INHIBITORY CONTROL PROCESSES DURING THE PREPARATION AND INITIATION OF MOTOR RESPONSES

By

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This thesis is dedicated to my Grandmother

FLORIKA GERTSAKIS

For her strength, love and joy for life.

Abstract of the Dissertation

The ability to stop ongoing movements or prevent unwanted movements is fundamental to behavioural control. This thesis addresses the neural processes underlying inhibitory control and how initiation and stop processes interact to control behaviour. We conducted four studies, employing various behavioural tasks that require humans to prepare to initiate a response with the possibility that it may have to be prevented or stopped from being initiated. In the first experiment we sought to determine whether the increase in reaction time (RT) during the performance of traditional stop-signal task was due to a decreased the amount of go-related preparatory activation. We used a startling acoustic stimulus (SAS) to determine whether the go-response could be triggered involuntarily, and investigated whether the latency of SAS responses were delayed when participants were instructed that they might have to stop their response compared to when they knew they would never stop (i.e., simple RT task). We found that the go-response was prepared in advance during the stop-signal task, but to a lesser degree as indicated by the slower SAS response latency, compared to when go trials were completed in the simple RT task. Thus, even the possibility of having to stop a response reduces the level of preparatory go-activation.

The second experiment tested the hypothesis that behavioural control during a stop-signal task is determined by an independent race between go- and stop-processes. In this experiment we used a SAS to manipulate initiation and inhibition by probing the go- and stop-response prior to and after the stop-signal respectively in a stop-signal task. We found that the go-response could be triggered by the SAS even 200 ms following the stop-signal suggesting that behavioural control during a stop-signal task is not determined by an independent race between go- and stop-activations, but rather by an interaction between go-activation and stop processes.

The third experiment investigated the effect of advance preparation on the ability to proactively and selectively inhibit a single limb in a bimanual response that had been cued to maybe stop. TMS was used to measure the excitability of the limb that was cued to maybe stop in comparison to the limb that was to continue with its response. In addition, a SAS was used to probe the preparatory state of the go-response in each limb. We found that increased preparatory go-activation of responses in both limbs overshadowed the neurophysiological evidence of proactive selective inhibition, while processes related to the selective stopping task appeared to suppress subcortical motor structures and the ability of the SAS to involuntarily trigger the prepared responses.

The fourth experiment sought to determine the role of the right inferior frontal gyrus (rIFG) and the pre-supplementary motor area (preSMA) in the inhibition of response initiation during a go/no-go task. We temporarily deactivated rIFG or preSMA using continuous theta burst stimulation (cTBS) and examined changes in inhibition, voluntary initiation, and the ability of a SAS to involuntary trigger the initiation of the response. We found that stimulation to both cortical sites impaired participant's ability to withhold movements during no-go trials. Notably, deactivating rIFG and preSMA did not affect voluntary initiation and did not enable the SAS to involuntarily trigger the response. These findings implicate the rIFG and the preSMA in the ability to inhibit responses during a go/no-go task, and suggests that preparation and initiation of the go-response occurs in response to the imperative stimulus, with inhibition only applied once the stimulus is identified as a no-go signal.

Taken together, these studies show that i) modulation of preparatory go-activation contributes to the ability to inhibit a motor response, ii) motor response inhibition is achieved by initiation activation being prevented from reaching threshold, iii) preparatory go-activation overshadows proactive inhibition, iv) inhibitory control depends on the integrity and recruitment of top-down inhibitory control to suppress initiation activation once a no-go stimulus is identified. This research speaks to the

interaction between initiation and inhibition processes and provides novel insight and evidence in support of an interactive model of inhibitory control.

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Glossary of Terms

BG: Basal ganglia

M1: Motor cortex

SMA: Supplementary motor area

preSMA: pre-supplementary motor area

rIFG: Right inferior frontal gyrus

STN: Subthalamic nucleus

GPi: Globus pallidus pars internal

GPe: Globus pallidus pars external

TMS: Transcranial magnetic stimulation

cTBS: Continuous theta burst stimulation

tDCS: Transcranial direct current stimulation

SAS: Startling acoustic stimulus

fMRI: Functional magnetic resonance imaging

EMG: Electromyography

ECoG: Electrocorticography

ERP: Event-related potential

RT: Reaction time

SSRT: Stop-signal reaction time

SSD: Stop-signal delay

CE: Corticospinal excitability

MEP: Motor evoked potential

MVF: Maximum voluntary force

RMT: Resting motor threshold

AMT: Active Motor threshold

ECR: Extensor carpi radialis

SCM: Sternocleidomastoid

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Statement of Contribution

I, Neil M. Drummond, was primarily responsible for the research program and experimental design for all of the included experiments, with input and oversight by my thesis committee consisting of Dr. Erin K. Cressman (Supervisor), Dr. Anthony N. Carlsen (Supervisor), Dr. Diane M. Ste-Marie, and Dr. Martin Bilodeau. Participant recruitment, experimental set-up, including programming, data collection, data analysis, data reduction, and statistical analysis were primarily completed by myself, Neil M. Drummond. Manuscript preparation for all chapters was completed by me, Neil M. Drummond, with input and/or editorial contributions from Dr. Erin K. Cressman and Dr. Anthony N. Carlsen.

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1. Chapter 1 General Introduction

Inhibiting unwanted movements, including stopping a pending or ongoing movement, is necessary to carry out actions as desired in a changing environment. Movement inhibition can occur either proactively by anticipating environmental changes, or reactively in response to sudden changes in the environment (Aron, 2011; Stuphorn & Emeric, 2012). Two behavioural tasks that have been used extensively in the literature to study inhibitory control are the stop-signal and go/no-go paradigms. Studies using neuroimaging during the performance of these tasks have identified a network of cortical and subcortical brain regions that are suggested to play an important role in proactive and reactive inhibitory control (Chambers, Garavan, & Bellgrove, 2009; Swick, Ashley, & Turken, 2011). This network includes the medial and lateral frontal cortex, basal ganglia (BG), and motor cortex (M1). Moreover, studies have emphasized critical roles for the pre-supplementary motor cortex (preSMA), right inferior frontal gyrus (rIFG), basal ganglia (BG), and the subthalamic nucleus (STN) in reactive stopping (Aron, 2011; Benis et al., 2014). In contrast, proactive control depends on activation in the preSMA, rIFG, and the striatum (Aron, 2011; Majid, Cai, Corey-Bloom, & Aron, 2013). While the neural circuitry and effect on inhibitory control differ between proactive and reactive inhibition, their underlying processes both ultimately converge on the motor cortex (M1) to prevent response output. Although many advances have been made in understanding the neural pathways and circuitry related to both forms of inhibitory control, it remains unclear how inhibition of a response competes or interacts with the initiation of a response to determine movement outcome. The overall goal of the projects outlined below is to determine the neural processes underlying inhibitory control and how go- and stop- processes interact to control behaviour.

Before outlining the questions of interest in more detail, the literature review will first describe the two behavioural tasks traditionally used to investigate inhibitory control (stop-signal task & go/no-go task). This will be followed by an examination of the literature pertaining to reactive and proactive

inhibitory control. I will then introduce two competing neural models of inhibitory control. To conclude, a description of how startle can be used as a novel tool to investigate inhibitory control will be provided, including how it has the potential to provide further insight into reactive and proactive inhibitory control and the mechanisms by which initiation and inhibition compete to control motor output.

1.1. Behavioural Tasks Used to Investigate Inhibitory Control

1.1.1. Stop-signal task

The stop-signal task incorporates processes related to both movement initiation and movement inhibition. Although many variations of the task exist, the core requirements are present across all task variations. Specifically, the stop-signal task requires participants to plan and initiate a movement as fast as possible in response to a go-signal, but inhibit this movement if following the go-signal a stop-signal is presented. The length of the delay between the go-signal and the stop-signal [i.e., stop-signal delay (SSD)] determines the success of stopping, such that a longer delay results in a decreased ability to withhold the movement.

The initiation or inhibition of the movement in a stop-signal task has been accounted for by a horse race model (Logan, Cowan, & Davis, 1984), which posits that two independent processes (a go and a stop process) race for control over the movement. The process that wins the race ultimately determines movement outcome. Based on this model, participants' performance during a stop-signal task can be used to calculate not only the speed of the initiation process (i.e., RT), but also the speed of the inhibitory process [i.e., stop signal reaction time (SSRT)], where the SSRT is determined by calculating the average time needed for the inhibitory process to stop/prevent the movement from being initiated (Logan et al., 1984). Based on numerous stop-signal task studies, the SSRT is on average 250 ms (Scangos & Stuphorn, 2010; Stuphorn & Emeric, 2012), indicating that if the SSD + 250 ms is less than the average response initiation time (RT), inhibition will win control over movement outcome.

1.1.2. Go/no-go task

In a typical go/no-go task only one stimulus is presented on each trial, either a go stimulus or a no-go stimulus. Participants are instructed to initiate the known response as fast as possible in response to the go stimulus. Thus, inhibitory performance is measured by the ability to withhold the response when the no-go stimulus is presented.

1.2. Reactive and Proactive control

Based on performance in the stop-signal and go/no-go tasks, it has been suggested that inhibitory control processes during the preparation and initiation of motor responses operate via two distinct modes: reactive control and proactive control (Aron, 2011; Braver, 2012; Stuphorn & Emeric, 2012). Reactive control has been described as a late correction mechanism, relying upon the detection of a stimulus that signals the stopping or changing of the planned or ongoing response (Braver, 2012). Conversely, it is proposed that proactive control uses task and goal-relevant information to bias the motor system prior to performing the movement, allowing advance preparation for the possibility of stopping (Braver, 2012). The following two sections will review the literature pertaining to the neural pathways and systems for reactive and proactive control.

1.2.1. Neural Pathways and Systems for Reactive Stopping

In brief, sensory information about the stop-signal is quickly sent to the prefrontal cortex, where inhibitory control arises and the stopping command begins to be generated. This command then likely targets the BG to prevent motor output at the level of the motor cortex. This network will now be laid out in more detail. Converging evidence suggests that two specific frontal regions are heavily involved in generating the inhibitory command; the rIFG, and the preSMA/SMA (see Aron, 2011; Stuphorn & Emeric, 2012 for a review). For instance, neuroimaging studies have repeatedly shown that both of these regions are highly active during stop-signal and go/no-go tasks, both of which are suggested to use

a reactive mode of inhibitory control (Aron, 2011; Stuphorn & Emeric, 2012). In addition, lesion studies and transcranial magnetic stimulation have shown that stopping an initiated response depends on the integrity of the rIFG, as well as the preSMA (reviewed by Aron, 2011; Chambers et al., 2009; Chikazoe, 2010; Levy & Wagner, 2011; Nachev, Kennard, & Husain, 2008). As individual nodes these two frontal areas are important for behavioural control, but also appear to be a part of a network or system for inhibitory control. Specifically, diffusion tensor imaging has shown strong structural connections between preSMA and IFG, as well as the BG (Aron, Behrens, Smith, Frank, & Poldrack, 2007; Ford, McGregor, Case, Crosson, & White, 2010; Forstmann et al., 2010).

