS. Bimodal stimuli have been shown to have greater effects on the early triggering of movement and enhancement of vigour in comparison to unimodal stimuli (Marinovic et al., 2015), which may explain the particularly disruptive effects of TMS in the high urgency condition of experiment one. Using a Brüel and Kjaer sound level meter at a distance of 15 cm tangentially to the centre of the coil, and the TMS unit set at 46% of MSO (mean RMT of participants in experiment one), the peak amplitude of the sound emitted by the coil was measured at 83.9dBA. The disruption of vigour that we observed in both of our experiments is similar to that which has been reported by Xu-Wilson et al. (2011), who identified a decrement in the vigour of saccades when TMS was applied shortly before or soon after saccade onset. The effect was observed regardless of the stimulation site over the skull, indicating that like our findings, the observed reduction of vigour could be attributed to the accessory sensory stimulation emitted by the coil discharge. The resemblance of those findings with the disruption of motor output in the absence of CS suppression we observed here may warrant further examination of the manifestation of preparatory inhibition during saccade preparation.

5.6.3.2 Modulation of the orbicularis oculi response with preparation urgency

The latency of OOc responses to the LAS in experiment two mirrored the pattern of inhibition observed in FDI MEPs during experiment one. Suppression of OOc latency was observed during low-urgency preparation but not during high urgency preparation. Amplitude of the OOc response, however, yielded a different pattern. OOc amplitude was suppressed for all foreperiod durations, with an increasing magnitude of suppression with increasing preparation time. One possible explanation for the divergence between OOc latency and amplitude we observed here is the fact that startling stimuli can elicit two separate eyeblink components – the auditory

eyeblink reflex and the auditory startle reflex. These can be difficult to distinguish from one another when analysing EMG records. The auditory eyeblink occurs at short latencies and is thought to be mediated by mesencephalic circuits (Brown et al., 1991). This precedes the auditory startle reflex which, when activated, results in a later onset OOc response along with a generalised skeletomotor response. Importantly, the auditory startle reflex is thought to originate from bulbopontine circuits, a pathway which is distinct from those associated with the auditory eyeblink reflex (Brown et al., 1991). Given the auditory eyeblink is the first component to occur temporally, measurement of OOc onset is most likely to capture this response. Measurement of OOc EMG amplitude on the other hand, may capture the peak of the auditory eyeblink reflex, the auditory startle reflex, or both, depending on which response was largest in a given trial. As such, the distinctive responses of OOc in terms of onset latency versus amplitude may reflect a differing effect of motor preparation on auditory eyeblink versus startle responses. The utility of examining OOc EMG in this context should also be noted. Our findings indicate that the startle response may provide an indication of the inhibition of motor pathways prior to movement initiation without the need for the presentation of electromagnetic stimulation, which may be unsuitable for some participants (Rossi et al., 2009).

5.6.4 The role of corticospinal suppression during preparation

Overall, the failure to observe evidence of premovement CS suppression in close temporal proximity of movement initiation, when urgency to move was high, brings to question the assumption that this phenomenon is an integral part of motor preparation. Rather, preparatory suppression of the CS tract may reflect a strategy employed by the motor system to protect the prepared response from interference. Evaluation of the behavioural effects we observed in contexts where preparatory CS inhibition was evident in comparison to when it was not may shed light on the potential strategic purposes of this phenomenon. The direction of force modulation we observed is opposite to that which we had predicted. We had hypothesised that a lack of preparatory CS inhibition would result in an enhancement of force in probe trials, rather than a reduction. If, as previously proposed, preparatory inhibition serves to keep preparatory activation below initiation threshold (Duque & Ivry, 2009), then we would expect to see effects in line with those that we hypothesised –

earlier triggering and enhanced force of movements, as a result of acoustic stimulation close to movement onset, when in the absence of preparatory inhibition. While we observed earlier triggering of movements overall in probe trials, which may again be attributed to the StartReact effect in both of our experiments (Valls-Solé et al., 1999; see also Kohfield, 1971; Nickerson, 1973), the magnitude of the early triggering of movement by the probe stimulus did not appear to differ between foreperiod lengths. The results we present here, and previous findings that preparatory inhibition is present before self-timed movements (Ibáñez et al., 2020), are then inconsistent with the impulse control hypothesis (Duque et al., 2010; Duque & Ivry, 2009).

While we cannot rule out the hypotheses that premovement suppression of the CS tract serves to suppress the initiation of competing response selections or to reduce background noise in motor circuits, our data do not completely fit with these explanations. Therefore, we propose an alternative hypothesis which may better fit the data we report here; preparatory CS inhibition serves as a strategy to protect the prepared movement from external interference. We consider two circumstances which may lead to failures to engage this strategy. First, when there is little time to engage in preparatory processes, the speeded initiation of movement is prioritised which precludes preparatory CS inhibition from taking place, in turn leaving the prepared movement prone to interference from external sources. Alternatively, when the level of motor preparation is low, the motor system may deem it unnecessary to engage in preparatory CS inhibition given there is not a sufficiently prepared movement to be protected from interference, hence leaving movements vulnerable to be disturbed by external sources when they have eventually reached a higher state of preparation closer to the time of initiation. We cannot conclusively rule the absence of CS inhibition, as the temporal location of CS inhibition may simply move to a later time point with increasing urgency. This, however, seems unlikely given CS excitability rise usually occurs 100 ms prior to action onset, leaving less than 150 ms for inhibitory processes to begin and then cease. Furthermore, there was evidence of subcortical suppression, indicating it would be possible for a fast-acting inhibition mechanism to manifest at this short timescale. Regardless, there was a direct effect on motor output in the situation when there was no evidence of CS inhibition 250 ms prior to action onset. The finding that time constraints impact CS inhibition to a

greater degree than that of startle circuits may support the notion that a more generalised inhibition initially acts on motor circuits which evolves into a more specified inhibition appropriate for the selected action. A global inhibition preceding a more specified one aligns with the dynamic cortico-basal ganglia loop proposed by Nambu (2004). This model proposes that during preparation, basal ganglia initially globally inhibit both the thalamus and motor cortical areas. A second signal from the basal ganglia then disinhibits the selected motor program, and finally, a third signal strongly inhibits competing response selections. Furthermore, this cortico-basal ganglia system has been suggested to be involved in the modulation of sensory information (Criaud et al., 2021). Activity evoked by a LAS or TMS pulse which interact with the second or third signals of this dynamic loop may activate processes that reduce the outflow of activity to the specified muscle. Such an effect may be inferred by the reduced force and vigour we observed in the absence of preparatory inhibition.

5.7 Conclusions

There is a transient suppression of CS excitability prior to movement onset when movement urgency is low and there is sufficient time to prepare a motor response. In contrast, when a movement must be rapidly prepared and initiated and there is little time to engage in preparatory processes, there is no evidence for CS suppression at the same temporal location. Furthermore, both a LAS and TMS pulse were found to impair movement force when presented in the absence of preparatory inhibition. The impairment of force we observed after TMS may be attributed to the accessory sensory stimulation provided by the TMS coil discharge. We conclude that preparatory inhibition may not be a physiologically necessary component of movement preparation, but rather, may reflect a strategy employed by the central nervous system which can serve to protect prepared movements from external interference.

CHAPTER SIX: GENERAL DISCUSSION

6.0 Overall summary

6.0.1 Mechanisms of the StartReact effect and motor preparation

The work presented in this thesis examined the types of movements that receive most benefit from intense sensory stimulation. The neural mechanisms underlying such stimulation are still a matter of debate. Initial accounts of the StartReact effect suggested intense sensory stimuli can activate a unique triggering circuit which bypasses the cerebral cortex, allowing for the extremely short RTs that are a hallmark of the phenomenon. Later proposals suggested that a fast transcortical pathway is activated by intense sensory stimulation, or that voluntary motor pathways are simply enhanced by the stimulation (Alibiglou & MacKinnon, 2012; Marinovic & Tresilian, 2016). The initial subcortical triggering hypothesis implies sufficient activity in subcortical startle circuits must be evoked for the StartReact effect to take place (Carlsen et al., 2004a). As such, under the startle triggering hypothesis, startle activity in SCM has been used as a neurophysiological means to discern StartReact triggered actions from voluntary ones.

In chapter two, several previously published datasets were reanalysed to evaluate whether startle activity is indeed a necessary condition for the StartReact effect to occur, as would be expected if triggering relies on startle circuits. Such subcortical triggering relies on several assumptions, which we addressed in chapter two. First, if a unique triggering circuit underlies the StartReact effect which is different from that of voluntary movements, then the data should be distributed bimodally, with a short-latency peak in the probability density function representing the subcortical triggering and a second longer-latency peak that represents voluntarily triggered movements. Our analysis failed to provide evidence that the data are distributed bimodally. Second, traditional analyses in the StartReact literature have examined differences between trials which occur with and without concomitant SCM activity. However, it was later suggested that startle activity is not necessary, nor sufficient, to observe the short RTs that are a hallmark of the StartReact effect (Marinovic & Tresilian, 2016). We examined whether this was the case by categorising trials into short and long RTs using CDFs and determining the proportion of trials in each category that occurred with concomitant startle activity. Using Bayesian methods, we determined that there is a task-dependent association of

startle activity with RT, which makes the traditional SCM-based method of analysis unreliable. Specifically, our analysis indicated that there are several types of movement which do not reliably show startle activity in trials with short RTs. Our analysis further suggested that similarly, for some movements, trials with long RTs often show coinciding startle activity. We also observed that those movements which did not show an association between RT and SCM activity all failed to show a statistically significant difference in RT between SCM+ and SCM- trials. This task-dependent association of RT with SCM activity can consequently affect both the manifestation of, as well as the ability to detect, StartReact on the basis of SCM activity. Therefore, analysis in StartReact research on the basis of startle activity can be unreliable. As such, we posed an alternative method of trial categorisation using CDFs of RT. Using this method, we found some evidence of certain tasks being more amenable to StartReact than others, but found little evidence of this effect in muscles which strongly differ in their cortical versus subcortical efferent connectivity. Under the startle triggering hypothesis, actions which engage the reticulospinal tract more strongly should be more amenable to StartReact. As such, we suggested that differences between actions in their triggering by the intense sensory stimulus may be due to the functionality of the movement, as has been previously proposed (Marinovic, de Rugy, et al., 2014).

In chapter three, we investigated how the efferent connectivity and force of a prepared action may impact its triggering by a LAS. Our analysis failed to detect a significant difference in RT between movements which differ in the strength of reticulospinal versus corticospinal contributions. Peak force and vigour, however, were facilitated more for the flexion movement, which may have stronger corticospinal connectivity (Godfrey et al., 2013; Koganemaru et al., 2010; McMillan et al., 2004; Park & Li, 2013; Quinn et al., 2018; Vallence et al., 2012). As such, we suggest this provides evidence for cortical involvement in the StartReact effect, given the production of force is highly correlated with M1 activity. Our analysis of the force enhancements provided by the LAS also indicated that the neural activity introduced to motor program circuits by the LAS is additive to the preparatory activation of the voluntary action, with these two processes summating to produce the final magnitude of the prepared action. These findings support cortical involvement in the StartReact effect and further highlight the utility of examining

indices of movement execution as a probe of the neural correlates of action preparation.

Modulation of the StartReact effect by maintaining isometric muscle contractions during preparation for action was examined in chapter four. We determined that contractions during preparation can provide some overall benefit on RT when they are maintained contralaterally to and congruently with the responding hand, at 10% of MVC. This type of contraction also provided the most benefit on peak force and vigour. Importantly, the enhancement of the StartReact effect by such contractions was muscle and laterally specific – no benefit was provided in a unilateral task or when the contraction was incongruent with the response. In chapter four, and later in this chapter, an outline of how these findings may suggest cortical involvement in the StartReact effect is provided.

The final experimental chapter examined how corticospinal suppression during action preparation - preparatory inhibition - may impact the StartReact effect. The time available to prepare and initiate an anticipatory action was either shortened or lengthened, and we demonstrated corticospinal suppression was not present 250 ms prior to action onset when preparation time was limited. The absence of preparatory inhibition showed little effect on RT, with no detectable change in the shortening of initiation time by the LAS or TMS pulse across the foreperiod lengths. However, we observed that in the absence of preparatory inhibition, sensory stimuli were disruptive to motor output. Rather than an enhancement of force and vigour as is typical in the StartReact effect, a LAS (as well as TMS pulse) reduced force and vigour when there was no detectable suppression of corticospinal excitability. This finding reflects the previous observation that a LAS inhibits the corticospinal tract when presented during low states of preparation, but excites the corticospinal tract when presented during high preparatory states (Marinovic, Tresilian, et al., 2014). Consistent with this state-dependent effect of a LAS on corticospinal excitability, the effects of a LAS on motor output appear to be state-dependent, with acoustic stimulation being suppressive of motor output in the absence of preparatory inhibition, while providing an enhancement of motor output when the system has advanced to a higher state of preparatory inhibition. These findings provide further evidence for a key role of the corticospinal tract in StartReact triggering. In light of disruptive effects of sensory stimuli in the absence of corticospinal suppression, we

provide an alternative account of preparatory inhibition, in which the suppression of corticospinal excitability may act to protect prepared responses from external interference. In addition, the dissociable suppressive effects observed in the corticospinal tract versus the suppression observed in subcortical startle circuits suggests a generalised suppression may precede a more specific inhibition which eventually acts on the specified effector.