PreSMA and rIFG are suggested to send information to subcortical structures to inhibit cortical M1 activation. The subcortical structure likely relaying inhibitory drive from preSMA/IFG to M1 during reactive inhibitory control is the subthalamic nucleus (STN), as it receives direct input from both preSMA and rIFG (Aron et al., 2007; Inase, Tokuno, Nambu, Akazawa, & Takada, 1999). Moreover, the STN also broadly excites the globus pallidus pars internal (GPi), which increases inhibition on thalamocortical output in a non-specific fashion (Aron, 2011; Gillies & Willshaw, 1998). Support for the role of the STN in the reactive stopping pathway has been shown with increased activation of the STN region on successful stops compared to failed stops during a stop-signal task (Aron & Poldrack, 2006), and altered stopping performance in Parkinson's disease patients when the STN was modulated with deep brain stimulation. This pathway for reactive inhibitory control has been termed the *hyperdirect* pathway (see Figure 1A) and has been suggested to result in a global non-specific nature of inhibition, such that the entire motor system is inhibited. Support for global inhibition comes from TMS studies demonstrating reduced corticospinal excitability even in limbs that are not relevant for task performance, such as bilateral inhibition (reduced MEP amplitude) in the hand and leg muscles even when inhibition of only a single hand is required or occurs (Badry et al., 2009; Greenhouse, Oldenkamp, & Aron, 2012). In sum, the

evidence supports a cortico- STN hyperdirect pathway for reactive stopping, which has global inhibitory effects on the M1.

1.2.2. Neural Pathways and Systems for Proactive Stopping

Although reactive stopping may be important in everyday life (e.g., preventing oneself from stepping in front of a New York taxi cab that comes racing down the street, or in sports requiring fast movements in response to a changing environment), it may be more beneficial to proactively anticipate the possibility of having to stop a specific response without affecting all other responses. To investigate proactive stopping, researchers have used a modified version of the stop-signal task called a selective stopping task. Essential to this selective stopping task is that advance information is given about which response/limb needs to be stopped, if and when a stop stimulus is presented - while for all other responses/limbs the stop stimulus should be ignored and the movement initiated. For example, during a dual-response stop-signal task (press buttons simultaneously with your left and right hand in response to a go-signal), a message stating “Maybe Stop Right” prior to the go-signal indicates that on that trial, should a stop-signal be presented, the participant would only have to stop the right hand response, but continue responding with the left hand.

Behavioural studies using TMS to index corticospinal excitability have found that unlike reactive stopping, which results in global suppression of the motor system (i.e., reduced MEP amplitude in all limbs regardless of task relevancy), proactive stopping is selective such that there is only inhibition (reduced MEP amplitude) in the task relevant limb that may have to be inhibited (Cai, Oldenkamp, & Aron, 2011; Greenhouse et al., 2012; Majid et al., 2013). However, this selective inhibition allowed by proactive control comes at a cost, as it has consistently been shown to result in longer stopping times during stop-signal trials (larger SSRT) and longer response initiation times (larger RT) during go trials (Greenhouse et al., 2012; Jahfari et al., 2011) compared to reactive inhibition.

Converging evidence suggests that proactive inhibition is carried out by a similar network to reactive inhibition with the use of the preSMA and rIFG, with recent investigations beginning to delineate the specific roles of the preSMA and rIFG in the inhibitory control network. Specifically, Swann and colleagues (2012) used fMRI and electrocorticography (ECoG) to investigate the roles of preSMA and rIFG in preparing to stop and stopping an action outright in a stop-signal task in which a cue was presented indicating to participants that they might have to stop (i.e., cue = stop-signal may be presented) or that they would not have to stop (i.e., cue = no stop-signal). Results from stop-signal cue trials revealed a serial activation onset pattern for preparing to stop, with preSMA activity beginning following cue onset with subsequent activation of rIFG only after go-signal onset. Inspection of activity following the stop-signal itself revealed that only rIFG was active, suggesting rIFG is involved in outright stopping of the movement. Based on these event and timing related activation patterns, Swann and colleagues (2012) suggest that the preSMA translates the stopping cue into a defined inhibitory command, and prepares the rIFG to implement the inhibitory command and stop behaviour via connections to subcortical structures, with final output to the motor cortex.

Under proactive inhibitory control, it is proposed that the output from preSMA and rIFG is sent to the striatum of the BG (Majid et al., 2013), through what is referred to as the *indirect* pathway (instead of STN in the hyperdirect pathway for reactive inhibition) (see Aron, 2011 for a review). Activation of preSMA & rIFG increases the activity of the striatum (specifically the caudate) which in turn decreases the activation of GPe (external). The decrease in GPe activation results in an increase in activation of a specific channel of the GPi either directly or via the STN. The increase in GPi activation results in the selective inhibition of a particular thalamocortical channel and consequent M1 inhibitory target (see Figure 1B for a schematic of the indirect pathway). It is the specific termination pattern of striatal neurons onto the GPe, and from GPe to GPi, that causes the focused (i.e., selective) inhibition,

unlike the effect of the hyperdirect inhibitory control pathway of STN on the GPi, which is very diffuse (i.e., global). Further support for this indirect pathway for proactive inhibition has been demonstrated by proactive stopping impairments found in patients with Huntington's disease (Majid et al., 2013), who experience degeneration/loss of striatal neurons contributing to the indirect pathway in the early stages of the disease (Albin, Young, & Penney, 1995; Starr, Kang, Heath, Shimamoto, & Turner, 2008; Vonsattel et al., 1985). Therefore, when given advance information about what response may need to be inhibited or controlled, the indirect pathway proactively constrains this response by suppressing those channels specific to its motor representation (Aron, 2011).

1.3. Neural models of inhibitory control

While the neural pathways and circuitry related to both forms of inhibitory control are relatively clear, the relationship between inhibitory and initiation activation remains unclear. For example, inhibition of motor output from M1 may be achieved by inhibition converging on the same pool of neurons as used by initiation to supress go-activation, or inhibition running in parallel to initiation and reaching response threshold first. Each possibility is currently accounted for by distinct neural models of inhibitory control.

The dominant theory in the field of inhibitory control has been the independent race model (Logan et al., 1984), which suggests that independent go- and stop-processes running in parallel race toward a single response threshold. Thus response outcome (initiation or inhibition) is determined by the activation signal that reaches threshold first. Recently however, alternative models which take into account recent neurophysiological findings have proposed that inhibitory control is explained via the interaction of go and stop processes (Boucher, Palmeri, Logan, & Schall, 2007; Dunovan, Lynch, Molesworth, & Verstynen, 2015). While several variations of an interactive model exist, they are conceptually similar, such that instead of parallel processes racing for control, response outcome is

determined by the modulation of a single go-activation towards or away from response threshold (Boucher et al., 2007; Dunovan et al., 2015). As such, inhibitory activation interacts with go-activation, which may or may not prevent it from reaching response threshold depending on the activation state of the go-process and the timing of inhibition. The differences between the parallel and interactive provide a distinct account of inhibitory control. Consider a water-holding vessel such as a bucket, with the top edge of the bucket representing response threshold. The independent model suggests the bucket has two chambers, one for a go tap to add water to and the other for the stop tap adding water to. The chamber that fills up first and starts overflowing is the winner, thus determining response outcome. In contrast, an interactive model suggests there is a single go tap filling a (single chamber) bucket up, with a stop drain that can deplete the amount of water in the bucket and prevent it from overflowing. The current research program looks to test the predictions put forth by the models, in particular the independent race model, and establish if inhibition and initiation processes proceed in a parallel or interactive fashion. The studies presented will combine various techniques with a SAS to investigate the evolution of activation within the motor system from a state of no-inhibition to a state of complete inhibition to gain insight into inhibitory control processes during the preparation and initiation of motor responses

1.4. Startle as a new tool to investigate stopping/inhibition

A startling acoustic stimulus (SAS >120 dB) has been used as a tool to investigate processes related to response preparation and initiation. When a response is known and prepared in advance, the presentation of a SAS not only results in the typical startle reflex (Brown et al., 1991; Landis, Hunt, & Strauss, 1939), but also has the ability to trigger the prepared response at latency too short to involve the voluntary initiation pathway (see Figure 1C). This effect has been defined as the “StartReact” effect (see Carlsen, Maslovat, & Franks, 2012 for a review). It has been suggested that the StartReact effect

arises when sufficient response preparation has occurred such that the SAS is able to increase response initiation and activation levels to an extent that will reach the required threshold and trigger the movement (Carlsen, Maslovat, et al., 2012). Specifically, Carlsen and colleagues suggest that "the SAS acts to facilitate RT by quickly and directly increasing activation of the initiation mechanism via a subcortically mediated ascending pathway such that the cortically stored response is triggered without the usual cortical processing" (p.26). The neuroanatomical correlates and pathway implicated in the StartReact effect is tightly coupled to the startle reflex pathway, which acts primarily through the reticular formation. After the SAS activates the reticular formation, descending activation is produced resulting in the startle reflex, as well as ascending activation, specifically ascending projections from the pontine reticular formation to the thalamus (see Figure 1D). Increased activation of the thalamus has been suggested to then provide the required input to the cortex to trigger the prepared movement via the primary motor cortex (i.e., give rise to the StartReact effect) (Carlsen, Maslovat, et al., 2012). Recent evidence further suggests that the startle neural activity leading to response initiation adds on to normal cortical initiation-related activation (Maslovat, Carter, Kennefick, & Carlsen, 2014). In terms of the aforementioned bucket analogy, the SAS appears to be able to temporarily increase the rate of flow from the "go" tap, and depending on how full the bucket is at that time, the additional influx of water fills the bucket up and either makes it overflow or not.

Given that the reactive and proactive fronto-BG-thalamic-M1 inhibitory pathways outlined in the sections above share common neural substrates with that of the startle/StartReact pathway (see Figure 1), startle may be able to be used as a tool to provide insight into inhibitory control processes. There is substantial evidence supporting a subcortically mediated startle reflex (Yeomans & Frankland, 1996), which would provide insight into the effects of inhibitory control on subcortical structures. Moreover, there is growing evidence to support a cortically mediated (M1) StartReact effect via

ascending activation from the thalamus (Alibiglou & MacKinnon, 2012; Stevenson et al., 2014). Thus, ascending startle activation to the thalamus may not only be additive with go-activation, but also additive with stop-activation. Therefore, we will use startle to provide insight into whether go- and stop-processes run in parallel, evidenced by startle indiscriminately propagating the initiation or inhibitory response depending on the state of each relative to threshold. Accordingly, the SAS would increase the rate of flow from both the go and stop taps, causing the initiation or inhibition of the response depending on which was closer to the top of the bucket. Alternatively, startle could provide evidence for a single pool of activation (i.e. interactive stop and go-processes), evidenced by startle only being able to propagate the initiation response depending on its activation level relative to threshold. Accordingly, the SAS would only be increasing the flow of the go tap while leaving the stop drain unaffected, and depending on how full the bucket is at that time and whether the stop drain is open or closed, the additional influx of water may cause the initiation of the response.

To date, startle has been used to investigate response initiation, however when applied in a paradigm that requires stopping it is expected to provide further insight into the dynamic interplay between initiating and stopping a movement. One previous study has used startle to investigate the stopping of an ongoing movement, as opposed to the initiation of a movement (Carlsen, Almeida, & Franks, 2012). Specifically, participants were instructed to perform a force offset task which involved the release of an isometric contraction in response to a control (82 dB) or startle (124 dB) auditory stimulus. Interestingly, results showed that startle was able to speed up the release of the contraction, similar to the typical speeding up of the initiation of a contraction. This suggests that for a force offset task the inhibitory command is a planned response, and can be elicited involuntarily by startle. Although this study provides evidence for the ability of startle to access an inhibitory response in a force offset

paradigm, it is far from clear if startle will have a similar effect in traditional inhibitory tasks such as a stop-signal or go/no-go task.