6.0.2 Potential therapeutic targets

Along with the mechanistic insights gained from the experiments reported here, the findings of this thesis may provide direction for practical applications of intense sensory stimulation. Our analysis of a range of different movement types in chapter two suggested that functionality of a prepared action may influence the benefit on RT that is derived from intense sensory stimulation. As such, functional movements which may engage a wider and more distributed network of neurons (Flament et al., 1993; Graziano, 2011; Graziano & Aflalo, 2007; Kouchtir-Devanne et al., 2012) may be more amenable to facilitation by an intense sensory stimulus (Marinovic, de Rugy, et al., 2014).

We have also shown that overall, movements of low force receive a proportionally greater benefit on force and vigour by the LAS in comparison to movements of higher force. This may be particularly beneficial in that more trials can be performed in a rehabilitative setting before muscle fatigue takes place. Furthermore, a sustained contraction at low to moderate levels of force can enhance the StartReact effect when the contraction is contralateral to the limb that is in preparation for action. These findings are applicable to neurological conditions such as stroke where impairment is often experienced on one side of the body. Engagement of the unimpaired side may therefore aid in the rehabilitative process.

Finally, given the increase of corticospinal suppression we observed with increasing preparation times, along with the disruptive effects of acoustic stimuli on motor output in the absence of such suppression, applications of the StartReact effect should ensure that sufficient time (i.e. at least 1400 ms) is allowed for preparation to take place. This would ensure the sensory stimulation can provide the most benefit to motor output. Further investigations are needed to confirm whether the findings we

present here are indeed similar in clinical groups where voluntary movement is impaired.

6.1 The dissociation between action initiation and magnitude

While we consistently observed the early initiation of movement in kind with the StartReact effect, we often observed little or no evidence that the magnitude of this shortening was modulated by the experimental conditions. For example, we did not find evidence for differences in RT shortening between muscle types in chapter three. While there was some benefit overall on RT in our CDF analysis in chapter four, the magnitude of the RT shortening by the LAS did not appear to be modified by the type of contraction maintained during preparation. Nor did we observe a modulation of RT shortening by foreperiod length in chapter five. In contrast, the enhancement of peak force and vigour by the LAS often showed a clear modulation by the experimental conditions. For example, peak force and vigour gains introduced by the LAS were greater for the flexor muscle in chapter three, were greater for the 10% MVC contralateral flexion contraction in chapter four, and there was a clear modulation of peak force and vigour in chapter five which depended on the level of preparatory inhibition.

There are a number of potential explanations that may contribute to this apparent dissociation between action initiation and magnitude. First, action initiation and execution may arise from entirely distinct neural signals, with execution being unrelated to the initiation process. Haith et al. (2016) previously found that RTs can be reduced from the usual voluntary RT by approximately 100 ms in a paradigm referred to as a forced-RT task. In light of these data, Haith et al. (2016) suggested that if preparation can be completed in a time period substantially shorter than the time taken for initiation to take place, then initiation must be a separate process which is fundamentally distinct from the preparation process. Similar to this, the signal that produces initiation may be independent of the signal that produces the features of action execution such as force and vigour. This may explain the differing benefit by the LAS we observed on RT and peak force/vigour.

Alternatively, RT may be more prone to a floor effect, limiting the ability to detect RT differences in comparison to force and vigour which may be affected to a lesser extent by a ceiling effect. However, this explanation appears to be unlikely

given we addressed this issue in chapter three by using a LAS at two intensities. We observed similar effects on RT regardless of whether the LAS was high or low intensity.

Another potential explanation, which was also described in chapter three, is based on the association of M1 activity with force output. For example, the LAS may evoke activity in M1 which adds to the magnitude of motor output but does not contribute to the rate of rise to initiation threshold. Finally, the activation model may be able to provide an account of the dissociation between action initiation and magnitude. If differences in StartReact magnitude depend on a modulation of the rate of rise of LAS-evoked activation, then differences between the rate of rise only become apparent at longer timescales after LAS presentation. **Figure 6.1** depicts potential activation changes in motor circuits over the course of preparation. Voluntary activation, high magnitude StartReact activation, and lower magnitude StartReact activation are shown in this figure. Both the high magnitude and lower magnitude StartReact activation are clearly different from voluntary activation in terms of initiation time (threshold crossing) and peak force (activation amplitude). However, when comparing the high and low magnitude StartReact activation slopes in **Figure 6.1A**, it is more difficult to distinguish the time of initiation. In this example, the LAS-induced activation is introduced close to movement onset, when the motor circuits are in a high state of preparation. In **Figure 6.1B**, LAS-induced activation is introduced earlier in preparation, which allows differences in the time-to-threshold between the two StartReact slopes to be more easily detected. Therefore, the divergence of high-magnitude and low-magnitude StartReact activation increases as a function of time. As such, the divergence of the rate of rise of activation only becomes apparent at a time point after initiation threshold has been crossed when a LAS is presented during high states of preparation (i.e. **Figure 6.1A**). Importantly, differences in amplitude between high and low magnitude StartReact activation can be detected regardless of whether the LAS was presented late in preparation (**Figure 6.1A**), or early in preparation (**Figure 6.1B**). Therefore, given the LAS was presented late in preparation for the experiments contained within this thesis, the change of slope for different movements may have been difficult to detect in terms of RT. This highlights the utility of examining aspects of movement other than RT. Force and vigour can provide further insights to motor

preparation that may be missed by simply examining the initiation of movement. While the StartReact effect has been previously demonstrated after intense sensory stimulation earlier in preparation, these studies have not compared different movement types in this protocol. Similarly, little attention has been placed on force and vigour in this context. Future investigations in the StartReact literature may therefore choose to present the intense sensory stimulus earlier in preparation when comparing the modulation of the StartReact effect (in the facilitation of both action initiation and magnitude) between different movement types.

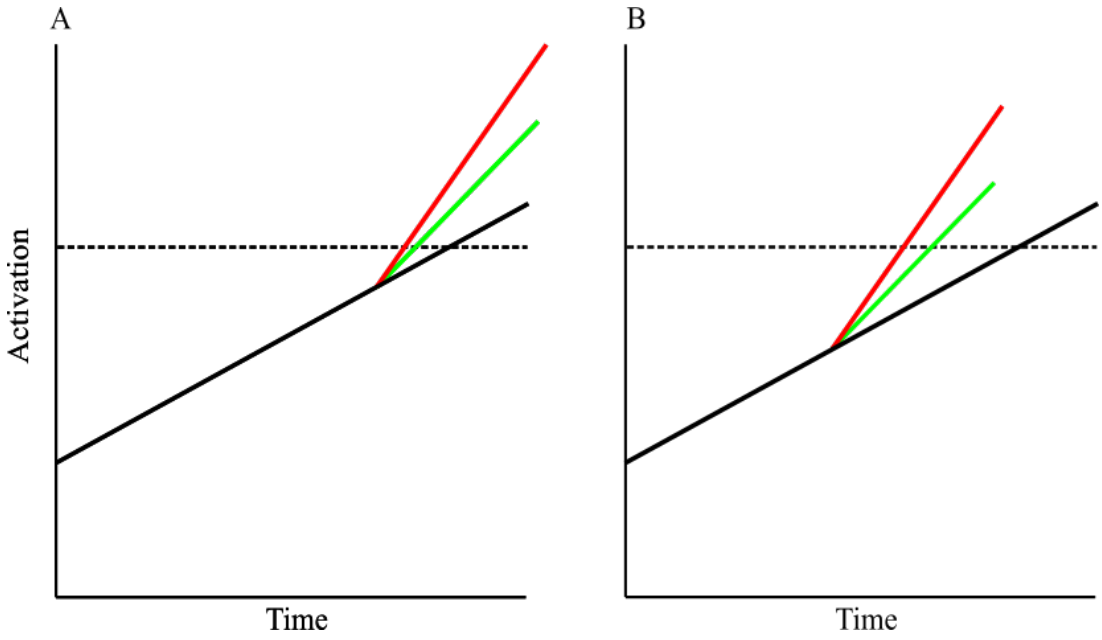


Figure 6.1. Activation introduced by the LAS at different time points. **A).** Preparatory activation (black line) builds up as preparation advances. The prepared action is initiated when activation exceeds initiation threshold (dashed line). When a LAS is presented late in preparation, it is difficult to differentiate the time of initiation between LAS-evoked activation of lower (green line) and higher (red line) magnitude. The amplitude of the activation, corresponding with peak force, is easier to differentiate between the two LAS-evoked activation magnitudes. **B).** When the same slopes of LAS-evoked activation occur earlier in preparation, it is easier to differentiate threshold crossing between high and low magnitude LAS activity.

6.2 The dissociation between eyeblink latency and magnitude

Similar to the different effects observed between the features of the voluntary motor output (i.e. RT and force), we observed a dissociation between OOc latency and amplitude in chapter five. OOc latency more closely reflected the pattern observed in the FDI MEP amplitude. Specifically, the latency of OOc responses demonstrated suppression during low-urgency preparation and a trend toward facilitation during high-urgency preparation. However, the amplitude of the OOc response in experiment two was reduced for all foreperiod lengths, although the magnitude of suppression was greater when movements were prepared with low urgency in comparison to when urgency to prepare movement was higher. This represents a dissociation between the latency and amplitude of OOc responses to the LAS.

Distinctions between startle-related activity of OOc in terms of the latency and amplitude of responses have been reported previously (Ison et al., 1973; Lipp et al., 2001; Pilz & Schnitzler, 1996). One possible explanation for the divergence between OOc latency and amplitude we observe here is the fact that startling stimuli can elicit two separate eyeblink components, which are often difficult to distinguish from one another when analysing EMG records. The auditory eyeblink reflex, which serves as an eye protective mechanism, occurs at short latencies and is thought to be mediated by mesencephalic circuits (Brown et al., 1991). This precedes the auditory startle reflex which, when activated, results in a later onset OOc response along with a generalised skeletomotor response. Importantly, the auditory startle reflex is thought to originate from bulbopontine circuits, a pathway of which is distinct from those associated with the auditory eyeblink reflex (Brown et al., 1991). Given the auditory eyeblink is the first component to occur temporally, measurement of OOc onset is most likely to capture this response. Measurement of OOc EMG amplitude on the other hand, may capture the peak of the auditory eyeblink reflex, the auditory startle reflex, or both, depending on which response was largest in a given trial. As such, the distinctive responses of OOc in terms of onset latency versus amplitude may reflect a differing effect of motor preparation on auditory eyeblink versus startle responses.

6.3 The cerebral cortex and StartReact triggering

Overall, the findings reported here make it difficult to refute the involvement of the cortex in the StartReact effect. A number of observations bring doubt to the startle

triggering hypothesis of the StartReact effect. For example, we failed to find evidence that startle activity is necessary for the StartReact effect to occur. Potential differences in the degree of facilitation derived from intense sensory stimulation that can be observed between different movement types may be attributed to the functionality of the movement employed. For example, intense sensory stimuli may contribute more to motor output when the prepared action engages a larger, and more functionally relevant cortical circuit (Marinovic, de Rugy, et al., 2014). Furthermore, the flexor muscle of the wrist, which has been suggested to have stronger functional corticospinal connectivity (Godfrey et al., 2013; Koganemaru et al., 2010; McMillan et al., 2004; Park & Li, 2013; Quinn et al., 2018; Vallence et al., 2012), received a greater benefit from the LAS in comparison to the extensor which likely receives less input from the corticospinal tract. The maintenance of a sustained muscle contraction contralateral to the responding hand enhanced the StartReact effect, which may occur through interhemispheric transmission of neural activity in motor circuits (Carson et al., 2004) via the uncrossed descending corticospinal fibres (Phillips & Porter, 1964), or through activation of the ipsilateral descending cortico-reticulo-propriospinal pathway (Bradnam et al., 2013). Finally, changes in corticospinal excitability over the course of preparation appeared to directly impact the StartReact effect – in the absence of preparatory inhibition, the enhancement of force and vigour by the LAS was reversed to an effect of disruption.

One pathway potentially implicated by our findings which has not been the focus of attention in the StartReact context is the rubrospinal tract (Kuypers & Brinkman, 1970; Lawrence & Kuypers, 1968; Lemon, 2008; see **Figure 1.4**). The rubrospinal tract receives significant input from the cortex and importantly, consistent with our findings, particularly contributes to modulations of force and velocity in flexors of the elbow and wrist (Lemon, 2008). If transmission (either directly or indirectly) of activity from startle-related areas (e.g. the PMRF) or from the auditory cortex to the magnocellular red nucleus, which gives rise to the rubrospinal tract, can be ascertained, then this may provide support for a key involvement of the rubrospinal tract in StartReact triggering. However, it should be noted that the rubrospinal tract is thought to be minor in humans (Rea, 2015). Another pathway which has received little attention in the StartReact literature, the cortico-reticulo-propriospinal tract, may also be implicated in the triggering of

prepared actions by intense sensory stimuli (Bradnam et al., 2013). As noted in chapter four, this tract is believed to be important in the functional recovery of movement after stroke and its role in triggering is supported by the findings of this thesis (Bradnam et al., 2013). While cortical involvement in StartReact triggering cannot be directly determined from these experiments, the findings of this thesis are in favour of cortical involvement in the StartReact effect, either through a fast transcortical pathway (e.g. see **Figure 1.2**) or simply by an enhancement of usual voluntary motor pathways. These findings provide novel insights of the mechanistic properties of the StartReact effect, as well as more generally of the process of motor preparation, initiation, and execution.

6.4 Facilitation via noradrenergic and dopaminergic systems

Finally, it should be considered how the enhancement of motor pathways by a LAS may occur through an increase in arousal and phasic catecholamine activity. High intensity sensory stimuli can evoke not only motor activity (both reflexive and voluntary), but can also produce a brief, wide-spread surge of arousal throughout the brain (Foote et al., 1980; Hackley & Valle-Inclán, 1999; Tona et al., 2016). Arousal induced by a LAS has the potential to contribute to the increase of force and vigour observed in the StartReact effect. This may occur through activation of locus coeruleus (LC). LC is a noradrenergic nucleus found in the brainstem which responds quickly to salient sensory stimuli (Aston-Jones et al., 1991) and is thought to be a major contributor to the arousal which is induced by intense sound (Foote et al., 1980; Hackley & Valle-Inclán, 1999; Mather & Harley, 2016; Szabadi, 2012; Tona et al., 2016). There are projections from LC to several cortical, subcortical, and spinal areas which are used to release noradrenaline throughout the central nervous system (Foote et al., 1983). Therefore, activation of LC by sensory stimuli can impact ongoing perceptual, cognitive, and motor activity. Importantly, the LC-noradrenaline system is thought to have significant interactions with the dopamine system (Ranjbar-Slamloo & Fazlali, 2020). For example, co-release of noradrenaline and dopamine occurs at noradrenergic terminals, and stimulation/suppression of LC has been found to produce parallel changes in both noradrenaline and dopamine concentration in the cortex of rats (Devoto et al., 2005a, 2005b).

While the effect of noradrenaline on motor output is unclear, dopamine has well-known effects on movement initiation and execution. In mice, dopamine neurons of the substantia nigra pars compacta increase phasic activity during action preparation, and when there are higher levels of activity in these dopamine neurons, there is a higher probability of initiating movement, movements are initiated at shorter latencies, and are executed with greater vigour (Da Silva et al., 2018). It should be noted that activation of LC has been found to take approximately 200 ms to reach its peak after the presentation of salient sensory stimuli in non-human primates (Joshi et al., 2016). In the context of the StartReact effect, where movements are quickly initiated after the presentation of a LAS, the time-course of LC activation is likely too long to impact the initiation of movement. Therefore, activation of LC by the LAS is unlikely to be a major contributor to the shortening of RT by the LAS. However, this time-course is compatible with force and vigour enhancements in the StartReact effect. Modulations of phasic LC activity by a LAS may therefore contribute to modulations of the facilitation of force and vigour, but not RT, which were observed throughout this thesis.

In addition, the motor symptoms of Parkinson's disease, which include impaired movement initiation and execution (e.g. bradykinesia) have been understood to be a result of a loss of dopamine neurons of the substantia nigra which modulate cortical-striatal loops (Dickson et al., 2009; Hoehn & Yahr, 1967; Jankovic, 2008). However, it has been suggested that LC degeneration is a major contributor to the progression of Parkinson's disease. For example, LC is one of the first brain structures to show degeneration in Parkinson's, with LC-related noradrenergic deficiency occurring earlier, to a greater extent, and having a stronger correlation with disease severity in comparison to substantia-nigra-related dopamine deficiency (Del Tredici et al., 2002; Vermeiren & De Deyn, 2017). In addition to the fact that LC noradrenergic neurons project directly onto the substantia nigra (Benarroch, 2009), this provides strong evidence to support the relationship of the noradrenergic and dopaminergic systems. Therefore, it is feasible that LC activation via a LAS could lead to phasic noradrenaline/dopamine release, which may contribute to facilitation of movement execution observed in the StartReact effect via an enhancement of voluntary motor pathways.

6.5 Future research directions

There are a number of implications for future research that arise from the findings of this thesis. For example, given the issues we present related to the examination of RT shortening on the basis of startle activity in chapter two, we suggest future research should include CDF analyses in order to evaluate entire RT distributions. Furthermore, throughout this thesis several suggestions are made regarding the practical applications of the StartReact effect. Further investigation may be required to confirm that the findings presented here in unimpaired individuals hold out in clinical populations. There are likely significant neural reorganisations within sensorimotor circuits that occur after neurological conditions that affect the voluntary control of movement, which may impact the patterns of facilitation observed between healthy and clinical populations (Latash & Greg Anson, 1996). However, the StartReact effect has been observed to be highly comparable between individuals whose voluntary movement is unimpaired versus those whose movement has been impaired after stroke (Honeycutt et al., 2014, Honeycutt et al., 2015; Marinovic et al., 2016). As such, the current findings would be expected to replicate in clinical samples. Future investigations may further examine whether the magnitude of facilitation provided in therapeutic settings is of practical significance.

Furthermore, future research may take into account the suggestion made previously in this chapter that the ability to detect differences between movement types in RT shortening by a LAS may be reduced as the voluntary activation in motor program circuits is increased (see **Figure 6.1**). We have highlighted the utility of examining peak force and vigour in this context. However, is it possible differences between different movement types in their triggering by a LAS may be more easily detected if stimulation is provided earlier in preparation. Therefore, our understanding of the StartReact effect may be strengthened if future research employs intense sensory stimulation earlier in preparation when comparing movement types. If in the comparison of different movement types, differences in RT shortening can be detected with earlier LAS presentation, and if these differences corroborate with peak force and vigour enhancements, this would provide strong evidence to support the activation model's account of the StartReact effect (see **Figure 6.1**).

The final point to note is that our examination of corticospinal suppression during action preparation in chapter five was limited in that our probe stimulus was presented at one time point, 250 ms prior to movement onset. While we detected no evidence of corticospinal suppression at this time point, we cannot rule out that a more rapid corticospinal suppression may begin > -250 ms prior to movement onset. It would be unlikely that this is the case, considering we observed subcortical suppression (as indexed by decreased OOc amplitudes after a LAS) at the same temporal location, indicating it is feasible for suppression of motor circuits to begin at such a short timescale. However, future investigations may examine corticospinal excitability at additional timepoints > -250 ms prior to movement onset in order to rule out this possibility.

REFERENCES

Alagona, G., Delvaux, V., Gérard, P., De Pasqua, V., Pennisi, G., Delwaide, P. J., Nicoletti, F., & Maertens De Noordhout, A. (2001). Ipsilateral motor responses to focal transcranial magnetic stimulation in healthy subjects and acute-stroke patients. *Stroke*, *32*(6), 1304–1309. https://doi.org/10.1161/01.STR.32.6.1304

Albert, J. H. (1997). Bayesian testing and estimation of association in a two-way contingency table. *Journal of the American Statistical Association*, *92*(438), 685. https://doi.org/10.2307/2965716

Alibiglou, L., & MacKinnon, C. D. (2012). The early release of planned movement by acoustic startle can be delayed by transcranial magnetic stimulation over the cortex. *Journal of Physiology*, *590*(4), 919–936. https://doi.org/10.1113/jphysiol.2011.219592

Anthony, B. J., & Putnam, L. E. (1985). Cardiac and blink reflex concomitants of attentional selectivity: A comparison of adults and young children. *Psychophysiology*, *22*(5), 508–516. https://doi.org/10.1111/j.1469-8986.1985.tb01641.x

Anzak, A., Tan, H., Pogosyan, A., & Brown, P. (2011). Doing better than your best: Loud auditory stimulation yields improvements in maximal voluntary force. *Experimental Brain Research*, *208*(2), 237–243. https://doi.org/10.1007/s00221-010-2474-1

Anzak, A., Tan, H., Pogosyan, A., Djamshidian, A., Ling, H., Lees, A., & Brown, P. (2011). Improvements in rate of development and magnitude of force with intense auditory stimuli in patients with Parkinson's disease. *European Journal of Neuroscience*, *34*(1), 124–132. https://doi.org/10.1111/j.1460-9568.2011.07735.x

Arikuni, T., Watanabe, K., & Kubota, K. (1988). Connections of area 8 with area 6 in the brain of the macaque monkey. *Journal of Comparative Neurology*, *277*(1), 21–40. https://doi.org/10.1002/cne.902770103

Ashe, J. (1997). Force and the motor cortex. *Behavioural Brain Research*, *86*(1), 1–15. https://doi.org/10.1016/S0166-4328(96)00145-3

Aston-Jones, G., Chiang, C., & Alexinsky, T. (1991). Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. *Progress in Brain Research*, *88*, 501–520. https://doi.org/10.1016/S0079-6123(08)63830-3

Baker, S. N. (2011). The primate reticulospinal tract, hand function and functional recovery. *Journal of Physiology*, *589*(23), 5603–5612. https://doi.org/10.1113/jphysiol.2011.215160

Bell, S. J., Lauer, A., Lench, D. H., & Hanlon, C. A. (2018). Visual attention affects the amplitude of the transcranial magnetic stimulation-associated motor-evoked potential: A preliminary study with clinical utility. *Journal of Psychiatric Practice*, *24*(4), 220–229. https://doi.org/10.1097/PRA.0000000000000321

Benarroch, E. E. (2009). The locus ceruleus norepinephrine system: Functional organization and potential clinical significance. *Neurology*, *73*(20), 1699–1704. https://doi.org/10.1212/WNL.0b013e3181c2937c

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bernstein, I. H., Clark, M. H., Edelstein, B. A., & Grant, D. A. (1969). Effects of an auditory signal on visual reaction time. *Journal of Experimental Psychology*, *80*(3), 567–569. https://doi.org/10.1037/h0027444

Binkofski, F., Fink, G. R., Geyer, S., Buccino, G., Gruber, O., Shah, N. J., Taylor, J. G., Seitz, R. J., Zilles, K., & Freund, H.-J. (2002). Neural activity in human primary motor cortex areas 4a and 4p is modulated differentially by attention to action. *Journal of Neurophysiology*, *88*(1), 514–519. https://doi.org/10.1152/jn.2002.88.1.514

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & Van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, *42*(1), 1–15. https://doi.org/10.1111/j.1469-8986.2005.00271.x

Bonato, P., D'Alessio, T., & Knaflitz, M. (1998). A statistical method for the

measurement of muscle activation intervals from surface myoelectric signal during gait. *IEEE Transactions on Biomedical Engineering*, *45*(3), 287–299. https://doi.org/10.1109/10.661154

Bradnam, L. V., Stinear, C. M., & Byblow, W. D. (2013). Ipsilateral motor pathways after stroke: Implications for noninvasive brain stimulation. *Frontiers in Human Neuroscience*, *7*. https://doi.org/10.3389/fnhum.2013.00184

Brochier, T., Boudreau, M. J., Paré, M., & Smith, A. M. (1999). The effects of muscimol inactivation of small regions of motor and somatosensory cortex on independent finger movements and force control in the precision grip. *Experimental Brain Research*, *128*(1–2), 31–40. https://doi.org/10.1007/s002210050814

Brown, P., Rothwell, J. C., Thompson, P. D., Britton, T. C., Day, B. L., & Marsden, C. D. (1991). New observations on the normal auditory startle reflex in man. *Brain*, *114*(4), 1891–1902. https://doi.org/10.1093/brain/114.4.1891

Burle, B., Vidal, F., Tandonnet, C., & Hasbroucq, T. (2004). Physiological evidence for response inhibition in choice reaction time tasks. *Brain and Cognition*, *56*(2), 153–164. https://doi.org/10.1016/j.bandc.2004.06.004

Campbell, A. D., Squair, J. W., Chua, R., Inglis, J. T., & Carpenter, M. G. (2013). First trial and start react effects induced by balance perturbations to upright stance. *Journal of Neurophysiology*, *110*(9), 2236–2245. https://doi.org/10.1152/jn.00766.2012

Carlsen, A. N., Chua, R., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2004a). Prepared movements are elicited early by startle. *Journal of Motor Behavior*, *36*(3), 253–264. https://doi.org/10.3200/JMBR.36.3.253-264

Carlsen, A. N., Chua, R., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2009). Differential effects of startle on reaction time for finger and arm movements. *Journal of Neurophysiology*, *101*(1), 306–314. https://doi.org/10.1152/jn.00878.2007