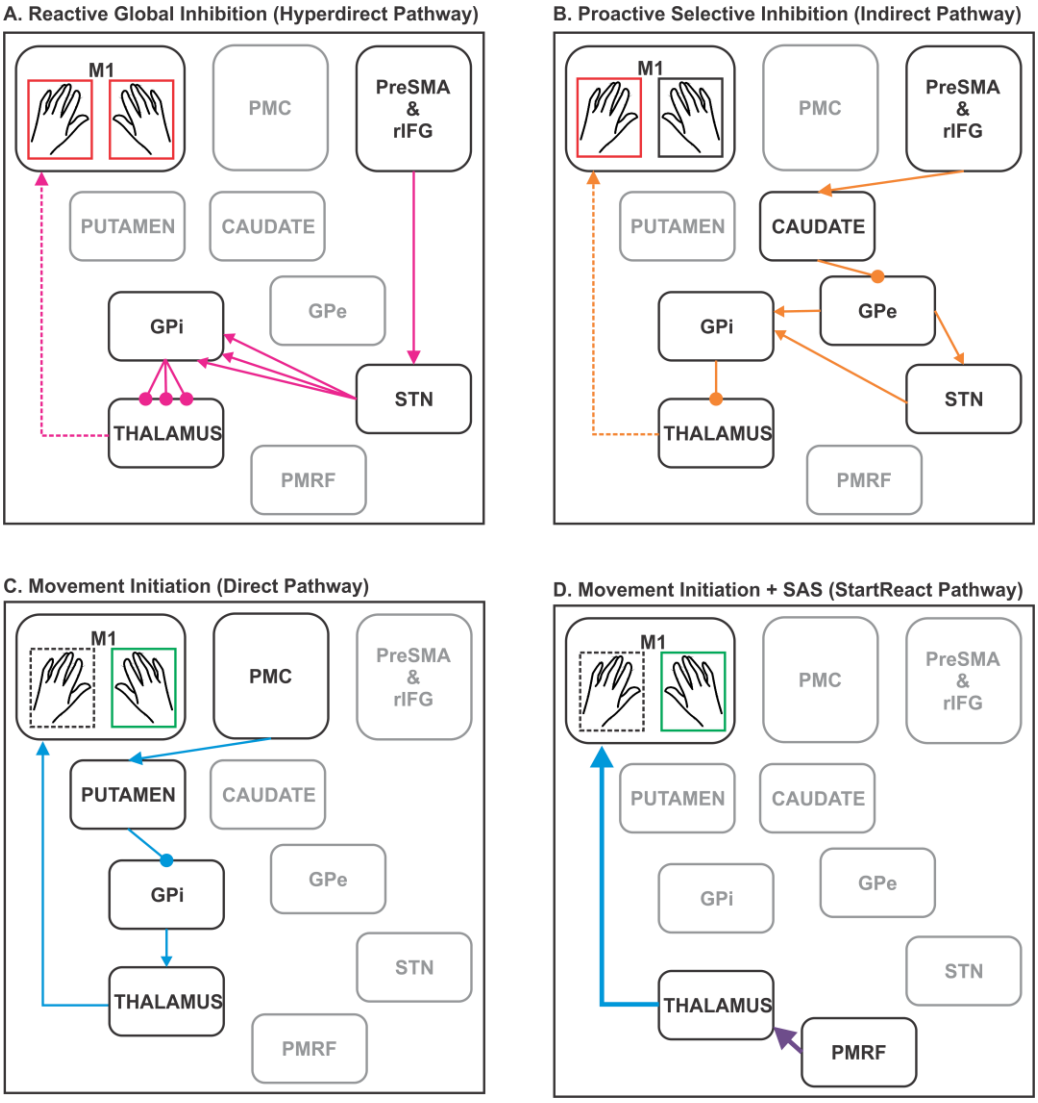

Figure 1.1 Schematic of the proposed fronto-basal ganglia-motor cortex circuits for inhibition and initiation. **A.** In reactive inhibition, the PreSMA & rIFG send input to the STN via the hyperdirect pathway. The increase in STN activation results in a broad increase in activation of the GPi which in turn results in global suppression of the thalamus, followed by inhibition of M1. **B.** Proactive selective inhibition of the left hand while concurrent preparation of a right hand response is occurring may be set-up via the indirect pathway. PreSMA & rIFG increase their input to the caudate, which in turn decreases the activation of GPe. The

decrease in GPe activation results in the increase in activation of a specific channel of the GPi either directly or via the STN. The specific increase in GPi activation results in the selective inhibition of thalamus and in turn selective inhibition of the left motor response. **C.** Participant initiates a right hand movement using the direct pathway. The PMC increases the activity of the putamen, and the putamen decreases the activity of the GPi. Decreasing activation in the GPi results in an increase in activation of the thalamus and in turn increased initiation-related activation to M1. **D.** Participant prepares to initiates a right hand movement when a startling acoustic stimulus (SAS) is presented with the go-signal. Presentation of a SAS results in the activation of the PMRF with ascending activation travelling to the thalamus and up to M1. The added activation from startle increases initiation-related activation to M1 causing the early initiation of the response. M1, primary motor cortex; PMC, premotor cortex; PreSMA, pre-supplementary motor area; rIFG, right inferior frontal gyrus; GPi, globus pallidus pars interna; GPe, globus pallidus pars externa; STN, subthalamic nucleus; PMRF, pontomedullary reticular formation.

1.5. Outline of Thesis

At present, there is no consensus in the literature on how inhibition and initiation compete or interact, what structures and processes are involved, and ultimately how humans are able to control motor behaviour during tasks which may require inhibition. In this PhD dissertation I detail four experiments that were designed to investigate the processes underlying inhibitory control when humans are preparing to initiate a motor response with the possibility of having to inhibit that response.

All experiments involved the collection of behavioural and neurophysiological measures. Experimental tasks included a stop-signal task (Chapters 2 and 3), a selective stop-signal task (Chapter 4), and a go/no-go task (Chapter 5). In all tasks, participants either had to inhibit a previously cued movement or determine if they should refrain from initiating a response. A secondary simple go-reaction time task, that didn't include inhibition, was included in all experiments to serve as a control and contrast to performance during the inhibitory task. Both electromyography (to measure muscle activation), and a startling acoustic stimulus [SAS: to examine/manipulate motor activation as shown by response triggering (StartReact effect)] were used in all experiments. Transcranial magnetic stimulation (TMS) was used in Chapter 4 to measure corticospinal excitability, and continuous theta burst stimulation (cTBS) was used in Chapter 5 to non-invasively and temporarily supress neuronal activation in cortical areas implicated in the inhibitory control network (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005).

As stated previously, the overall goal of this dissertation was to gain a better understanding of the processes that underlie inhibitory control by examining the interaction between initiation and inhibition. The main objectives (**bolded**) and hypotheses (*italicized*) for each experiment (Chapters 2 to 5) are outlined briefly below.

Experiment 1: In Chapter 2 I investigated whether **the preparatory state of the go-response was reduced during the performance of a stop-signal task due to the possibility of having to inhibit the response**. A SAS was presented concurrent with the go-signal during performance of a simple RT task and a stop-signal task to determine whether the go-response was sufficiently prepared to be triggered involuntarily by startle in both tasks, and whether there were differences between tasks. It was hypothesized that *if the increase in RT typically observed during the stop-signal task compared to a simple RT task is due to lack of response preparation, the SAS would not result in short-latency RT's. If however, longer RTs are a result of a reduced amount of preparatory activation in the stop-signal task, the presentation of a SAS would trigger the early release of the prepared response, albeit at a longer latency compared with the same response triggered during a simple RT task.*

Experiment 2: The experiment outlined in Chapter 3 investigated **the time course of initiation and inhibitory related activation during the performance of a stop-signal task**. Specifically, a SAS was presented before the stop-signal, with the stop-signal and at three time points after the stop-signal to determine whether response outcome could be manipulated. *It was hypothesized that if performance during a stop-signal task is governed by independent go- and stop-activations racing towards a threshold, then SAS related activation should increase activation of the initiation or inhibition process and thus influence response outcome in a predictable manner depending on the timing. Specifically, a SAS before and concurrent with the stop-signal should only propagate the go-response (increasing probability of responding), whereas a SAS after the stop-signal should propagate the stop-response (increasing the probability of stopping).*

Experiment 3: In Chapter 4 I investigated **how advance preparation of known responses affects proactive selective inhibition during the performance of a simple RT selective stopping task**, as previous experiments have been performed in a choice RT paradigm where the ability to prepare responses ahead of the go-signal was limited. One group performed a simple selective stopping task (response known in advance) and the other performed a choice selective stopping task (response unknown in advance). The neurophysiological effect of proactive selective inhibition in advance of the go-signal was examined using single pulse TMS, and a SAS was delivered coincident with the go-signal during the simple selective stopping task to investigate the preparatory state of the limb cued to maybe stop (stop-cued) and the limb that was to continue responding (non-cued). In contrast to the choice task, *it was hypothesized that the TMS during the performance of a simple selective stopping task would reveal an increase in corticospinal excitability (CE) related to advanced preparation of the responses. However, the TMS results were still expected to replicate the selective decrease in the stop-cued response compared to the non-cued response. In accordance with the expected proactive selective inhibition of the stop-cued response, we hypothesized that the presentation of a SAS concurrent with the go-signal during the simple selective stopping task would only result in the involuntary triggering of the non-cued response.*

Experiment 4: The experiment discussed in Chapter 5 examines **the inhibitory role of the rIFG and preSMA during the performance of a go/no-go task, and asks whether inhibition on preparatory go-activation prevents SAS from triggering the prepared go-response.** Continuous theta burst stimulation (cTBS) was applied to the rIFG and preSMA to cause a transient (~40 min) suppression in the underlying neurons (Huang et al., 2005). The experiment consisted of three phases; a pre-cTBS phase in which participants completed a go/no-go and simple RT tasks, followed by offline cTBS of either rIFG or

preSMA, then a post-cTBS phase which was identical to the pre-cTBS. *It was hypothesized that deactivating inhibitory centres (rIFG or preSMA) would impair inhibitory control which would result in the triggering of the prepared response by the SAS. These findings would indicate which cortical area(s) are responsible for inhibiting go-activation and preventing overt movement.*

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2. Chapter 2

Startle reveals decreased response preparatory activation during a stop-signal task

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2.1. Abstract

In a stop-signal task participants are instructed to initiate a movement in response to a go-signal, but to inhibit this movement if an infrequent stop-signal is presented after the go. Reaction time (RT) in a stop-signal task is typically longer compared to a simple-RT task, which may be attributed to a reduced readiness to initiate the response caused by the possibility of having to inhibit the response. The purpose of this experiment was to probe the preparatory activation level of the motor response during a stop-signal task using a startling acoustic stimulus (SAS), which has been shown to involuntarily trigger sufficiently prepared responses at a short latency. Participants completed two separate tasks; a simple-RT task, followed by a stop-signal RT task. During both tasks, a SAS (120dB) was pseudo-randomly presented concurrent with the go-signal. As expected, RT during the simple-RT task was significantly shorter than during the stop-signal task. A significant reduction in RT was noted when a SAS was presented during the simple-RT task, however, during the stop-signal task a SAS resulted in either a significant speeding *or moderate* delay in RT. Additionally, the subset of SAS trial responses with the shortest RT latencies produced during the stop-signal task were also delayed compared to the short latency SAS trial responses observed during the simple-RT task. Despite evidence that a response was prepared in advance of the go-signal during a stop-signal task it appears that the amount of preparatory activation was reduced compared to that achieved during a simple-RT task.