Carlsen, A. N., Chua, R., Inglis, T. J., Sanderson, D. J., & Franks, I. M. (2004b). Can prepared responses be stored subcortically? *Experimental Brain Research*, *159*(3), 301–309. https://doi.org/10.1007/s00221-004-1924-z

Carlsen, A. N., Chua, R., Summers, J. J., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2009). Precues enable multiple response preprogramming: Evidence from startle. *Psychophysiology*, *46*(2), 241–251. https://doi.org/10.1111/j.1469-8986.2008.00764.x

Carlsen, A. N., Dakin, C. J., Chua, R., & Franks, I. M. (2007). Startle produces early response latencies that are distinct from stimulus intensity effects. *Experimental Brain Research*, *176*(2), 199–205. https://doi.org/10.1007/s00221-006-0610-8

Carlsen, A. N., Hunt, M. A., Inglis, J. T., Sanderson, D. J., & Chua, R. (2003). Altered triggering of a prepared movement by a startling stimulus. *Journal of Neurophysiology*, *89*(4), 1857–1863. https://doi.org/10.1152/jn.00852.2002

Carlsen, A. N., & MacKinnon, C. D. (2010). Motor preparation is modulated by the resolution of the response timing information. *Brain Research*, *1322*, 38–49. https://doi.org/10.1016/j.brainres.2010.01.076

Carlsen, A. N., & Maslovat, D. (2019). Startle and the StartReact effect. *Journal of Clinical Neurophysiology*, *36*(6), 452–459. https://doi.org/10.1097/WNP.0000000000000582

Carlsen, A. N., Maslovat, D., & Franks, I. M. (2012). Preparation for voluntary movement in healthy and clinical populations: Evidence from startle. *Clinical Neurophysiology*, *123*(1), 21–33. https://doi.org/10.1016/j.clinph.2011.04.028

Carlsen, A. N., Maslovat, D., Lam, M. Y., Chua, R., & Franks, I. M. (2011). Considerations for the use of a startling acoustic stimulus in studies of motor preparation in humans. *Neuroscience and Biobehavioral Reviews*, *35*(3), 366–376. https://doi.org/10.1016/j.neubiorev.2010.04.009

Carlsen, A. N., Nagelkerke, P., Garry, M., Hodges, N., & Franks, I. M. (2000). Using the startle paradigm to investigate the nature of a prepared response. *Journal of Sport and Exercise Psychology*, *22*, 24.

Carson, R. G. (1995). The dynamics of isometric bimanual coordination. *Experimental Brain Research*, *105*(3), 465–476. https://doi.org/10.1007/BF00233046

Carson, R. G., Riek, S., Mackey, D. C., Meichenbaum, D. P., Willms, K., Forner, M., & Byblow, W. D. (2004). Excitability changes in human forearm

corticospinal projections and spinal reflex pathways during rhythmic voluntary movement of the opposite limb. *Journal of Physiology*, *560*(3), 929–940. https://doi.org/10.1113/jphysiol.2004.069088

Castellote, J. M., & Kofler, M. (2018). StartReact effects in first dorsal interosseous muscle are absent in a pinch task, but present when combined with elbow flexion. *PLoS ONE*, *13*(7), e0201301. https://doi.org/10.1371/journal.pone.0201301

Castellote, J. M., Kofler, M., Mayr, A., & Saltuari, L. (2017). Evidence for startle effects due to externally induced lower limb movements: Implications in neurorehabilitation. *BioMed Research International*, *2017*, 1–13. https://doi.org/10.1155/2017/8471546

Chen, R., Yaseen, Z., Cohen, L. G., & Hallett, M. (1998). Time course of corticospinal excitability in reaction time and self- paced movements. *Annals of Neurology*, *44*(3), 317–325. https://doi.org/10.1002/ana.410440306

Chen, Y. T., Li, S., Zhou, P., & Li, S. (2016). Different effects of startling acoustic stimuli (SAS) on TMS-induced responses at rest and during sustained voluntary contraction. *Frontiers in Human Neuroscience*, *10*, 8. https://doi.org/10.3389/fnhum.2016.00396

Chen, Y. T., Li, S., Zhou, P., & Li, S. (2019). The effects of conditioning startling acoustic stimulation (SAS) on the corticospinal motor system: A SAS–TMS study. *Experimental Brain Research*, *237*(8), 1973–1980. https://doi.org/10.1007/s00221-019-05569-0

Cheney, P. D., & Fetz, E. E. (1980). Functional classes of primate corticomotoneuronal cells and their relation to active force. *Journal of Neurophysiology*, *44*(4), 773–791. https://doi.org/10.1152/jn.1980.44.4.773

Choudhury, S., Shobhana, A., Singh, R., Sen, D., Anand, S. S., Shubham, S., Baker, M. R., Kumar, H., & Baker, S. N. (2019). The relationship between enhanced reticulospinal outflow and upper limb function in chronic stroke patients. *Neurorehabilitation and Neural Repair*, *33*(5), 375–383. https://doi.org/10.1177/1545968319836233

Clough, J. F. M., Kernell, D., & Phillips, C. G. (1968). The distribution of

monosynaptic excitation from the pyramidal tract and from primary spindle afferents to motoneurones of the baboon's hand and forearm. *The Journal of Physiology*, *198*(1), 145–166. https://doi.org/10.1113/jphysiol.1968.sp008598

Coppens, M. J. M., Roelofs, J. M. B., Donkers, N. A. J., Nonnekes, J., Geurts, A. C. H., & Weerdesteyn, V. (2018). A startling acoustic stimulus facilitates voluntary lower extremity movements and automatic postural responses in people with chronic stroke. *Journal of Neurology*, *265*(7), 1625–1635. https://doi.org/10.1007/s00415-018-8889-5

Criaud, M., Anton, J. L., Nazarian, B., Longcamp, M., Metereau, E., Boulinguez, P., & Ballanger, B. (2021). The human basal ganglia mediate the interplay between reactive and proactive control of response through both motor inhibition and sensory modulation. *Brain Sciences*, *11*(5). https://doi.org/10.3390/brainsci11050560

Da Silva, J. A., Tecuapetla, F., Paixão, V., & Costa, R. M. (2018). Dopamine neuron activity before action initiation gates and invigorates future movements. *Nature*, *554*(7691), 244–248. https://doi.org/10.1038/nature25457

Dai, T. H., Liu, J. Z., Sahgal, V., Brown, R. W., & Yue, G. H. (2001). Relationship between muscle output and functional MRI-measured brain activation. *Experimental Brain Research*, *140*(3), 290–300. https://doi.org/10.1007/s002210100815

Davis, M., Gendelman, D. S., Tischler, M. D., & Gendelman, P. M. (1982). A primary acoustic startle circuit: Lesion and stimulation studies. *Journal of Neuroscience*, *2*(6), 791–805. https://doi.org/10.1523/jneurosci.02-06-00791.1982

Davranche, K., Tandonnet, C., Burle, B., Meynier, C., Vidal, F., & Hasbroucq, T. (2007). The dual nature of time preparation: Neural activation and suppression revealed by transcranial magnetic stimulation of the motor cortex. *European Journal of Neuroscience*, *25*(12), 3766–3774. https://doi.org/10.1111/j.1460-9568.2007.05588.x

de Noordhout, A. M., Rapisarda, G., Bogacz, D., Gérard, P., De Pasqua, V., Pennisi, G., & Delwaide, P. J. (1999). Corticomotoneuronal synaptic connections in

normal man. An electrophysiological study. *Brain*, *122*(7), 1327–1340. https://doi.org/10.1093/brain/122.7.1327

de Rugy, A., Loeb, G. E., & Carroll, T. J. (2012). Muscle coordination is habitual rather than optimal. *Journal of Neuroscience*, *32*(21), 7384–7391. https://doi.org/10.1523/JNEUROSCI.5792-11.2012

Deacon, T. W. (1992). Cortical connections of the inferior arcuate sulcus cortex in the macaque brain. *Brain Research*, *573*(1), 8–26. https://doi.org/10.1016/0006-8993(92)90109-M

Dean, L. R., & Baker, S. N. (2017). Fractionation of muscle activity in rapid responses to startling cues. *Journal of Neurophysiology*, *117*(4), 1713–1719. https://doi.org/10.1152/jn.01009.2015

Del Tredici, K., Rüb, U., De Vos, R. A. I., Bohl, J. R. E., & Braak, H. (2002). Where does Parkinson disease pathology begin in the brain? *Journal of Neuropathology and Experimental Neurology*, *61*(5), 413–426. https://doi.org/10.1093/jnen/61.5.413

Derosiere, G. (2018). A dynamical system framework for theorizing preparatory inhibition. *Journal of Neuroscience*, *38*(14), 3391–3393. https://doi.org/10.1523/JNEUROSCI.0069-18.2018

Dettmers, C., Ridding, M. C., Stephan, K. M., Lemon, R. N., Rothwell, J. C., & Frackowiak, R. S. J. (1996). Comparison of regional cerebral blood flow with transcranial magnetic stimulation at different forces. *Journal of Applied Physiology*, *81*(2), 596–603. https://doi.org/10.1152/jappl.1996.81.2.596

Devoto, P., Flore, G., Saba, P., Fà, M., & Gessa, G. L. (2005a). Stimulation of the locus coeruleus elicits noradrenaline and dopamine release in the medial prefrontal and parietal cortex. *Journal of Neurochemistry*, *92*(2), 368–374. https://doi.org/10.1111/j.1471-4159.2004.02866.x

Devoto, P., Flore, G., Saba, P., Fà, M., & Gessa, G. L. (2005b). Co-release of noradrenaline and dopamine in the cerebral cortex elicited by single train and repeated train stimulation of the locus coeruleus. *BMC Neuroscience*, *6*(1), 1–11. https://doi.org/10.1186/1471-2202-6-31

Di Lazzaro, V., Oliviero, A., Profice, P., Insola, A., Mazzone, P., Tonali, P., &

Rothwell, J. C. (1999). Direct demonstration of interhemispheric inhibition of the human motor cortex produced by transcranial magnetic stimulation. *Experimental Brain Research*, *124*(4), 520–524. https://doi.org/10.1007/s002210050648

Dickson, D. W., Braak, H., Duda, J. E., Duyckaerts, C., Gasser, T., Halliday, G. M., Hardy, J., Leverenz, J. B., Del Tredici, K., Wszolek, Z. K., & Litvan, I. (2009). Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *The Lancet Neurology*, *8*(12), 1150–1157. https://doi.org/10.1016/S1474-4422(09)70238-8

Duque, J., Greenhouse, I., Labruna, L., & Ivry, R. B. (2017). Physiological markers of motor inhibition during human behavior. *Trends in Neurosciences*, *40*(4), 219–236. https://doi.org/10.1016/j.tins.2017.02.006

Duque, J., & Ivry, R. B. (2009). Role of corticospinal suppression during motor preparation. *Cerebral Cortex*, *19*(9), 2013–2024. https://doi.org/10.1093/cercor/bhn230

Duque, J., Labruna, L., Cazares, C., & Ivry, R. B. (2014). Dissociating the influence of response selection and task anticipation on corticospinal suppression during response preparation. *Neuropsychologia*, *65*, 287–296. https://doi.org/10.1016/j.neuropsychologia.2014.08.006

Duque, J., Lew, D., Mazzocchio, R., Olivier, E., & Ivry, R. B. (2010). Evidence for two concurrent inhibitory mechanisms during response preparation. *Journal of Neuroscience*, *30*(10), 3793–3802. https://doi.org/10.1523/JNEUROSCI.5722-09.2010

Duque, J., Mazzocchio, R., Dambrosia, J., Murase, N., Olivier, E., & Cohen, L. G. (2005). Kinematically specific interhemispheric inhibition operating in the process of generation of a voluntary movement. *Cerebral Cortex*, *15*(5), 588–593. https://doi.org/10.1093/cercor/bhh160

Fernandez-Del-Olmo, M., Bello, O., Lopez-Alonso, V., Marquez, G., Sanchez, J. A., Morenilla, L., & Valls-Solé, J. (2013). The effects of startle and non-startle auditory stimuli on wrist flexion movement in Parkinson's disease. *Neuroscience Letters*, *548*, 56–60. https://doi.org/10.1016/j.neulet.2013.05.069

Fetz, E. E., & Cheney, P. D. (1980). Postspike facilitation of forelimb muscle activity by primate corticomotoneuronal cells. *Journal of Neurophysiology*, *44*(4), 751–772. https://doi.org/10.1152/jn.1980.44.4.751

Fisher, K. M., Zaaimi, B., & Baker, S. N. (2012). Reticular formation responses to magnetic brain stimulation of primary motor cortex. *Journal of Physiology*, *590*(16), 4045–4060. https://doi.org/10.1113/jphysiol.2011.226209

Fisher, R. J., Sharott, A., Kuhn, A. A., & Brown, P. (2004). Effects of combined cortical and acoustic stimuli on muscle activity. *Experimental Brain Research*, 1–6.