2.2. Introduction

In a stop-signal task participants are instructed to plan and initiate a response as fast as possible to a go-signal, but inhibit this planned movement in response to an infrequent stop-signal presented at variable delays after the go (Logan, Cowan, & Davis, 1984). Similar to a simple reaction time (RT) task, in a stop-signal task only one response is initiated in response to the go-signal. Since the seminal work of Donders (1969), significant evidence has supported the proposal that when a single response is required, it can be selected and planned in advance of the go-signal (Carlsen, Maslovat, & Franks, 2012; Leuthold, Sommer, & Ulrich, 2004). When interpreted within the context of a neural activation model (Hanes & Schall, 1996), this advance preparation allows the activation level related to the motor response to be maintained close to the threshold needed for the response to be initiated - thus reducing the time to achieve threshold following presentation of the go-signal and resulting in a fast RT. Yet for stop-signal RT tasks results have consistently shown that RT on go-trials is longer in comparison to RT in a simple-RT task (by approximately 100 - 200 ms) despite a theoretical capability for advance response preparation that should be similar to that for a simple-RT task (Verbruggen & Logan, 2009). The increase in RT observed in a stop-signal task may arise as a result of a reduced amount of preparatory activation related to the motor response, due to the possibility of having to inhibit the response. Indeed, the amount of advance preparatory activation has previously been shown to affect performance in RT tasks. For example, lateralized readiness potential (LRP) amplitude measured prior to the go-signal, which is viewed as an index of motor preparatory activation (Coles, 1989; Kutas & Donchin, 1980), has demonstrated that larger LRPs correspond with faster RTs (Gratton, Coles, Sirevaag, Eriksen, & Donchin, 1988; Leuthold, Sommer, & Ulrich, 1996).

Another way to probe the preparatory activation level of the motor response during a stop-signal task would be to use a loud (120 dB) startling acoustic stimulus (SAS). During a simple-RT task it has

been shown that a SAS not only causes a reflexive startle response (Brown et al., 1991; Landis, Hunt, & Strauss, 1939) but if a motor response is sufficiently prepared a SAS can also trigger the prepared action involuntarily, producing very short RTs while preserving the kinematics and EMG features of the movement (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004b; Carlsen et al., 2012; Castellote, Kumru, Queralt, & Valls-Solé, 2007; Siegmund, Inglis, & Sanderson, 2001; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999; Valls-Solé et al., 1995). This phenomenon is suggested to arise as a result of the SAS increasing the activation in neural circuits related to the motor response beyond the threshold necessary for initiation. The neural mechanism underlying this effect is currently a matter of debate, with studies supporting both a subcortical storage and release mechanism (Castellote & Valls-Sole, 2015; Honeycutt & Perreault, 2012; Nonnekes et al., 2014; Sanegre, Castellote, Haggard, & Valls-Solé, 2004; Valls-Solé et al., 1999), as well as a mechanism involving the subcortically mediated triggering of a cortically stored motor command (Alibiglou & MacKinnon, 2012; Maslovat, Carter, Kennefick, & Carlsen, 2014; Stevenson et al., 2014). This early involuntary response initiation is not seen, however, in circumstances where there is a limited ability to prepare the response in advance (e.g., a choice-RT task), (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004a; Maslovat, Carlsen, & Franks, 2012). Thus a SAS can be used to probe response preparation prior to a go-signal by examining whether the expected response is triggered at a short latency. The latency at which the response is triggered by the SAS may also provide insight into the amount of preparatory activation, with decreased preparation associated with longer control and SAS response latencies (Drummond, Cressman, & Carlsen, 2015).

In the current experiment a SAS was presented concurrent with the go-signal to determine whether the response was prepared in advance of the go-signal during a stop-signal task. It was hypothesised that if the longer go-trial RTs observed during a stop-signal task were due to an inability to prepare the response, the SAS would not result in short latency RTs. If however, longer RTs on go-trials were the

result of a *reduced* level of preparation, the presentation of a SAS would trigger the early release of the prepared response, albeit at a longer latency compared to the same response triggered during a simple-RT task.

2.3. Materials and Methods

2.3.1. Participants

Data were collected from fourteen healthy participants (7M, 7F; mean age = 26.0 years, SD = 3.6) with no sensory or motor dysfunction. However, four participants failed to show a reflexive startle response (see *Data reduction* section for details) in the majority of the startle trials in the simple-RT task and thus their data were excluded from the analyses (see Carlsen, Maslovat, Lam, Chua, & Franks, 2011 for more details regarding recommended inclusion criteria). Data are presented from the remaining ten participants (5M, 5F; mean age = 25.7 years, SD = 3.7). All participants provided written informed consent and had normal or corrected to normal vision. The study was approved by and conducted in accordance with the ethical guidelines set by the University of Ottawa's Research Ethics Board and conformed to the latest revision of the declaration of Helsinki.

2.3.2. Apparatus and task

Participants sat facing a 24-inch LCD computer monitor with their right arm resting in a manipulandum that restricted movement to wrist flexion and extension. The forearm was parallel to the floor with the palm facing inwards and secured using Velcro straps placed proximal to the wrist and distal to the elbow. Participants completed two separate tasks in a serial fashion which were performed in a single session lasting approximately 1 hour 30 minutes. First, a simple-RT task with instructions to react as fast as possible to a visual go-signal (see *Instrumentation and stimuli* section for details) by performing a targeted 20° wrist extension movement from a neutral position (wrist neither flexed nor extended). Participants then completed a stop-signal task with instructions to react as fast as possible to

the go-signal but try to withhold the response if a stop-signal was presented. Participants were told that the probability of being able to stop when a stop-signal appeared was approximately 50%, therefore they should not wait for a stop-signal to appear. Feedback was provided on the computer monitor after each trial consisting of RT on that trial and accuracy with respect to the target. Points were given by means of a payoff matrix, such that in the simple-RT task the payoff was designed to solely reward fast RTs (≤ 200 ms), while in the stop-signal task the payoff was designed to equally reward fast RTs (≤ 350 ms) and correct responses (i.e., going on go-trials and stopping on stop-trials). A displacement RT greater than 500 ms in either task resulted in a deduction of points and a "Too slow!" message displayed on the screen. The simple-RT was always administered before the stop-signal task to avoid any influence of experience with a previous stop-signal task on the preparatory activation during simple-RT task performance (Monsell, 2003; Waters-Metenier, Husain, Wiestler, & Diedrichsen, 2014).

2.3.3. Instrumentation and stimuli

To start each trial, a white square (8 cm x 8 cm) was presented in the middle of the screen and a visual warning signal "GET READY!" was displayed for 1000 ms. This was followed by a variable foreperiod (2000-2500 ms), and the presentation of the imperative go-signal (the square turned green). For the simple-RT task participants performed 10 practice trials followed by 20 testing trials.

Stimulus presentation during the stop-signal task was similar to the simple-RT task, with the exception that on 25% of trials, a stop-signal (the square turned red) was presented following the go-signal. The time between the go- and stop-signals (stop-signal delay [SSD]) was dynamically varied based on individual responses using a tracking procedure: SSD started at 200 ms and increased or decreased by 25 ms following a successful or failed stop respectively (Logan, Schachar, & Tannock, 1997). Thus, the tracking procedure ensured an individualized SSD, thereby compensating for differences between

participants in task performance (see Verbruggen & Logan, 2009 for a review). Participants performed 10 practice trials in the stop-signal task followed by 100 testing trials.

A SAS consisting of a 120 dB, 25 ms, white noise waveform (equal power from 1 HZ to 22 kHz), was presented concurrent with the go-signal via a loudspeaker (MG Electronics M58-H, rise time <1 ms) located behind the participant's head in 25% of the simple-RT trials (5/20 trials) and 20% of the *go-trials* in the stop-signal task (15/75 go-trials [or 15/100 total trials]). The SAS was always presented on go-trials since we expected a response to be initiated regardless of the effect of the SAS. This design avoids the issue of classifying any possible involuntary triggering of the response by SAS on a stop-trial as an "error." Moreover, go-trials where a SAS was presented were always preceded immediately by another go-trial in order to prevent a preceding stop-trial from influencing preparation in the subsequent SAS trial, as several studies have found that go RT is modulated when preceded by a stop-signal trial (see Verbruggen & Logan, 2009 for a review). Acoustic stimulus intensity was confirmed using a precision sound level meter located at the same distance from the loudspeaker to the ears (30 cm, Cirrus Research CR: 162C, A-weighted, impulse response mode). Participants were told that during the testing trials a loud auditory stimulus would be presented randomly, however, this noise was irrelevant to the task and they should continue performing the task as instructed. The SAS was presented pseudo-randomly such that no two consecutive trials included a SAS, no SAS was presented in the first two trials, and no SAS was presented before, on, or after a stop-trial. Given the trial type breakdown there was an increase in probability of a stop-trial or SAS trial following two or more consecutive go-trials. However, because participants were unaware of how many SAS, stop, and go-trials there were, it is unlikely that participants were able to predict when a SAS or stop-trial would occur.

Surface electromyography (EMG) data were collected from the muscle bellies of the right extensor carpi radialis longus (ECR), right flexor carpi radialis (FCR), and left sternocleidomastoid (SCM; as an

indication of a startle reflex) using bipolar preamplified surface electrodes (Delsys DE 2.1, Delsys Inc.) connected to an external amplifier system (Delsys Bagnoli-8). Wrist angular position data were collected using a potentiometer attached to the central axis of the manipulandum. On each trial, band-passed (20-450 Hz) EMG and raw position data were digitally sampled at 4 kHz (National Instruments PCIe-6321) for 3 s beginning 1 s prior to the go-signal using a customized program written with LabVIEW software (National Instruments Inc.) and stored for offline analysis.

2.3.4. Data reduction

Surface EMG burst onsets in ECR and SCM were defined as the point at which the filtered EMG (2^{nd} order elliptic filter) first began a sustained (>20 ms duration) rise 2 standard deviations above baseline levels (mean EMG activity 100 ms prior to the go-signal onset). To distinguish startle-related SCM activity from other SCM activity, SCM onset had to occur within a time window between 30 ms and 170 ms following SAS onset (indicative of the reflexive startle response, see Carlsen et al., 2011). Similar to Kumru et al. (2006) who presented a startle in a go/no-go RT task, the startle SCM time window was extended to 170 ms following the SAS in order to determine whether inhibitory processes related to stop-signal task performance affected the onset and size of the startle response. The magnitude of the SCM response was quantified as the integrated EMG profile over the first 100 ms of muscle activity (Q100). The proportion of go-trials resulting in a startle response (SCM+) within simple-RT and stop-signal tasks was calculated by dividing the observed number of SCM+ SAS trials by the total number of SAS trials. The proportion of SAS SCM+ “early” responses was calculated by dividing the number of observed “early” SCM+ responses by the total number of SCM+ SAS trials (see *Statistical analyses* and *Response latency* sections below for details regarding “early” responses).

Premotor RT was defined as the time between the go-signal and EMG onset in the ECR muscle. Go-trials during the simple-RT and stop-signal task with a premotor RT greater than 3 standard deviations

above the participant's mean were considered erroneous and removed from the analysis (.75% and 1.5% of trials respectively). Peak displacement was defined as the greatest displacement achieved during the movement, and final position corresponded to the angular position of the wrist with respect to the home position at the first time point at which angular velocity fell below 8°/second and remained below for at least 150 ms. A response with a peak displacement greater than or equal to 2° was defined as an initiated response, whereas anything less than 2° was considered a stop response. Additional measures which were specific to the Stop-signal task included probability of successful stopping and mean SSD (time between go-signal and stop-signal). To estimate the duration of the stop process, the mean method was used (see Logan et al., 1984), which calculates stop signal RT (SSRT) by subtracting the mean of the inhibition function (i.e., SSD where *p*-respond = .5) from the mean RT observed in control go-trials on a per-participant basis.