Flament, D., Goldsmith, P., Buckley, C. J., & Lemon, R. N. (1993). Task dependence of responses in first dorsal interosseous muscle to magnetic brain stimulation in man. *The Journal of Physiology*, *464*(1), 361–378. https://doi.org/10.1113/jphysiol.1993.sp019639

Foote, S. L., Aston-Jones, G., & Bloom, F. E. (1980). Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proceedings of the National Academy of Sciences of the United States of America*, *77*(5 I), 3033–3037. https://doi.org/10.1073/pnas.77.5.3033

Foote, S. L., Bloom, F. E., & Aston Jones, G. (1983). Nucleus locus ceruleus: New evidence of anatomical and physiological specificity. *Physiological Reviews*, *63*(3), 844–914. https://doi.org/10.1152/physrev.1983.63.3.844

Furubayashi, T., Ugawa, Y., Terao, Y., Hanajima, R., Sakai, K., Machii, K., Mochizuki, H., Shiio, Y., Uesugi, H., Enomoto, H., & Kanazawa, I. (2000). The human hand motor area is transiently suppressed by an unexpected auditory stimulus. *Clnical Neurophysiology*, 178-183. doi:10.1016/S1388-2457(99)00200-X.

Godfrey, S. B., Lum, P. S., Chan, E., & Harris-Love, M. L. (2013). Cortical effects of repetitive finger flexion- vs. extension-resisted tracking movements: A TMS study. *Journal of Neurophysiology*, *109*(4), 1009–1016. https://doi.org/10.1152/jn.00143.2012

Graziano, M. S. A. (2011). New insights into motor cortex. *Neuron*, *71*(3), 387–388.

https://doi.org/10.1016/j.neuron.2011.07.014

Graziano, M. S. A., & Aflalo, T. N. (2007). Mapping behavioral repertoire onto the cortex. *Neuron*, *56*(2), 239–251. https://doi.org/10.1016/j.neuron.2007.09.013

Greenhouse, I., Sias, A., Labruna, L., & Ivry, R. B. (2015). Nonspecific inhibition of the motor system during response preparation. *Journal of Neuroscience*, *35*(30), 10675–10684. https://doi.org/10.1523/JNEUROSCI.1436-15.2015

Gunel, E., & Dickey, J. (1974). Bayes factors for independence in contingency tables. *Biometrika*, *61*(3), 545. https://doi.org/10.2307/2334738

Habibollahi Saatlou, F., Rogasch, N. C., McNair, N. A., Biabani, M., Pillen, S. D., Marshall, T. R., & Bergmann, T. O. (2018). MAGIC: An open-source MATLAB toolbox for external control of transcranial magnetic stimulation devices. *Brain Stimulation*, *11*(5), 1189–1191. https://doi.org/10.1016/j.brs.2018.05.015

Hackley, S. A., & Valle-Inclán, F. (1999). *Accessory stimulus effects on response selection: Does arousal speed decision making?* http://direct.mit.edu/jocn/article-pdf/11/3/321/1758556/089892999563427.pdf

Haith, A. M., Pakpoor, J., & Krakauer, J. W. (2016). Independence of movement preparation and movement initiation. *Journal of Neuroscience*, *30*, 3007–3015. https://doi.org/10.1523/JNEUROSCI.3245-15.2016

Hammond, G. R. (1973). Lesions of pontine and medullary reticular formation and prestimulus inhibition of the acoustic startle reaction in rats. *Physiology and Behavior*, *10*(2), 239–243. https://doi.org/10.1016/0031-9384(73)90304-1

Hanes, D. P., & Schall, J. D. (1996). Neural control of voluntary movement initiation. *Science*, *274*(5286), 427–430. https://doi.org/10.1126/science.274.5286.427

Hannah, R., Cavanagh, S. E., Tremblay, S., Simeoni, S., & Rothwell, J. C. (2018). Selective suppression of local interneuron circuits in human motor cortex contributes to movement preparation. *Journal of Neuroscience*, *38*(5), 1264–1276. https://doi.org/10.1523/JNEUROSCI.2869-17.2017

Hartigan, P. M. (1985). Computation of the dip statistic to test for unimodality.

Applied Statistics, *34*(3), 320. https://doi.org/10.2307/2347485

Hasbroucq, T., Kaneko, H., Akamatsu, M., & Possamaï, C. A. (1997). Preparatory inhibition of cortico-spinal excitability: A transcranial magnetic stimulation study in man. *Cognitive Brain Research*, *5*(3), 185–192. https://doi.org/10.1016/S0926-6410(96)00069-9

Hasbroucq, T., Kaneko, H., Akamatsu, M., & Possamaï, C. A. (1999). The time-course of preparatory spinal and cortico-spinal inhibition: An H-reflex and transcranial magnetic stimulation study in man. *Experimental Brain Research*, *124*(1), 33–41. https://doi.org/10.1007/s002210050597

Hershenson, M. (1962). Reaction time as a measure of intersensory facilitation. *Journal of Experimental Psychology*, *63*(3), 289–293. https://doi.org/10.1037/h0039516

Hoehn, M. M., & Yahr, M. D. (1967). Parkinsonism: Onset, progression, and mortality. *Neurology*, *17*(5), 427–442. https://doi.org/10.1212/wnl.17.5.427

Honeycutt, C. F., Kharouta, M., & Perreault, E. J. (2013). Evidence for reticulospinal contributions to coordinated finger movements in humans. *Journal of Neurophysiology*, *110*(7), 1476–1483. https://doi.org/10.1152/jn.00866.2012

Honeycutt, C. F., & Perreault, E. J. (2012). Planning of ballistic movement following stroke: Insights from the startle reflex. *PLoS ONE*, *7*(8), e43097. https://doi.org/10.1371/journal.pone.0043097

Honeycutt, C. F., Tresch, U. A., & Perreault, E. J. (2014). Startling acoustic stimuli elicit rapid hand extension following stroke. *Biosystems and Biorobotics*, *7*, 431–435. https://doi.org/10.1007/978-3-319-08072-7_65

Honeycutt, C. F., Tresch, U. A., & Perreault, E. J. (2015). Startling acoustic stimuli can evoke fast hand extension movements in stroke survivors. *Clinical Neurophysiology*, *126*(1), 160–164. https://doi.org/10.1016/j.clinph.2014.05.025

Hori, A., Yasuhara, A., Naito, H., & Yasuhara, M. (1986). Blink reflex elicited by auditory stimulation in the rabbit. *Journal of the Neurological Sciences*, *76*(1), 49–59. https://doi.org/10.1016/0022-510X(86)90141-3

Hyndman, R. J., & Fan, Y. (1996). Sample quantiles in statistical packages. *The American Statistician*, *50*(4), 361. https://doi.org/10.2307/2684934

Ibáñez, J., Hannah, R., Rocchi, L., & Rothwell, J. C. (2020). Premovement suppression of corticospinal excitability may be a necessary part of movement preparation. *Cerebral Cortex*, *30*(5), 2910–2923. https://doi.org/10.1093/cercor/bhz283

Ilic, T. V, Pötter-Nerger, M., Holler, I., Siebner, H. R., Ilic, N. V, Deuschl, G., & Volkmann, J. (2011). Startle stimuli exert opposite effects on human cortical and spinal motor system excitability in leg muscles. *Physiol. Res*, *60*, 101–106. https://doi.org/10.33549/physiolres.932182

Jankelowitz, S. K., & Colebatch, J. G. (2004). The acoustic startle reflex in ischemic stroke. *Neurology*, *62*(1), 114–116. https://doi.org/10.1212/01.WNL.0000101711.48946.35

Jankovic, J. (2008). Parkinson's disease: Clinical features and diagnosis. *Journal of Neurology, Neurosurgery and Psychiatry*, *79*(4), 368–376. https://doi.org/10.1136/jnnp.2007.131045

Jaskowski, P., & Verleger, R. (1993). A clock paradigm to study the relationship between expectancy and response force. *Perceptual and Motor Skills*, *77*(1), 163–174. https://doi.org/10.2466/pms.1993.77.1.163

Jeffreys, H. (1961). *Theory of probability*. Oxford University Press.

Jones, F. P., & Kennedy, J. L. (1951). An Electromyographic Technique for Recording the Startle Pattern. *Journal of Psychology: Interdisciplinary and Applied*, *32*(1), 63–68. https://doi.org/10.1080/00223980.1951.9916084

Joshi, S., Li, Y., Kalwani, R. M., & Gold, J. I. (2016). Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex. *Neuron*, *89*(1), 221–234. https://doi.org/10.1016/j.neuron.2015.11.028

Kawashima, R., Matsumura, M., Sadato, N., Naito, E., Waki, A., Nakamura, S., Matsunami, K., Fukuda, H., & Yonekura, Y. (1998). Regional cerebral blood flow changes in human brain related to ipsilateral and contralateral complex hand movements - A PET study. *European Journal of Neuroscience*, *10*(7), 2254–2260. https://doi.org/10.1046/j.1460-9568.1998.00237.x

Kennefick, M., Maslovat, D., & Carlsen, A. N. (2014). The time course of corticospinal excitability during a simple reaction time task. *PLoS ONE*, *9*(11), e113563. https://doi.org/10.1371/journal.pone.0113563

Kiper, P., Szczudlik, A., Venneri, A., Stozek, J., Luque-Moreno, C., Opara, J., Baba, A., Agostini, M., & Turolla, A. (2016). Computational models and motor learning paradigms: Could they provide insights for neuroplasticity after stroke? An overview. *Journal of the Neurological Sciences*, *369*, 141–148.

Kirkpatrick, N. J., Ravichandran, V. J., Perreault, E. J., Schaefer, S. Y., & Honeycutt, C. F. (2018). Evidence for startle as a measurable behavioral indicator of motor learning. *PLoS ONE*, *13*(5). https://doi.org/10.1371/journal.pone.0195689

Klein, P.-A., Duque, J., Labruna, L., & Ivry, R. B. (2016). Comparison of the two cerebral hemispheres in inhibitory processes operative during movement preparation. *NeuroImage*, *125*, 220–232. https://doi.org/https://doi.org/10.1016/j.neuroimage.2015.10.007

Kofler, M., Müller, J., & Valls-Solè, J. (2006). Auditory startle responses as a probe of brainstem function in healthy subjects and patients with movement disorders. *Supplements to Clinical Neurophysiology*, *58*, 232–248. https://doi.org/10.1016/S1567-424X(09)70072-8

Koganemaru, S., Mima, T., Thabit, M. N., Ikkaku, T., Shimada, K., Kanematsu, M., Takahashi, K., Fawi, G., Takahashi, R., Fukuyama, H., & Domen, K. (2010). Recovery of upper-limb function due to enhanced use-dependent plasticity in chronic stroke patients. *Brain*, *133*(11), 3373–3384. https://doi.org/10.1093/brain/awq193

Kohfield, D. L. (1971). Simple reaction time as a function of stimulus intensity in decibels of light and sound. *Journal of Experimental Psychology*, *88*, 251–257. https://doi.org/10.1037/h0030891

Kouchtir-Devanne, N., Capaday, C., Cassim, F., Derambure, P., & Devanne, H. (2012). Task-dependent changes of motor cortical network excitability during precision grip compared to isolated finger contraction. *Journal of Neurophysiology*, *107*(5), 1522–1529. https://doi.org/10.1152/jn.00786.2011

Kuhn, A. A., Sharott, A., Trottenberg, T., Kupsch, A., & Brown, P. (2004). Motor cortex inhibition induced by acoustic stimulation. *Experimental Brain Research*, 120–124. https://doi.org/10.1007/s00221-004-1883-4

Kuhn, Y. A., Keller, M., Ruffieux, J., & Taube, W. (2017). Adopting an external focus of attention alters intracortical inhibition within the primary motor cortex. *Acta Physiologica*, *220*(2), 289–299. https://doi.org/10.1111/apha.12807

Kumru, H., Urra, X., Compta, Y., Castellote, J. M., Turbau, J., & Valls-Solé, J. (2006). Excitability of subcortical motor circuits in Go/noGo and forced choice reaction time tasks. *Neuroscience Letters*, *406*(1–2), 66–70. https://doi.org/10.1016/j.neulet.2006.07.012

Kumru, H., & Valls-Solé, J. (2006). Excitability of the pathways mediating the startle reaction before execution of a voluntary movement. *Experimental Brain Research*, *169*(3), 427–432. https://doi.org/10.1007/s00221-005-0156-1

Kuypers, H. G. J. M., & Brinkman, J. (1970). Precentral projections to different parts of the spinal intermediate zone in the rhesus monkey. *Brain Research*, *24*(1), 29–48. https://doi.org/10.1016/0006-8993(70)90272-6

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models . *Journal of Statistical Software*, *82*(13). https://doi.org/10.18637/jss.v082.i13