2.3.5. Statistical analyses

In order to investigate the effect of a startling stimulus on kinematic and EMG variables, only SAS trials where a startle response was observed in SCM (i.e., SCM+) were included in these analyses (see Carlsen et al., 2011 for rationale). Premotor RT was analyzed using a 2 stimulus (control vs. SAS) x 2 task (simple-RT vs. stop-signal task) repeated measures analysis of variance (RM ANOVA). Premotor RT for SAS trials in the stop-signal task was found to be distributed bimodally (see *Response latency* section in Results below), thus premotor RTs were classified based on whether or not they were faster than each participant's own fastest control RT in the stop-signal task (see *Response latency* section below for details). These responses were separated out and classified as "early" responses. For the simple-RT task, all SAS trials with SCM activity (SCM+) were considered "early" responses. Premotor RT and response kinematics (final position and peak displacement) for these "early" responses were compared to control trials using 2 stimulus (control vs. SAS) x 2 task (simple-RT vs. stop-signal) RM ANOVAs. The proportion

of go-trials in which the SAS elicited an EMG response in the SCM (SCM+) and the proportion of SCM+ trials that resulted in the "early" release of a movement were compared between the simple-RT and stop-signal tasks using Student's paired t-tests. Prior to analyses, proportion data were subjected to an arcsine square root transformation (Osborne, 2010). Differences with a probability < .05 were considered to be significant and Tukey's Honestly Significant Difference post-hoc tests were administered to determine the locus of any significant differences. Partial eta squared (η^2_p) and *r* values are reported to provide estimates of effect size.

2.4. Results

2.4.1. Stop-signal task performance

Across participants the probability of successful stopping was found to be 37.3 % (*SD* = 5.4). As well, the mean SSD was 105 ms (*SD* = 47) while mean SSRT was 291 ms (*SD* = 57). While simply descriptive, these data demonstrate that participants were performing the task correctly and are in line with norms for manual stop-signal task performance (Stuphorn & Emeric, 2012).

2.4.2. Startle response

Analysis of the proportion of SAS trials in which a startle response was elicited revealed that the SAS led to a greater proportion of SCM+ responses during the simple-RT task compared to the stop-signal task, $t(9) = 2.649$, $p = .027$, $r = .66$, (see Figure 2.1, grey bars). In fact, the mean within-subject difference between the proportion of SCM+ trials during the simple-RT task (*M* = .94, *SD* = .09) and stop-signal task (*M* = .75, *SD* = .27) was .19 (*SD* = .23). Analysis of the onset and size (Q100) of the startle response revealed no differences between the simple and stop-signal tasks, $t(9) = 1.212$, $p = .256$, $r = .37$, and $t(9) = 0.906$, $p = .389$, $r = .08$, respectively.

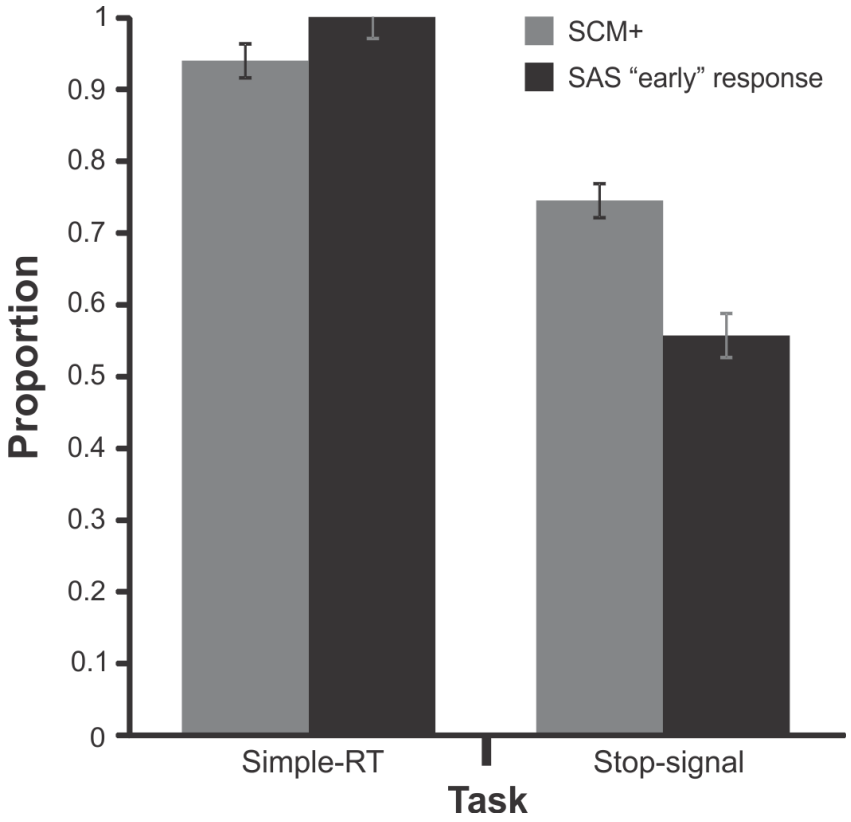

Figure 2.1. Mean proportion of startle trials that resulted in a startle response (SCM+: Grey) and mean proportions of trials showing a startle response that resulted in the “early” release of the response (SAS “Early” response: Black) as a function of task. See *Response latency* section for definition of an “early” response. Error bars denote within-subject 95% confidence intervals of comparisons between the simple-RT and stop-signal tasks (Morey, 2008).

2.4.3. Response latency

Analysis of response latency confirmed significant main effects for task, $F(1,9) = 38.841$, $p < .001$, $\eta^2_p = .812$, and stimulus, $F(1,9) = 28.871$, $p < .001$, $\eta^2_p = .762$, as well as a significant interaction, $F(1,9) = 5.011$, $p = .05$, $\eta^2_p = .358$ (see Figure 2.2). Post-hoc analysis of the interaction revealed that control RT during the simple-RT task ($M = 211.42$ ms, $SD = 21.45$) was significantly faster ($p = .01$) than control RT during the stop-signal task ($M = 302.60$ ms, $SD = 30.66$). In addition, RT in SAS trials during the simple-RT task ($M = 89.09$ ms, $SD = 16.07$) was significantly faster ($p < .01$) than control RT in the simple-RT task. Furthermore, SAS trials in the simple-RT task demonstrated significantly faster ($p < .01$) RTs than SAS trials during the stop-signal task ($M = 251.70$ ms, $SD = 101.48$). No significant difference was observed between SAS RT and control RT in the stop-signal task ($p = .18$).

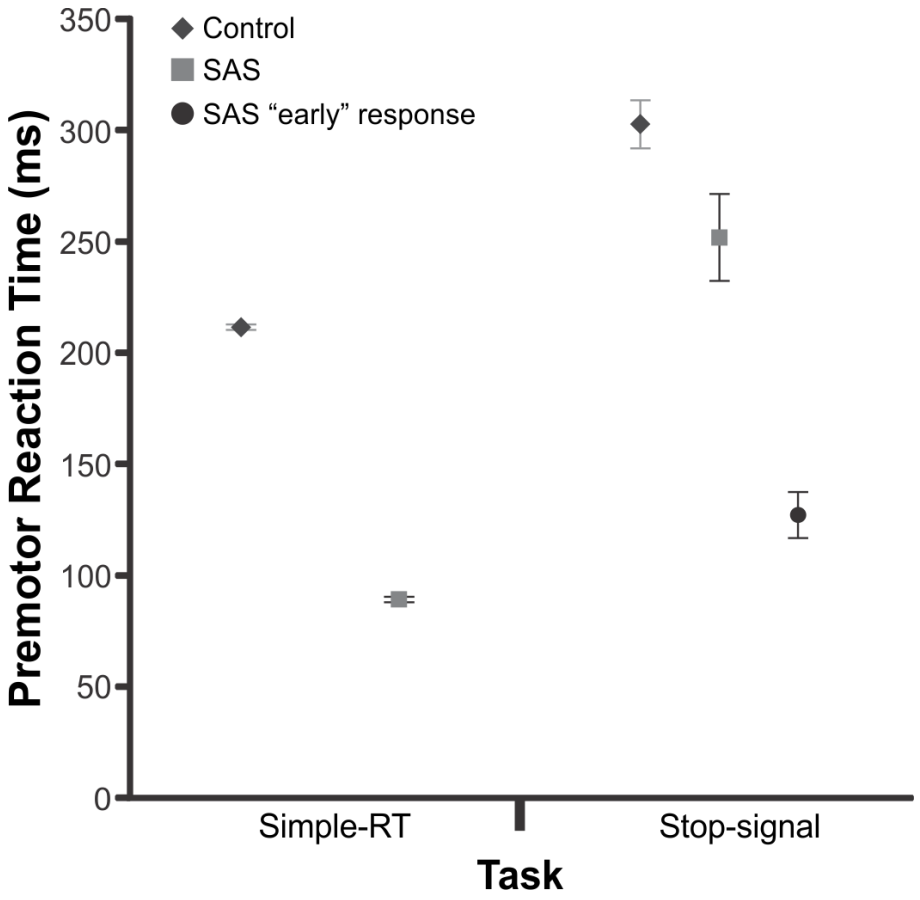

Figure 2.2. Mean premotor reaction times for each task separated by trial type. Control trials are shown in dark grey (diamonds), startle trials (SAS) are shown in light grey (squares), and stop-signal task SAS trials that resulted in the early release of the movement (SAS "early" response) is shown in black (circle). See *Response latency* section for definition of an "early" response (see also Fig 2.3 black line). Error bars denote within-subject 95% confidence intervals (Morey, 2008) computed within task (i.e., simple-RT and stop-signal RT tasks, respectively).

A large amount of variability was observed in the SAS RT data during the stop-signal task (see Figure 2.3, light grey lines), thus an analysis was undertaken to assess and quantify bimodality in the data. A continuous non-linear regression was performed on individual participant data which was transformed into z-scores relative to each participant's own mean control RT during the stop-signal task (Figure 2.4) (see Frankland & Zumbo, 2002 for details). The analyses revealed a significant bimodal distribution, $F(1,14) = 55.556$, $p < .001$, $R^2 = .947$, $SS_{error} = .003$. The first identified distribution had a mean z-score of -2.023 (*SD* = 0.477) and the second distribution had a mean z-score of 1.144 (*SD* = 1.204). Results indicate that the presentation of the SAS had a dichotomous effect on RT during the stop-signal task, with responses falling into either a fast or a (moderately) delayed response distribution relative to control RT. Trials were separated into two groups for each participant based on whether the observed RT fell above or below that participant's fastest RT in control trials, those below were defined as "early" responses (individual cut-off shown in Figure 2.3 by horizontal black line) and were compared to the RT of all SAS trials from the simple-RT task. It was reasoned that these early responses may be more reflective of an involuntarily triggered response by the SAS during the stop-signal task and would thus allow for a more equitable comparison to SAS-trial RTs seen in the simple-RT task. Analysis again revealed main effects for both task $F(1,9) = 43.193$, $p < .001$, $\eta^2_p = .828$, and stimulus, $F(1,9) = 871.012$, $p < .001$, $\eta^2_p = .990$, as well as a significant interaction, $F(1,9) = 31.286$, $p < .001$, $\eta^2_p = .777$. Post-hoc analysis revealed that all means were significantly different (all *p* values < .01), such that "early" SAS RTs (*M* = 127.07 ms, *SD* = 32.57) was significantly faster than control RT during both simple-RT and stop-signal tasks. However, the early SAS RTs in the stop-signal task were nevertheless significantly slower than the SAS RTs in the simple-RT task (see Figure 2.2).

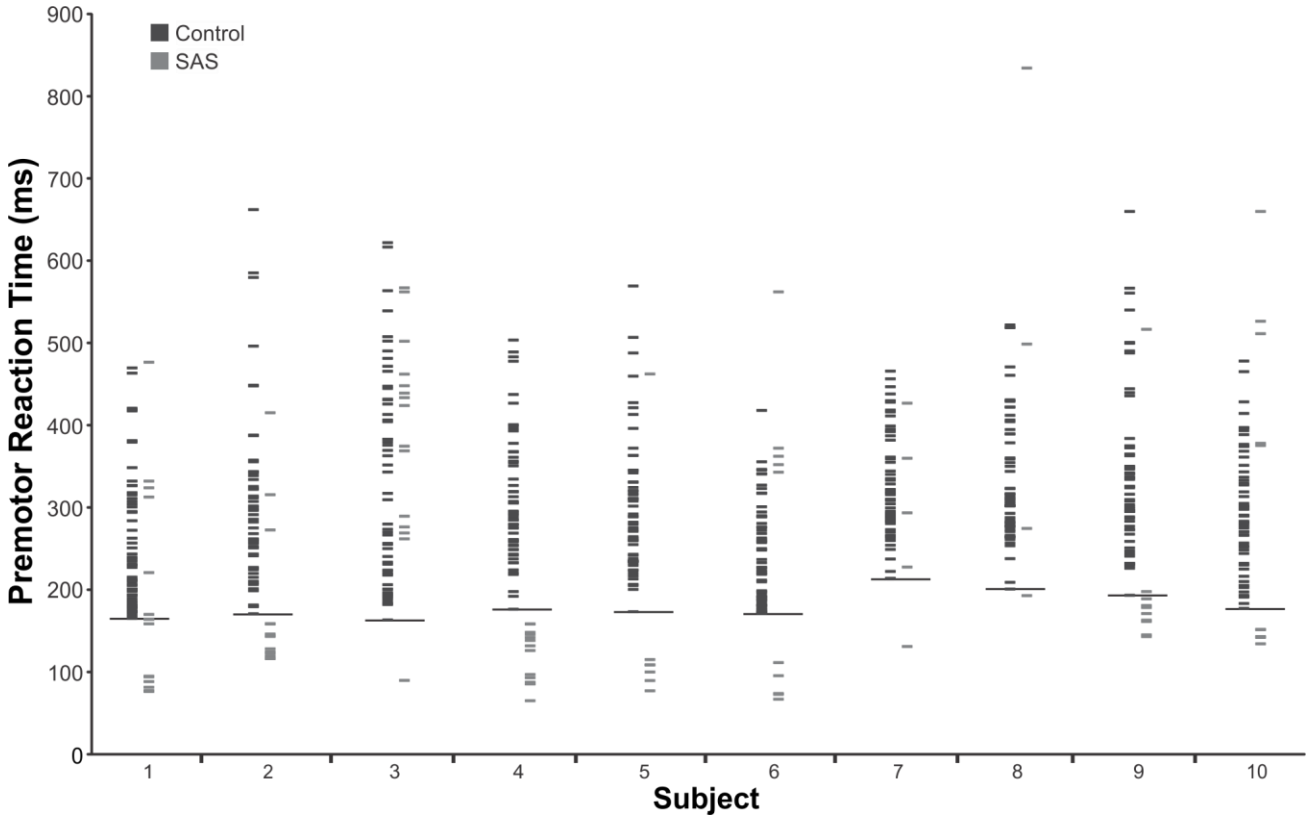

Figure 2.3. Individual trial premotor reaction time observed during the stop-signal task for each participant. Control trials are shown in dark grey, and startle trials (SAS) are shown in light grey. Black horizontal lines indicate participants' fastest control trial reaction time, and were used as a cut-off to define "early" responses (see *Response latency* section for details).

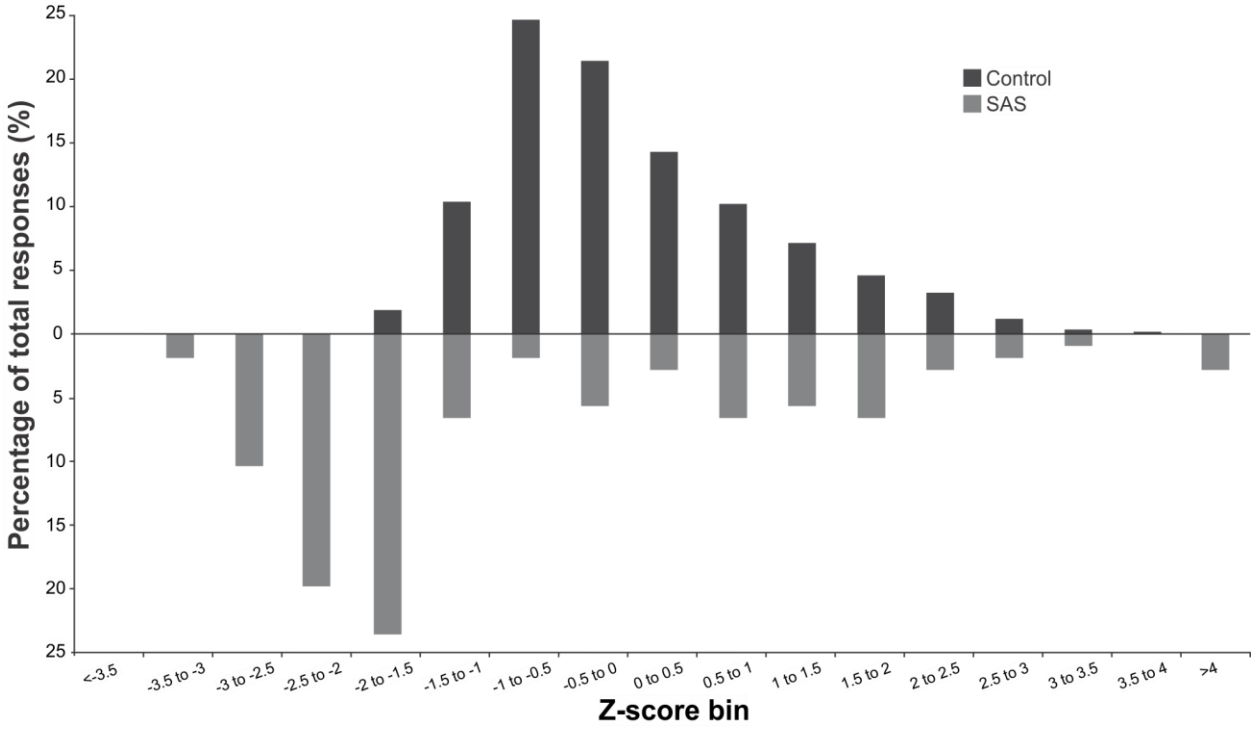

Figure 2.4. Percentage of total responses as a function of z-score bin for control trials (dark grey top panel) and startle trials (SAS: Light grey bottom panel). Note the bimodal distribution within SAS data, with the SAS either reducing RT (*M* z-score = -2.023) or delaying RT (*M* z-score = 1.144) relative to mean control RT.

Analysis of the proportion of the SAS trials which resulted in the early release of the response revealed that a greater proportion occurred during the simple-RT task (M = 1.0, SD = 0.0) compared to the stop-signal task (M = .56, SD = .30), $t(9) = 5.697$, $p < .001$, r = .88 (see Figure 2.1, black bars).

2.4.4. Response kinematics

"Early" responses observed in response to a SAS were analyzed to determine if they exhibited any differences in kinematics compared to control trial go-responses for both the simple-RT and stop-signal tasks. Analysis of final position revealed a significant main effect of task, $F(1,9) = 14.957$, $p = .004$, η^2_p = .624, as well as a significant interaction, $F(1,9) = 7.593$, $p = .022$, $\eta^2_p = .458$, but no main effect of stimulus, $F(1,9) = .583$, $p = .465$, $\eta^2_p = .061$. Post-hoc analyses revealed that the final position achieved during the stop-signal task control trials (M = 22.70°, SD = 2.27) and early SAS responses (M = 18.18°, SD = 3.62) were not different, but indicated that final position for early SAS responses were significantly smaller ($p < .01$) than the final position achieved during simple-RT task control trials (M = 27.97°, SD = 7.09) and simple-RT SAS responses (M = 30.26°, SD = 7.27). Analysis of peak displacement also revealed a significant main effect of task, $F(1,9) = 21.938$, $p = .001$, $\eta^2_p = .709$, and a significant interaction, $F(1,9) = 19.213$, $p = .002$, $\eta^2_p = .681$, but no main effect of stimulus, $F(1,9) = .751$, $p = .409$, $\eta^2_p = .077$. Post-hoc comparisons revealed no differences ($p = .23$) in peak displacement between control and early SAS responses in the stop-signal task (M = 25.86°, SD = 2.17), however, all others were significantly different ($p < .05$), such that peak displacement was always smaller in the stop-signal task compared to the simple-RT task (M = 36.99°, SD = 4.56) and peak displacement was larger during simple-RT task SAS responses (M = 40.21°, SD = 4.63).

2.5. Discussion

The purpose of the present study was to investigate preparatory activation of the motor response during a stop-signal task by presenting a startling acoustic stimulus (SAS) concurrent with the go-signal. Previous work has demonstrated that with sufficient response preparation, a SAS can involuntarily trigger the "early" release of the prepared movement (Carlsen et al., 2004b; Carlsen et al., 2012; Castellote et al., 2007; Siegmund et al., 2001; Valls-Solé et al., 1999; Valls-Solé et al., 1995). The results from the simple-RT task in our experiment replicate these findings, demonstrating significant speeding (Δ 122 ms) of premotor RT during SAS trials compared to control (see Figure 2.2). Furthermore, these SAS RTs were of sufficiently short latency (89 ms) that it is unlikely that normal voluntary cortical processes were employed in initiation of the response (Carlsen et al., 2012). It appears that in the simple-RT task preparatory response activation was sufficiently high to allow for short latency involuntary triggering by the SAS. Further support for this high level of advance preparation during the simple-RT task comes from the high proportion of startle responses and "early" responses observed (Figure 2.1). Results from the stop-signal task indicate that participants were performing the task correctly, and as expected, premotor RT in the control trials (303 ms) was significantly slower compared to that in the simple-RT task (211 ms). In contrast to the simple-RT task, no significant shortening in mean response latency was observed between SAS and control trials in the stop-signal task (see Figure 2.2), suggesting that the response was not (highly) prepared in advance. However, further analysis of these stop-signal task SAS trials revealed the presence of two RT distributions within the data, consisting of 1) fast (or "early"), and 2) moderately delayed responses (Figure 2.3 & 2.4). The "early" SAS response distribution indicates that on a trial-to-trial basis the response was sometimes sufficiently prepared to be triggered early by the SAS. However, these early SAS-triggered responses during the stop-signal task were nevertheless elicited later (*M* = 127 ms) compared to the SAS triggered responses in the simple-RT

task (*M* = 89 ms), which suggests responses were prepared, but at a decreased level of preparatory activation compared to the simple-RT task. Kinematic analyses revealed no differences in the overt responses produced between SAS and control trials within each task, suggesting that the "early" startle-triggered response was indeed the planned "go" motor response. Differences in kinematics found between tasks are likely due to practice effects; that is, because the simple-RT task was carried out first (see *Apparatus and task* section, above), participants became more accurate and efficient at preparing and performing the 20° targeted response as time progressed, resulting in performance benefits during the second (stop-signal) task. The stop-signal task also exhibited a decrease in the proportion of startle reflex responses observed in SCM, suggesting that during some stop-signal task trials preparation was dramatically reduced such that it may be considered absent. Together, these findings suggest that longer RTs typically observed on "go" trials during the stop-signal task compared to a simple-RT task may be attributed to a decrease in response preparatory activation.