Latash, M. L., & Greg Anson, J. (1996). What are "normal movements" in atypical populations? *Behavioral and Brain Sciences*, *19*(1), 55–106. https://doi.org/10.1017/s0140525x00041467

Lawrence, D. G., & Kuypers, H. G. J. M. (1968). The functional organizatuin of the motor system in the monkey. *Brain*, *91*(1), 1–14. https://doi.org/10.1093/brain/91.1.1

Lebon, F., Greenhouse, I., Labruna, L., Vanderschelden, B., Papaxanthis, C., & Ivry, R. B. (2016). Influence of delay period duration on inhibitory processes for response preparation. *Cerebral Cortex*, *26*(6), 2461–2470. https://doi.org/10.1093/cercor/bhv069

Lecas, J. C., Requin, J., Anger, C., & Vitton, N. (1986). Changes in neuronal activity of the monkey precentral cortex during preparation for movement. *Journal of*

Neurophysiology, *56*(6), 1680–1702. https://doi.org/10.1152/jn.1986.56.6.1680

Lemon, R. N. (2008). Descending pathways in motor control. *Annual Review of Neuroscience*, *31*, 195–218. https://doi.org/10.1146/annurev.neuro.31.060407.125547

Lemon, R. N., Johansson, R. S., & Westling, G. (1995). Corticospinal control during reach, grasp, and precision lift in man. *Journal of Neuroscience*, *15*(9), 6145–6156. https://doi.org/10.1523/jneurosci.15-09-06145.1995

Lenth, R. (2019). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R Package Version 1.3.4. https://cran.r-project.org/package=emmeans

Leocani, L., Cohen, L. G., Wassermann, E. M., Ikoma, K., & Hallett, M. (2000). Human corticospinal excitability evaluated with transcranial magnetic stimulation during different reaction time paradigms. *Brain*, *123*(6), 1161–1173. https://doi.org/10.1093/brain/123.6.1161

Leow, L. A., Uchida, A., Egberts, J. L., Riek, S., Lipp, O. V., Tresilian, J., & Marinovic, W. (2018). Triggering mechanisms for motor actions: The effects of expectation on reaction times to intense acoustic stimuli. *Neuroscience*, *393*, 226–235. https://doi.org/10.1016/j.neuroscience.2018.10.008

Leow, L., Tresilian, J. R., Uchida, A., Koester, D., Spingler, T., Riek, S., & Marinovic, W. (2021). Acoustic stimulation increases implicit adaptation in sensorimotor adaptation. *European Journal of Neuroscience*, ejn.15317. https://doi.org/10.1111/ejn.15317

Liang, M., Mouraux, A., & Iannetti, G. D. (2013). Bypassing primary sensory cortices-a direct thalamocortical pathway for transmitting salient sensory information. *Cerebral Cortex*, *23*(1), 1–11. https://doi.org/10.1093/cercor/bhr363

Liepert, J., Dettmers, C., Terborg, C., & Weiller, C. (2001). Inhibition of ipsilateral motor cortex during phasic generation of low force. *Clinical Neurophysiology*, *112*(1), 114–121. https://doi.org/10.1016/s1388-2457(00)00503-4

Lipp, O. V., Kaplan, D. M., & Purkis, H. M. (2006). Reaction time facilitation by acoustic task-irrelevant stimuli is not related to startle. *Neuroscience Letters*, *409*(2), 124–127. https://doi.org/10.1016/j.neulet.2006.09.025

Lipp, O. V, Blumenthal, T. D., & Adam, A. R. (2001). Attentional modulation of blink startle at long, short, and very short lead intervals. *Biological Psychology*, *58*(2), 89–103. https://doi.org/10.1016/S0301-0511(01)00109-0

Liu, J., Sheng, Y., & Liu, H. (2019). Corticomuscular coherence and its applications: A review. *Frontiers in Human Neuroscience*, *13*(3), 1–16. https://doi.org/10.3389/fnhum.2019.00100

Luce, R. D. (2008). *Response times: Their role in inferring elementary mental organization*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780195070019.001.0001

MacKay, W. A., & Bonnet, M. (1990). CNV, stretch reflex and reaction time correlates of preparation for movement direction and force. *Electroencephalography and Clinical Neurophysiology*, *76*(1), 47–62. https://doi.org/10.1016/0013-4694(90)90057-Q

MacKinnon, C. D., Allen, D. P., Shiratori, T., & Rogers, M. W. (2013). Early and unintentional release of planned motor actions during motor cortical preparation. *PLoS ONE*, *8*(5), e63417. https://doi.org/10.1371/journal.pone.0063417

MacKinnon, C. D., Bissig, D., Chiusano, J., Miller, E., Rudnick, L., Jager, C., Zhang, Y., Mille, M. L., & Rogers, M. W. (2007). Preparation of anticipatory postural adjustments prior to stepping. *Journal of Neurophysiology*, *97*(6), 4368–4379. https://doi.org/10.1152/jn.01136.2006

Maier, M. A., Bennett, K. M. B., Hepp-Reymond, M. C., & Lemon, R. N. (1993). Contribution of the monkey corticomotoneuronal system to the control of force in precision grip. *Journal of Neurophysiology*, *69*(3), 772–785. https://doi.org/10.1152/jn.1993.69.3.772

Marinovic, W., Brauer, S. G., Hayward, K. S., Carroll, T. J., & Riek, S. (2016). Electric and acoustic stimulation during movement preparation can facilitate movement execution in healthy participants and stroke survivors. *Neuroscience Letters*, *618*, 134–138. https://doi.org/10.1016/j.neulet.2016.03.009

Marinovic, W., Cheung, F. L. Y., Riek, S., & Tresilian, J. R. (2014). The effect of attention on the release of anticipatory timing actions. *Behavioral*

Neuroscience, *128*(5), 548–555. https://doi.org/10.1037/bne0000007

Marinovic, W., de Rugy, A., Lipp, O. V., & Tresilian, J. R. (2013). Responses to loud auditory stimuli indicate that movement-related activation builds up in anticipation of action. *Journal of Neurophysiology*, *109*(4), 996–1008. https://doi.org/10.1152/jn.01119.2011

Marinovic, W., de Rugy, A., Riek, S., & Tresilian, J. (2014). The early release of actions by loud sounds in muscles with distinct muscle connectivity. *Experimental Brain Research*, *232*(12), 3797-3802. https://doi.org/10.1007/s00221-014–4074.

Marinovic, W., Flannery, V., & Riek, S. (2015). The effects of preparation and acoustic stimulation on contralateral and ipsilateral corticospinal excitability. *Human Movement Science*, *42*, 81–88. https://doi.org/10.1016/j.humov.2015.05.003

Marinovic, W., Milford, M., Carroll, T., & Riek, S. (2015). The facilitation of motor actions by acoustic and electric stimulation. *Psychophysiology*, *52*(12), 1698–1710. https://doi.org/10.1111/psyp.12540

Marinovic, W., Poh, E., de Rugy, A., & Carroll, T. J. (2017). Action history influences subsequent movement via two distinct processes. *ELife*, *6*, e26713. https://doi.org/10.7554/eLife.26713

Marinovic, W., Reid, C. S., Plooy, A. M., Riek, S., & Tresilian, J. R. (2011). Corticospinal excitability during preparation for an anticipatory action is modulated by the availability of visual information. *Journal of Neurophysiology*, *105*(3), 1122–1129. https://doi.org/10.1152/jn.00705.2010

Marinovic, W., Tresilian, J., Chapple, J. L., Riek, S., & Carroll, T. J. (2017). Unexpected acoustic stimulation during action preparation reveals gradual re-specification of movement direction. *Neuroscience*, *348*, 23–32. https://doi.org/10.1016/j.neuroscience.2017.02.016

Marinovic, W., & Tresilian, J. R. (2016). Triggering prepared actions by sudden sounds: Reassessing the evidence for a single mechanism. *Acta Physiologica*, *217*(1), 13–32. https://doi.org/10.1111/apha.12627

Marinovic, W., Tresilian, J. R., de Rugy, A., Sidhu, S., & Riek, S. (2014).

Corticospinal modulation induced by sounds depends on action preparedness. *Journal of Physiology*, *592*(1), 153–169. https://doi.org/10.1113/jphysiol.2013.254581

Maslovat, D., Franks, I. M., Leguerrier, A., & Carlsen, A. N. (2015). Responses to startling acoustic stimuli indicate that movement-related activation is constant prior to action: A replication with an alternate interpretation. *Physiological Reports*, *3*(2), e12300. https://doi.org/10.14814/phy2.12300

Maslovat, D., Kennedy, P. M., Forgaard, C. J., Chua, R., & Franks, I. M. (2012). The effects of prepulse inhibition timing on the startle reflex and reaction time. *Neuroscience Letters*, *513*(2), 243–247. https://doi.org/10.1016/j.neulet.2012.02.052

Mather, M., & Harley, C. W. (2016). The Locus Coeruleus: Essential for Maintaining Cognitive Function and the Aging Brain. *Trends in Cognitive Sciences*, *20*(3), 214–226. https://doi.org/10.1016/j.tics.2016.01.001

McInnes, A. N., Castellote, J. M., Kofler, M., Honeycutt, C. F., Lipp, O. V., Riek, S., Tresilian, J. R., & Marinovic, W. (2021). Cumulative distribution functions: An alternative approach to examine the triggering of prepared motor actions in the StartReact effect. *European Journal of Neuroscience*, *53*(5), 1545–1568. https://doi.org/10.1111/ejn.14973

McInnes, A. N., Corti, E. J., Tresilian, J. R., Lipp, O. V., & Marinovic, W. (2020). Neural gain induced by startling acoustic stimuli is additive to preparatory activation. *Psychophysiology*, *57*(3), e13493. https://doi.org/10.1111/psyp.13493

McMillan, S., Nougier, V., & Byblow, W. D. (2004). Human corticospinal excitability during a precued reaction time paradigm. *Experimental Brain Research*, *156*(1), 80–87. https://doi.org/10.1007/s00221-003-1772-2

Metoki, N., Sato, Y., Satoh, K., Okumura, K., & Iwamoto, J. (2003). Muscular atrophy in the hemiplegic thigh in patients after stroke. *American Journal of Physical Medicine and Rehabilitation*, *82*, 862–865.

Moller, A. R. (2003). *Sensory systems: Anatomy, physiology and pathophysiology*. Gulf Professional Publishing.

https://books.google.com.au/books?hl=en&lr=&id=Sla-9M7Di08C&oi=fnd&pg=PP2&ots=MM98U0UFZT&sig=rht-a4kkPZmumj6omRjrT4yvq1o&redir_esc=y#v=onepage&q&f=false

Morey, R. D., Jeffrey, N. R., Jamil, T., Urbanek, S., Forner, K., & Ly, A. (2018). *Computation of Bayes factors for common designs*. https://cran.r-project.org/web/packages/BayesFactor/BayesFactor.pdf

Muellbacher, W., Facchini, S., Boroojerdi, B., & Hallett, M. (2000). Changes in motor cortex excitability during ipsilateral hand muscle activation in humans. *Clinical Neurophysiology*, *111*(2), 344–349. https://doi.org/10.1016/S1388-2457(99)00243-6

Nelson, A., Schneider, D. M., Takatoh, J., Sakurai, K., Wang, F., & Mooney, R. (2013). A circuit for motor cortical modulation of auditory cortical activity. *Journal of Neuroscience*, *33*(36), 14342–14353. https://doi.org/10.1523/JNEUROSCI.2275-13.2013

Nguyen, A. T., Jacobs, L. A., Tresilian, J. R., Lipp, O. V., & Marinovic, W. (2020). Preparatory suppression and facilitation of voluntary and involuntary responses to loud acoustic stimuli in an anticipatory timing task. *Psychophysiology*, e13730. https://doi.org/10.1111/psyp.13730

Nguyen, A. T., Jacobs, L. A., Tresilian, J. R., Lipp, O. V., & Marinovic, W. (2021). Preparatory suppression and facilitation of voluntary and involuntary responses to loud acoustic stimuli in an anticipatory timing task. *Psychophysiology*, *58*(2), e13730. https://doi.org/10.1111/psyp.13730

Nickerson, R. S. (1973). Intersensory facilitation of reaction time: Energy summation or preparation enhancement? *Psychological Review*, *80*(6), 489–509. https://doi.org/10.1037/h0035437

Niemi, P., & Näätänen, R. (1981). Foreperiod and simple reaction time. *Psychological Bulletin*, *89*(1), 133–162. https://doi.org/10.1037/0033-2909.89.1.133

Nonnekes, J., Carpenter, M. G., Inglis, J. T., Duysens, J., & Weerdesteyn, V. (2015). What startles tell us about control of posture and gait. *Neuroscience & Biobehavioral Reviews*, *53*, 131–138.

https://doi.org/https://doi.org/10.1016/j.neubiorev.2015.04.002

Nonnekes, J., de Kam, D., Nijhuis, L. B. O., van Geel, K., Bloem, B. R., Geurts, A., & Weerdesteyn, V. (2015). StartReact effects support different pathophysiological mechanisms underlying freezing of gait and postural instability in parkinson's disease. *PLOS ONE*, *10*(3), e0122064. https://doi.org/10.1371/journal.pone.0122064