2.5.1. Reduced preparatory activation

A startle response (i.e., SCM+) was elicited in a majority of stop-signal SAS trials (75%) suggesting that the motor system was engaged in at least some advance preparation (Carlsen et al., 2012; Waters-Metenier et al., 2014). However, a null effect of SAS on RT when a startle reflex was elicited (SAS trials vs. control trials) during the stop-signal task suggests that the level of preparation was too low for the voluntary response to be consistently triggered early by the SAS. Upon closer inspection, the RT data revealed that the SAS had two contrasting effects. As seen in Figure 2.3, premotor RT observed during SAS trials within each participant was not stereotyped; instead, responses appeared to fall into one of two distributions. Analysis of z-score transformed SAS RT data confirmed the presence of bimodality within the RT distribution, revealing a "fast response" distribution occurring at a mean of -2.02 SDs from

control RT and a “moderately delayed response” distribution occurring at a mean of +1.14 SD (Figure 2.4). The presence of this fast-response distribution suggests that on a number of trials the level of preparation was sufficiently high to enable startle to involuntarily trigger the prepared response. Since the additional activation that startle provides to the motor system is presumably constant across SAS trials (Maslovat et al., 2014), the latency at which responses are involuntarily triggered by startle can provide insight into the level of preparatory activation achieved. It was hypothesized that if the increased RT observed during the performance of a stop-signal task was the result of a *reduced* level of voluntary preparation, SAS triggered responses during the stop-signal task would have a longer RT compared to the same responses triggered during a simple-RT task. Indeed, results showed that “early” SAS triggered responses during the stop-signal task (*M* = 127 ms) were elicited later compared to the SAS triggered responses in the simple-RT task (*M* = 89 ms) indicative of a decreased amount of preparatory activation during the stop-signal task (see Figure 2.2). In addition to differences in startle-triggered response latency, evidence of reduced preparation during the stop-signal task can be seen by the reduced proportion of SAS trials that resulted in the early triggering of the response during the stop-signal task (56%). In contrast, the simple-RT task showed a high level of advance preparation as evidenced by a high probability of the SAS triggering an early release of the response (100%) (Figure 2.1).

Modulation of preparatory activation between tasks is likely a result of the strategy chosen to comply with task demands. The goal in the simple-RT task was to initiate a response as fast as possible, and given that the go-signal was always presented, participants were able to hold preparatory activation very close to threshold in order to decrease the time needed to initiate the response when the go-signal was inevitably presented - resulting in fast RTs. In contrast, instructions in the stop-signal task were to

initiate a response as fast as possible in the go-trials and withhold a response in the stop-trials, requiring participants to balance the speed of the go-response with the possibility of having to inhibit it - resulting in slower RTs. Our results suggest that during the stop-signal task, the level of preparatory activation was not held as close to initiation threshold by way of a strategy to deal with the potential of having to inhibit response initiation. In this way, the time needed for go-activation to reach threshold would be increased in order to allow inhibitory processes sufficient time to inhibit response output if necessary. In support of these results, Ko and colleagues recently (2015) showed that EEG-derived measures of preparatory activation corresponded with stopping success during a choice selective-stopping task. Specifically, during stop trials in which participants failed to stop there was a larger amount of voluntary preparatory activation compared to trials in which participants successfully stopped (Ko et al., 2015). Thus our results support the suggestion that the ability to inhibit motor output not only depends on the speed and strength of inhibitory processes, but the amount of voluntary preparatory activation related to the go-response.

Alternatively, the decrease in proportion of early responses as well as the increased SAS RT observed in the stop-signal task startle trials may be due to inhibition imposed by the motor system, rather than a reduced preparatory activation level per se. Two distinct mechanisms for inhibitory control have been proposed to exist: reactive and proactive inhibition (Aron, 2011). Reactive inhibition has been described as a late correction mechanism, relying upon the detection of a stimulus that signals the stopping of a planned or ongoing response resulting in global non-specific suppression of the motor system suggested to be implemented via the hyperdirect pathway (see Aron, 2011 for pathway details). In contrast, proactive inhibition is thought to use task and goal-relevant information to inhibit the motor system prior to performing the movement, allowing one to *plan for* the possibility of stopping (Braver,

2012). Proactive inhibition results in the suppression of response channels specific to the motor representation which may be or is being stopped, which is suggested to be implemented via the indirect pathway (see Aron, 2011 for pathway details).

According to these definitions, a proactive inhibitory mechanism would have been invoked to account for the present results because preparatory activation was probed with a SAS concurrent with the go-signal (i.e., prior to any possibility of being able to react to any stop-signal). However, several lines of evidence suggest that reactive inhibition was likely used for stopping the response (if necessary) in the current stop-signal task. First, proactive inhibition is typically only observed during the performance of a *selective* stop-signal task, where advance information is provided indicating that one limb in a bimanual response may have to be stopped in the upcoming trial (e.g., "maybe stop right") (Cai, Oldenkamp, & Aron, 2011; Majid, Cai, George, Verbruggen, & Aron, 2012). In contrast, reactive inhibition is typically observed during the performance of more traditional stop-signal tasks, such as the one used in the current study, which do not provide a precue (Majid et al., 2012). Although there is some evidence that both reactive and proactive inhibition might be engaged in the same traditional stop-signal task, proactive inhibition has only been seen on trials directly following stop-signal trials, which showed longer response times (Chen, Scangos, & Stuphorn, 2010). Given that in the current study the SAS was never presented following a stop-signal trial, this limits the potential for proactive inhibition to have influenced SAS RT results. Second, proactive inhibition has consistently been shown to result in longer RTs during go-trials and longer stopping times (i.e., increased SSRT) during stop-signal trials compared to reactive inhibition (Greenhouse, Oldenkamp, & Aron, 2012; Jahfari et al., 2011). Based on task demands of the current stop-signal task (i.e., react as fast as possible to the go-signal but try to inhibit/withhold the response if a stop-signal was presented), the use of a reactive mode of inhibition

would be strategically beneficial for task performance compared to proactive inhibition in the current task.

In other inhibitory tasks such as a go/no-go task, it may be more strategically beneficial to use proactive inhibition to inhibit the motor system in advance of the imperative stimulus to prevent a false go response on no-go trials. Although speculative, results from go/no-go studies employing a SAS to probe preparatory activation concurrent with the go-signal (Carlsen et al., 2008; Kumru et al., 2006) are more consistent with the use of proactive inhibition as opposed to a more reactive mode of inhibition in the current study. Specifically, during a go/no-go task presenting a SAS resulted in a significant decrease in the magnitude of the startle response (area of SCM EMG activity) compared to a choice RT task (Kumru et al., 2006). In addition, in a go/no-go task a SAS was not effective for involuntarily triggering the early release of the response (Carlsen et al., 2008). These results suggest that a high level of proactive inhibition may be placed on the motor system in these types of tasks in order to facilitate the withholding of a response in the case of a no-go stimulus.

In contrast to the SAS results obtained in a go/no-go task (Carlsen et al., 2008; Kumru et al., 2006), the present experiment found no differences in the onset or integrated area (Q100) of the startle response between simple and stop-signal tasks and showed that a SAS can often involuntarily trigger the early release of the response (albeit with a delayed onset and reduced frequency compared to a simple-RT task. This again suggests a reactive mode of inhibitory control was likely used in the present task as opposed to proactive inhibition. These differences in the effects arising from a SAS between stop-signal and go/no-go tasks suggest that different modes of inhibition are likely used for each type of task (reactive inhibition in typical stop-signal tasks vs. proactive in go/no-go tasks). Importantly, reactive inhibition alone cannot account for the delay in “early” SAS RTs observed in the stop-signal task

compared to the simple-RT task, as inhibition would only occur after the presentation of a stop-signal and thus could not influence the SAS trials (Badry et al., 2009; Majid et al., 2012). Taken together, the current evidence suggests that performance during a typical stop-signal task is likely governed by a decrease in voluntary response preparatory activation prior to the go-signal followed by a primarily reactive mode of inhibitory control in response to a stop-signal.

2.5.2. Absence of response preparation

While presenting a SAS during the stop-signal task often resulted in a startle response in SCM, it nevertheless occurred significantly less often (-19 %) compared to during the simple-RT task (Figure 2.1). This decrease in the incidence of SCM activation provides additional insight into the processes occurring prior to response initiation, as well as the source of the increase in RT observed during the stop-signal task. One potential explanation for the decrease in the proportion of startle responses observed between tasks is that because the stop-signal task was always performed following the simple-RT task, participants may have habituated to the SAS over the course of the experiment. This account is unlikely because unlike previous studies documenting startle habituation (Abel, Waikar, Pedro, Hemsley, & Geyer, 1998; Valls-Solé, Valldeoriola, Tolosa, & Nobbe, 1997), we found no reduction in the size (Q100) of the startle response between the simple-RT and stop-signal tasks.

The more probable explanation for the decreased incidence of SCM activation observed during the stop-signal task is that participants were simply not preparing (or at least not enough preparatory activation had accrued) prior to the go-signal. Previous studies have shown that readiness to perform a response during a simple-RT task prevents habituation of the reflexive startle reaction, presumably due to enhanced excitability of the motor pathway (see Carlsen et al., 2011 for a review; Valls-Solé et al., 1997). In contrast, a diminished probability of eliciting a startle response in SCM can be found in tasks in

which advance response specific motor-preparation is largely limited (e.g., choice-RT task). Evidence for an absence of advance preparation can be seen in the distribution of control RTs during the stop-signal task, with a large within-participant range observed (*mean* RT range = 357 ms, *SD* = 85) compared to that observed in the simple-RT task (71 ms, *SD* = 27). This increased within-participant range observed during the stop-signal task compared to the simple-RT task is likely due to larger variations in the amount of preparation trial-to-trial. Support for this assertion comes from previous studies that have shown that variations in baseline preparatory activation levels and the rate of rise of activation can account for the variability in RT (see Gold & Shadlen, 2007; Munoz & Everling, 2004 for review). Moreover, preparatory activation level has been suggested to be more predictive than rate of rise of trial-to-trial variability of RTs (Connolly, Goodale, Goltz, & Munoz, 2005; Dorris & Munoz, 1998; Dorris, Pare, & Munoz, 1997; Everling & Munoz, 2000; Lecas, Requin, Anger, & Vitton, 1986; Riehle & Requin, 1993). Thus in the present study the decreased incidence of observing a reflexive startle response together with the large range of control trial RTs observed during performance of the stop-signal task suggests that preparation prior to the go-signal was not homogenous across trials and provides evidence that on a small proportion of trials participants did not sufficiently prepare the go-response in advance. This dramatic reduction of advance preparation on a small proportion of trials provides a parsimonious explanation for the positively skewed data typically observed during the performance of the stop-signal task (Verbruggen & Logan, 2009).