Nonnekes, J., de Niet, M., Weerdesteyn, V., Geurts, A. C., Oude Nijhuis, L. B., de Bot, S. T., Pasman, J. W., van de Warrenburg, B. P. C., & Bloem, B. R. (2014). Startreact restores reaction time in HSP: Evidence for subcortical release of a motor program. *Journal of Neuroscience*, *34*(1), 275–281. https://doi.org/10.1523/JNEUROSCI.2948-13.2014

Nonnekes, J., Scotti, A., Oude Nijhuis, L. B., Smulders, K., Queralt, A., Geurts, A. C. H., Bloem, B. R., & Weerdesteyn, V. (2013). Are postural responses to backward and forward perturbations processed by different neural circuits? *Neuroscience*, *245*, 109–120. https://doi.org/10.1016/j.neuroscience.2013.04.036

Oliveira, M. A., Hsu, J., Park, J., Clark, J. E., & Shim, J. K. (2008). Age-related changes in multi-finger interactions in adults during maximum voluntary finger force production tasks. *Human Movement Science*, *27*(5), 714–727. https://doi.org/10.1016/j.humov.2008.04.005

Ossanna, M. R., Zong, X., Ravichandran, V. J., & Honeycutt, C. F. (2019). Startle evokes nearly identical movements in multi-jointed, two-dimensional reaching tasks. *Experimental Brain Research*, *237*, 71–78. https://doi.org/10.1007/s00221-018-5399-8

Palmer, E., & Ashby, P. (1992). Corticospinal projections to upper limb motoneurones in humans. *The Journal of Physiology*, *448*(1), 397–412. https://doi.org/10.1113/jphysiol.1992.sp019048

Park, W. H., & Li, S. (2013). Responses of finger flexor and extensor muscles to transcranial magnetic stimulation during isometric force production tasks. *Muscle and Nerve*, *48*(5), 739–744. https://doi.org/10.1002/mus.23804

Perez, M. A., Butler, J. E., & Taylor, J. L. (2014). Modulation of transcallosal

inhibition by bilateral activation of agonist and antagonist proximal arm muscles. *Journal of Neurophysiology*, *111*(2), 405–414. https://doi.org/10.1152/jn.00322.2013

Perez, M. A., & Cohen, L. G. (2008). Mechanisms underlying functional changes in the primary motor cortex ipsilateral to an active hand. *Journal of Neuroscience*, *28*(22), 5631–5640. https://doi.org/10.1523/JNEUROSCI.0093-08.2008

Petrides, M., & Pandya, D. N. (1988). Association fiber pathways to the frontal cortex from the superior temporal region in the rhesus monkey. *Journal of Comparative Neurology*, *273*(1), 52–66. https://doi.org/10.1002/cne.902730106

Petrides, Michael, & Pandya, D. N. (2006). Efferent association pathways originating in the caudal prefrontal cortex in the macaque monkey. *Journal of Comparative Neurology*, *498*(2), 227–251. https://doi.org/10.1002/cne.21048

Phillips, C. G., & Porter, R. (1964). The pyramidal projection to motoneurones of some muscle groups of the baboon's forelimb. *Progress in Brain Research*, *12*(C), 222–245. https://doi.org/10.1016/S0079-6123(08)60625-1

Pieron, H. (1914). Recherches sur les lois de variation des temps de latence sensorielle en fonction des intensités excitatrices. *Année Psychol*, *22*, 17–96.

Pins, D., & Bonnet, C. (1996). On the relation between stimulus intensity and processing time: Piéron's law and choice reaction time. *Perception & Psychophysics*, *58*, 390–400. https://doi.org/10.3758/BF03206815

Quinn, L., Miljevic, A., Rurak, B. K., Marinovic, W., & Vallence, A. M. (2018). Differential plasticity of extensor and flexor motor cortex representations following visuomotor adaptation. *Experimental Brain Research*, *236*(11), 2945–2957. https://doi.org/10.1007/s00221-018-5349-5

R Core Team. (2019). *R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.* https://www.r-project.org/

Rahimi, M., & Honeycutt, C. F. (2020a). StartReact increases the probability of muscle activity and distance in severe/moderate stroke survivors during two-dimensional reaching task. *Experimental Brain Research*, *238*(5), 1219–1227. https://doi.org/10.1007/s00221-020-05797-9

Rahimi, M., & Honeycutt, C. F. (2020b). StartReact increases the probability of muscle activity and distance in severe/moderate stroke survivors during two-dimensional reaching task. *Experimental Brain Research*, *238*(5), 1219–1227. https://doi.org/10.1007/s00221-020-05797-9

Ranjbar-Slamloo, Y., & Fazlali, Z. (2020). Dopamine and noradrenaline in the brain; Overlapping or dissociate functions? *Frontiers in Molecular Neuroscience*, *12*(21), 334. https://doi.org/10.3389/fnmol.2019.00334

Ratcliff, R. (1979). Group reaction time distributions and an analysis of distribution statistics. *Psychological Bulletin*, *86*(3), 446–461. https://doi.org/10.1037/0033-2909.86.3.446

Rea, P. (2015). Essential Clinical Anatomy of the Nervous System. In *Essential Clinical Anatomy of the Nervous System*. Elsevier Inc. https://doi.org/10.1016/C2014-0-01830-8

Romanski, L. M., Tian, B., Fritz, J., Mishkin, M., Goldman-Rakic, P. S., & Rauschecker, J. P. (1999). Dual streams of auditory afferents target multiple domains in the primate prefrontal cortex. *Nature Neuroscience*, *2*(12), 1131–1136. https://doi.org/10.1038/16056

Roshan, L., Paradiso, G. O., & Chen, R. (2003). Two phases of short-interval intracortical inhibition. *Experimental Brain Research*, *151*(3), 330–337. https://doi.org/10.1007/s00221-003-1502-9

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., Avanzini, G., Bestmann, S., Berardelli, A., Brewer, C., Canli, T., Cantello, R., Chen, R., Classen, J., Demitrack, M., Di Lazzaro, V., Epstein, C. M., George, M. S., Fregni, F., Ilmoniemi, R., Jalinous, R., … Ziemann, U. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology*, *120*(12), 2008–2039. https://doi.org/10.1016/j.clinph.2009.08.016

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., Dimitrijević, M. R., Hallett, M., Katayama, Y., Lücking, C. H., Maertens de Noordhout, A. L., Marsden, C. D., Murray, N. M. F., Rothwell, J. C., Swash, M., & Tomberg, C. (1994). Non-invasive electrical and magnetic stimulation of

the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalography and Clinical Neurophysiology*, *91*(2), 79–92. https://doi.org/10.1016/0013-4694(94)90029-9

Rossini, P. M., Zarola, F., Stalberg, E., & Caramia, M. (1988). Pre-movement facilitation of motor-evoked potentials in man during transcranial stimulation of the central motor pathways. *Brain Research*, *458*(1), 20–30. https://doi.org/https://doi.org/10.1016/0006-8993(88)90491-X

Rothwell, J. C. (2006). Chapter 18 The startle reflex, voluntary movement, and the reticulospinal tract. *Supplements to Clinical Neurophysiology*, *58*(C), 223–231. https://doi.org/10.1016/S1567-424X(09)70071-6

Selvanayagam, V. S., Riek, S., De Rugy, A., & Carroll, T. J. (2016). Strength Training Biases Goal-Directed Aiming. *Medicine and Science in Sports and Exercise*, *48*(9), 1835–1846. https://doi.org/10.1249/MSS.0000000000000956

Serranová, T., Jech, R., Martí, M. J., Modreanu, R., Valldeoriola, F., Sieger, Z., Růžička, E., & Valls-Solé, J. (2012). A loud auditory stimulus overcomes voluntary movement limitation in cervical dystonia. *PloS One*, *7*(10). https://doi.org/10.1371/JOURNAL.PONE.0046586

Serrien, D. J., Strens, L. H. A., Cassidy, M. J., Thompson, A. J., & Brown, P. (2004). Functional significance of the ipsilateral hemisphere during movement of the affected hand after stroke. *Experimental Neurology*, *190*(2), 425–432. https://doi.org/10.1016/j.expneurol.2004.08.004

Shibuya, K., Kuboyama, N., & Tanaka, J. (2014). Changes in ipsilateral motor cortex activity during a unilateral isometric finger task are dependent on the muscle contraction force. *Physiological Measurement*, *35*(3), 417–428. https://doi.org/10.1088/0967-3334/35/3/417

Shim, J. K., Oliveira, M. A., Hsu, J., Huang, J. F., Park, J., & Clark, J. E. (2007). Hand digit control in children: Age-related changes in hand digit force interactions during maximum flexion and extension force production tasks. *Experimental Brain Research*, *176*(2), 374–386. https://doi.org/10.1007/s00221-006-0629-x

Starr, A., Caramia, M., Zarola, F., & Rossini, P. M. (1988). Enhancement of motor cortical excitability in humans by non-invasive electrical stimulation appears prior to voluntary movement. *Electroencephalography and Clinical Neurophysiology*, *70*(1), 26–32. https://doi.org/10.1016/0013-4694(88)90191-5

Stevenson, A. J., Chiu, C., Maslovat, D., Chua, R., Gick, B., Blouin, J. S., & Franks, I. M. (2014). Cortical involvement in the StartReact effect. *Neuroscience*, *269*, 21–34. https://doi.org/10.1016/j.neuroscience.2014.03.041.

Stinear, C. M., Walker, K. S., & Byblow, W. D. (2001). Symmetric facilitation between motor cortices during contraction of ipsilateral hand muscles. *Experimental Brain Research*, *139*(1), 101–105. https://doi.org/10.1007/s002210100758

Strens, L. H. A., Fogelson, N., Shanahan, P., Rothwell, J. C., & Brown, P. (2003). The ipsilateral human motor cortex can functionally compensate for acute contralateral motor cortex dysfunction. *Current Biology*, *13*(14), 1201–1205. https://doi.org/10.1016/S0960-9822(03)00453-6

Szabadi, E. (2012). Modulation of physiological reflexes by pain: Role of the locus coeruleus. *Frontiers in Integrative Neuroscience*, *6*, 94. https://doi.org/10.3389/fnint.2012.00094

Teasdale, N., Bard, C., Fleury, M., Young, D. E., & Proteau, L. (1993). Determining movement onsets from temporal series. *Journal of Motor Behavior*, *25*(2), 97–106. https://doi.org/10.1080/00222895.1993.9941644

Thach, W. T. (1978). Correlation of neural discharge with pattern and force of muscular activity, joint position, and direction of intended next movement in motor cortex and cerebellum. *Journal of Neurophysiology*, *41*(3), 654–676. https://doi.org/10.1152/jn.1978.41.3.654

Tona, K. D., Murphy, P. R., Brown, S. B. R. E., & Nieuwenhuis, S. (2016). The accessory stimulus effect is mediated by phasic arousal: A pupillometry study. *Psychophysiology*, *53*(7), 1108–1113. https://doi.org/10.1111/psyp.12653

Touge, T., Taylor, J. L., & Rothwell, J. C. (1998). Reduced excitability of the cortico-spinal system during the warning period of a reaction time task. *Electroencephalography and Clinical Neurophysiology - Electromyography*

and Motor Control, *109*(6), 489–495. https://doi.org/10.1016/S0924-980X(98)00050-2

Tresch, U. A., Perreault, E. J., & Honeycutt, C. F. (2014). Startle evoked movement is delayed in older adults: Implications for brainstem processing in the elderly. *Physiological Reports*, *2*(6), e12025. https://doi.org/10.14814/phy2.12025

Tresilian, J. R., & Plooy, A. M. (2006). Effects of acoustic startle stimuli on interceptive action. *Neuroscience*, *142*(2), 579–594. https://doi.org/10.1016/j.neuroscience.2006.06.029

Uematsu, A., Obata, H., Endoh, T., Kitamura, T., Hortobágyi, T., Nakazawa, K., & Suzuki, S. (2010). Asymmetrical modulation of corticospinal excitability in the contracting and resting contralateral wrist flexors during unilateral shortening, lengthening and isometric contractions. *Experimental Brain Research*, *206*(1), 59–69. https://doi.org/10.1007/s00221-010-2397-x

Ulrich, R., Miller, J., & Schröter, H. (2007). Testing the race model inequality: An algorithm and computer programs. *Behavior Research Methods*, *39*(2), 291–302. https://doi.org/10.3758/BF03193160

Ulrich, R., Rinkenauer, G., & Miller, J. (1998). Effects of stimulus duration and intensity on simple reaction time and response force. *Journal of Experimental Psychology: Human Perception and Performance*, *24*(3), 915–928. https://doi.org/10.1037/0096-1523.24.3.915

Valldeoriola, F., Valls-Solé, J., Tolosa, E., Ventura, P. J., Nobbe, F. A., & Martí, M. J. (1998). Effects of a startling acoustic stimulus on reaction time in different parkinsonian syndromes. *Neurology*, *51*(5), 1315–1320. https://doi.org/10.1212/wnl.51.5.1315

Vallence, A. M., Hammond, G. R., & Reilly, K. T. (2012). Increase in flexor but not extensor corticospinal motor outputs following ischemic nerve block. *Journal of Neurophysiology*, *107*(12), 3417–3427. https://doi.org/10.1152/jn.01118.2010

Valls-Solé, J., Kofler, M., Kumru, H., Castellote, J. M., & Sanegre, M. T. (2005). Startle-induced reaction time shortening is not modified by prepulse inhibition. *Experimental Brain Research*, *165*(4), 541–548.

https://doi.org/10.1007/s00221-005-2332-8

Valls-Solé, J., Kumru, H., & Kofler, M. (2008). Interaction between startle and voluntary reactions in humans. *Experimental Brain Research*, *187*(4), 497–507. https://doi.org/10.1007/s00221-008-1402-0

Valls-Solé, J., Rothwell, J. C., Goulart, F., Cossu, G., & Muñoz, E. (1999). Patterned ballistic movements triggered by a startle in healthy humans. *Journal of Physiology*, *516*(3), 931–938. https://doi.org/10.1111/j.1469-7793.1999.0931u.x

Valls-Solé, J., Solé, A., Valldeoriola, F., Muñoz, E., Gonzalez, L. E., & Tolosa, E. S. (1995). Reaction time and acoustic startle in normal human subjects. *Neuroscience Letters*, *195*(2), 97–100. https://doi.org/10.1016/0304-3940(94)11790-P

Valls-Solé, J., Valldeoriola, F., Molinuevo, J. L., Cossu, G., & Nobbe, F. (1999). Prepulse modulation of the startle reaction and the blink reflex in normal human subjects. *Experimental Brain Research*, *129*(1), 49–56. https://doi.org/10.1007/s002210050935

Van Elswijk, G., Kleine, B. U., Overeem, S., & Stegeman, D. F. (2007). Expectancy induces dynamic modulation of corticospinal excitability. *Journal of Cognitive Neuroscience*, *19*(1), 121–131. https://doi.org/10.1162/jocn.2007.19.1.121

Vermeiren, Y., & De Deyn, P. P. (2017). Targeting the norepinephrinergic system in Parkinson's disease and related disorders: The locus coeruleus story. *Neurochemistry International*, *102*, 22–32. https://doi.org/10.1016/j.neuint.2016.11.009

Wade, D. T., Langton-Hewer, R., Wood, V. A., Skilbeck, C. E., & Ismail, H. M. (1983). The hemiplegic arm after stroke: Measurement and recovery. *Journal of Neurology Neurosurgery and Psychiatry*, *46*(6), 521–524. https://doi.org/10.1136/jnnp.46.6.521

Whelan, R. (2008). Effective analysis of reaction time data. *Psychological Record*, *58*(3), 475–482. https://doi.org/10.1007/BF03395630

Wickens, J., Hyland, B., & Anson, G. (1994). Cortical cell assemblies: A possible mechanism for motor programs. *Journal of Motor Behavior*, *26*(2), 66–82.

https://doi.org/10.1080/00222895.1994.9941663

Xu-Wilson, M., Tian, J., Shadmehr, R., & Zee, D. S. (2011). TMS perturbs saccade trajectories and unmasks an internal feedback controller for saccades. *Journal of Neuroscience*, *31*(32), 11537–11546. https://doi.org/10.1523/JNEUROSCI.1584-11.2011

Yeomans, J. S., & Frankland, P. W. (1995). The acoustic startle reflex: Neurons and connections. *Brain Research Reviews*, *21*(3), 301–305.

Yoon, T., Geary, R. B., Ahmed, A. A., & Shadmehr, R. (2018). Control of movement vigor and decision making during foraging. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(44), E10476–E10485. https://doi.org/10.1073/pnas.1812979115

Yu, W. S., van Duinen, H., & Gandevia, S. C. (2010). Limits to the control of the human thumb and fingers in flexion and extension. *Journal of Neurophysiology*, *103*(1), 278–289. https://doi.org/10.1152/jn.00797.2009

Ziemann, U., Ishii, K., Borgheresi, A., Yaseen, Z., Battaglia, F., Hallett, M., Cincotta, M., & Wassermann, E. M. (1999). Dissociation of the pathways mediating ipsilateral and contralateral motor-evoked potentials in human hand and arm muscles. *The Journal of Physiology*, *518*(3), 895–906. https://doi.org/10.1111/j.1469-7793.1999.0895p.x

Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Appendix - Copyright Permissions

Figure 1.2.

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

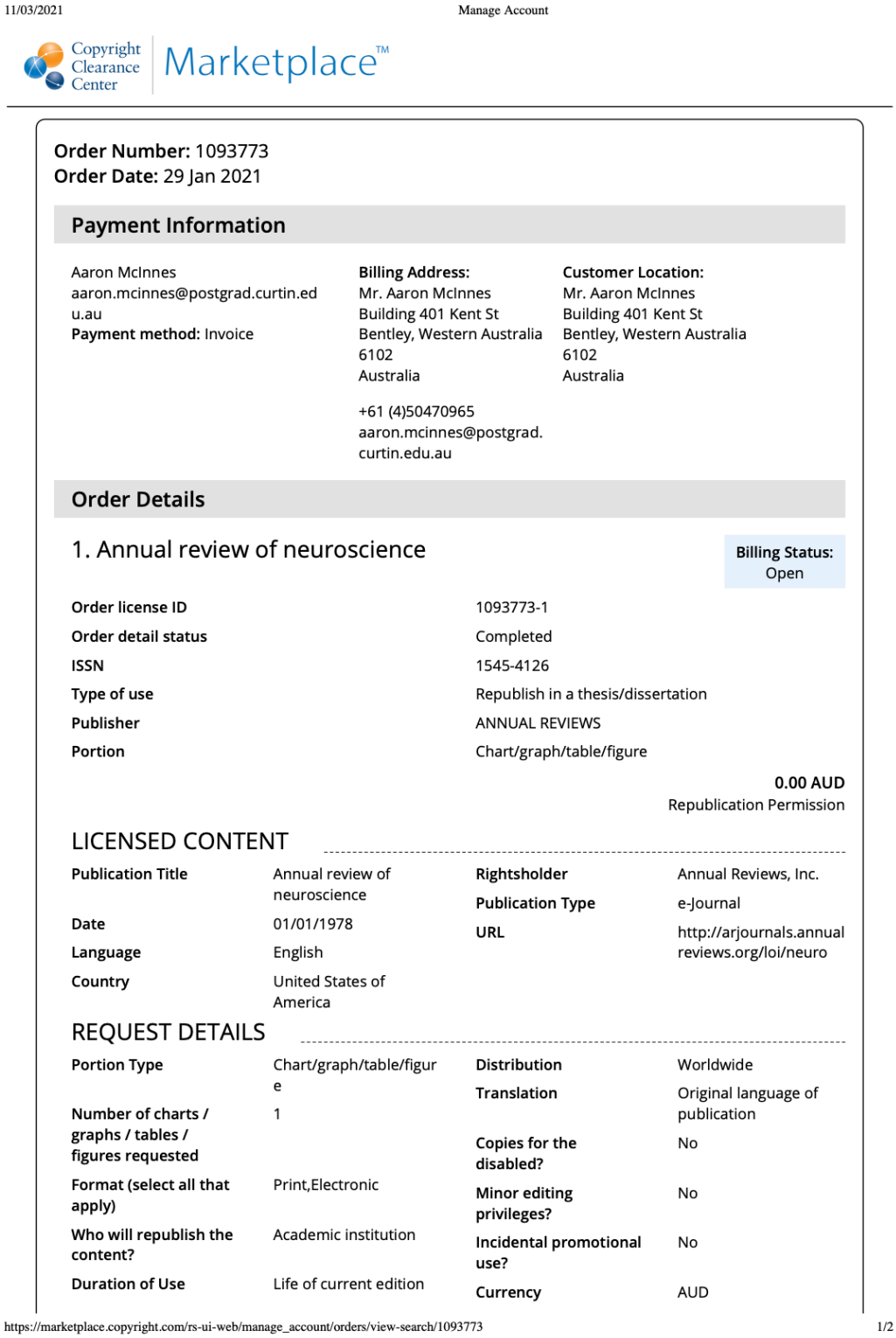
Mar 11, 2021

This Agreement between Mr. Aaron McInnes ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4997940136482
License date	Jan 28, 2021
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Acta Physiologica
Licensed Content Title	Triggering prepared actions by sudden sounds: reassessing the evidence for a single mechanism
Licensed Content Author	J. R. Tresilian, W. Marinovic
Licensed Content Date	Dec 12, 2015
Licensed Content Volume	217
Licensed Content Issue	1
Licensed Content Pages	20
Type of use	Dissertation/Thesis
Requestor type	University/Academic

Format	Print and electronic
Portion	Figure/table
Number of figures/tables	1
Will you be translating?	No
Title	Use the force: Augmenting neural excitability via unexpected acoustic stimulation and movement vigour
Institution name	Curtin University
Expected presentation date	Mar 2021
Portions	Figure 3
Requestor Location	Mr. Aaron McInnes Building 401 Kent St Bentley, Western Australia 6102 Australia Attn: Mr. Aaron McInnes
Publisher Tax ID	EU826007151
Total	0.00 AUD

Figure 1.4



11/03/2021 Manage Account

Copyright Clearance Center | Marketplace™

Order Number: 1093773
Order Date: 29 Jan 2021

Payment Information

Aaron McInnes
aaron.mcinnes@postgrad.curtin.edu.au
Payment method: Invoice

Billing Address:
Mr. Aaron McInnes
Building 401 Kent St
Bentley, Western Australia 6102
Australia

+61 (4)50470965
aaron.mcinnes@postgrad.curtin.edu.au

Customer Location:
Mr. Aaron McInnes
Building 401 Kent St
Bentley, Western Australia 6102
Australia

Order Details

1. Annual review of neuroscience

Billing Status: Open

Order license ID	1093773-1
Order detail status	Completed
ISSN	1545-4126
Type of use	Republish in a thesis/dissertation
Publisher	ANNUAL REVIEWS
Portion	Chart/graph/table/figure

0.00 AUD
Republication Permission

LICENSED CONTENT

Publication Title	Annual review of neuroscience	**Rightsholder**	Annual Reviews, Inc.
		Publication Type	e-Journal
Date	01/01/1978	**URL**	http://arjournals.annualreviews.org/loi/neuro
Language	English		
Country	United States of America		

REQUEST DETAILS

Portion Type	Chart/graph/table/figure	**Distribution**	Worldwide
		Translation	Original language of publication
Number of charts / graphs / tables / figures requested	1	**Copies for the disabled?**	No
Format (select all that apply)	Print,Electronic	**Minor editing privileges?**	No
Who will republish the content?	Academic institution	**Incidental promotional use?**	No
Duration of Use	Life of current edition	**Currency**	AUD

Lifetime Unit Quantity	Up to 499
Rights Requested	Main product

NEW WORK DETAILS

Title	Use the force: Augmenting neural excitability via unexpected acoustics stimulation and movement vigour.	**Institution name**	Curtin University
		Expected presentation date	2021-03-19
Instructor name	Aaron McInnes		

ADDITIONAL DETAILS

The requesting person / organization to appear on the license	Aaron McInnes

REUSE CONTENT DETAILS

Title, description or numeric reference of the portion(s)	Figure 1	**Title of the article/chapter the portion is from**	Descending pathways in motor control
Editor of portion(s)	N/A	**Author of portion(s)**	Roger N. Lemon
Volume of serial or monograph	Botond Roska and Huda Y. Zoghbi	**Issue, if republishing an article from a serial**	31
Page or page range of portion	199	**Publication date of portion**	2008-04-04

Total Items: 1

Subtotal: 0.00 AUD

Order Total: 0.00 AUD

Chapter Two

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Mar 11, 2021

This Agreement between Mr. Aaron McInnes ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4997941507769
License date	Jan 28, 2021
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	European Journal of Neuroscience
Licensed Content Title	Cumulative distribution functions: An alternative approach to examine the triggering of prepared motor actions in the StartReact effect
Licensed Content Author	Welber Marinovic, James R. Tresilian, Stephan Riek, et al
Licensed Content Date	Sep 30, 2020
Licensed Content Volume	0
Licensed Content Issue	0
Licensed Content Pages	24
Type of use	Dissertation/Thesis

Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title	Use the force: Augmenting neural excitability via unexpected acoustic stimulation and movement vigour
Institution name	Curtin University
Expected presentation date	Mar 2021
Requestor Location	Mr. Aaron McInnes Building 401 Kent St Bentley, Western Australia 6102 Australia Attn: Mr. Aaron McInnes
Publisher Tax ID	EU826007151
Total	0.00 AUD