2.6. Conclusion

In summary, the use of a startling acoustic stimulus (SAS) provides novel insight regarding the motor preparatory state of the “go” response during a stop-signal task. Results from SAS trials during the performance of a stop-signal task indicate that while participants were often preparing a go-

response, the level of that voluntary preparatory activation was likely reduced compared to that achieved during a simple-RT task. These findings suggest that longer RTs typically observed during a stop-signal task may be attributed to a decrease in preparatory activation of the voluntary response. Reducing the level of preparatory activation relative to threshold for response initiation may be one strategy to deal with the potential of having to inhibit response initiation. Participants appear to trade off preparatory activation resulting in more time to allow inhibitory processes to inhibit response output if a stop-signal is presented. On a small proportion of stop-signal trials this preparation appears to be dramatically reduced such that it may be considered absent. This heterogeneity in preparatory strategy also provides a neural explanation for the range and distribution of RTs typically observed in the stop-signal task literature.

2.7. References

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3. Chapter 3

Go-activation endures following the presentation of a stop-signal: Evidence from startle

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3.1. Abstract

Logan & Cowen (1984) have proposed that in a stop-signal task (SST), independent go- and stop-processes "race" to control behaviour. If the go-process wins, an overt response is produced, while if the stop-process wins, the response is withheld. One prediction that follows from this proposal is that if the activation associated with one process is enhanced, it would be more likely to win the race. We looked to determine whether response outcome, specifically initiation and inhibition, could be manipulated by using a startling acoustic stimulus (SAS), which has been shown to provide added response activation. In the present study participants were to respond to a visual go-stimulus; however, if a subsequent stop-signal appeared they were to inhibit the response. The stop-signal was presented at a delay corresponding to a probability of responding of 0.4 (determined from a baseline block of trials). On stop-trials a SAS was presented either simultaneous with the go-signal or stop-signal, or 100, 150, or 200 ms following the stop-signal. Results showed that presenting a SAS during stop-trials led to an increase in probability of responding when presented with or following the stop-signal. The latency of SAS responses at the stop-signal+150 ms and stop-signal+200 ms probe times suggests that these responses would have been expected to be voluntarily inhibited but instead were involuntarily initiated by the SAS. Thus, results demonstrate that go-activation endures even 200 ms following a stop-signal and remains accessible well after the response has been inhibited, providing evidence against a winner take all race between independent go- and stop-processes.

3.2. Introduction

A stop-signal task is primarily used to examine one's ability to inhibit an ongoing voluntary movement upon presentation of a stop-signal and thus incorporates processes related to both movement initiation and movement inhibition. Specifically, participants are to plan and initiate a movement as fast as possible in response to a go-signal, but inhibit this movement if a stop-signal is presented following the go-signal. In general, the length of the delay between the go-signal and the stop-signal (i.e., stop-signal delay; SSD) determines one's success of stopping, such that a longer delay results in a decreased ability to withhold the movement.

An independent horse race model (Logan, Cowan, & Davis, 1984) has been put forward to explain and predict why some movements are initiated and others inhibited in a stop-signal task. Specifically, Logan and colleagues posit that two independent processes (a "go" process and a "stop" process) race for control over the movement and the process that crosses a threshold first wins the race and ultimately determines response outcome. When described within a neural accumulator framework (Hanes & Schall, 1996), these go and stop processes reflect two independent pools of neural activation which increase towards a single threshold of neural activity that once achieved, determines response output (i.e., initiation or inhibition) (Boucher, Palmeri, Logan, & Schall, 2007; Logan, Yamaguchi, Schall, & Palmeri, 2015). For example, go-activation builds in response to the go-signal to initiate the motor response, and inhibitory-activation builds in response to the stop-signal to stop/prevent motor output. In accordance with the independent race model, the response-activation that crosses the threshold first determines the resultant behavioural outcome.

While there is an extensive amount of research and literature detailing the neural processes underlying movement initiation (e.g., Hallett, 2007; Hanes & Schall, 1996), much less is known about the processes underlying inhibition. It is generally accepted that the primary motor cortex (M1) is a key

cortical node in voluntary motor control and distinct patterns of neural activation within M1 have been shown to represent unique motor responses, coding such movement parameters as direction and speed (Crammond & Kalaska, 1996; Fabbri, Caramazza, & Lingnau, 2010; Georgopoulos, 2014; Kalaska, Caminiti, & Georgopoulos, 1983; Moran & Schwartz, 1999; Riehle & Requin, 1989). Once an absolute threshold in activation is achieved in M1, the motor response is sent downstream to the muscles to initiate movement execution (Hanes & Schall, 1996).

While neural data is in support of M1 activation leading to the initiation of the "go" response in the horse race model, it is unclear whether there exists an equivalent and independent activation pattern specific to an inhibitory "stop" response in M1. Recent literature indicates that inhibition may also consist of a distinct pattern of neural activation, suggesting that stopping or inhibiting a movement may in fact be a planned response rather than simply an absence of motor activation (Badry et al., 2009; Carlsen, Almeida, & Franks, 2012; Smith, Jamadar, Provost, & Michie, 2013; van den Wildenberg et al., 2010). Evidence for a M1 coded inhibitory response has been shown by stereotyped "stopping" event-related potentials (ERPs), recorded using electrocorticography over premotor cortex and M1 during a stop-signal task (Mattia et al., 2012). These "stopping" ERPs were selectively expressed during stop-signal trials, and their onsets preceded the end of the stop process, demonstrating activation in M1 specific to stopping a motor response. Furthermore, the presentation of a startling acoustic stimulus (SAS) has been shown to involuntarily trigger the active stopping of an ongoing isometric contraction in a force offset task (Carlsen, Almeida, et al., 2012). This limited evidence provides support for an independent stop process as posited by the horse race model. Furthermore, it suggests that this inhibition may consist of an independent response represented within M1 neuronal activation patterns, which is initiated in a similar way as a go-response.

The purpose of this experiment was to determine whether response outcome, specifically initiation and inhibition, can be manipulated during a stop-signal task. To do this, a startling acoustic stimulus (SAS) was used to probe the state of go- and stop-responses in M1, by artificially adding activation to the motor system. Startle research has consistently demonstrated that if there is sufficient activation related to the motor response, the presentation of a SAS is able to involuntary trigger the initiation of the planned response, a phenomenon termed the StartReact effect (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004; Carlsen, Maslovat, & Franks, 2012; Castellote, Kumru, Queralt, & Valls-Solé, 2007; Siegmund, Inglis, & Sanderson, 2001; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999; Valls-Solé et al., 1995). Recent evidence suggests this involuntary response triggering is a result of SAS-related activation adding to existing cortical activation levels which increases the activation beyond the threshold required for motor output (Alibiglou & MacKinnon, 2012; Maslovat, Carter, Kennefick, & Carlsen, 2014; Maslovat, Drummond, Carter, & Carlsen, 2015). Therefore, if inhibitory responses are prepared, specified and initiated in a similar manner to go-responses, then SAS activation should also sum with the activation related to the stop-response such that it involuntarily triggers the inhibitory response output.

In the current experiment a SAS was used to probe the activation level of the go- and/or stop-response(s) at various time points: simultaneous with the go-signal, simultaneous with the stop-signal, and either 100, 150, or 200 ms following the stop-signal. A stop-signal task with a single known response was chosen to allow for the possibility of advance preparation of the go-response prior to the go-signal, and a stop-signal delay (SSD) corresponding to an individualized probability of responding of 0.4 in a baseline block of trials was chosen in order to favour the stop-response (i.e., inhibitory activation is more likely to reach threshold before initiation activation). It was hypothesized that when a SAS is delivered with the go- or stop-signal, it can only provide additional activation to the go-response. Thus,

the SAS is expected to result in the early triggering of the movement (i.e., the StartReact effect) and an increased probability of responding compared to that predicted under normal voluntary control (i.e., *p*-respond 0.4). According to the independent horse race model and the neural evidence supporting an M1 coded inhibitory response, at some delay following the stop-signal, activation related to the stop-response will begin. It was hypothesized that when the SAS is delivered following the stop-signal it would provide additional activation to both the initiation and inhibitory responses. Thus, given that the stop-signal is delivered at a delay favouring the stop-response the SAS is expected to result in the early triggering of the inhibitory response which would be expressed by a decreased probability of responding compared to non-SAS stop trials.

3.3. Materials and Method

3.3.1. Participants

Data were collected from thirteen healthy participants (8M, 5F; M_{age} = 26 years, *SD* = 5.8) with no sensory or motor dysfunction. Two participants failed to show a reflexive startle response (see Data reduction and analysis section for details) in the majority of the startle trials in the simple-RT task and thus their data were excluded from the analyses (see Carlsen, Maslovat, Lam, Chua, & Franks, 2011 for more details regarding recommended inclusion criteria). Data are presented from the remaining eleven participants (7M, 4F; M_{age} = 26 years, *SD* = 6.2). All participants provided written informed consent and had normal or corrected to normal vision. The study was approved by and conducted in accordance with the ethical guidelines set by the University of Ottawa's Research Ethics Board and conformed to the latest revision of the declaration of Helsinki.

3.3.2. Experimental set-up

Participants sat in a chair facing a computer monitor with their right arm placed in a custom made force measurement device. Their torso was restrained using a harness to limit the contribution of force generation solely to the right wrist. Their arm was supported and secured in a neutral position (semi-pronated with the palm perpendicular to the floor) such that the dorsal surface of the hand was in contact with a solid plate, restricting movement to concentric wrist flexion and isometric wrist extension. The plate was attached to a force transducer allowing measurement of force produced during an isometric wrist extension.

3.3.3. Procedure

Prior to behavioural testing, each participant's maximum voluntary isometric extension force was determined. Participants familiarized themselves with the test procedures of the voluntary force production task during several warm-up contractions. Participants then completed a maximal voluntary force (MVF) test, which consisted of 3 MVF trials, with 1 minute of recovery between trials (for a detailed protocol see Sahaly, Vandewalle, Driss, & Monod, 2001). MVF was then determined by calculating the average peak force across the three trials.

Participants then performed a simple-RT task, consisting of 10 practice trials followed by 20 testing trials. Participants were instructed to initiate an isometric wrist extension with a target force of 40% of their MVF as fast as possible to a visual go-signal. A target wrist extension force of 40 % of MVF was chosen to provide a sufficient agonist burst for analysis without resulting in fatigue during the performance of the experiment. To start the trial, a white square (8 cm x 8 cm) was displayed on a blank black screen indicating to the participant to "get ready". During this time period, 1 second of real-time force feedback was provided graphically on the monitor, as participants were instructed not to exert any force prior to the go-signal. Following a variable foreperiod of 2-4 seconds, the square turned green (go-

signal), indicating to the participant to initiate the response. Following the response, performance feedback was displayed on the monitor for 3 seconds, which included their reaction time as well as a message indicating whether their response was “Correct” or “Incorrect”. A correct response was when force onset occurred between 50 ms and 500 ms following the go-signal. Participants received 25 points for responding correctly and up to 25 points for responding quickly (1 point for every 1 ms below 200 ms). During the simple-RT task a white noise SAS (120 dB, 25 ms, with equal power from 1Hz to 22 kHz) was presented pseudo-randomly on five of the 20 testing trials concurrent with the go-signal via a loudspeaker (MG Electronics M58-H, rise time <1 ms) located behind the participant’s head. Stimulus intensity and frequency content was confirmed using a precision sound level meter located at the same distance from the loudspeaker to the ears (30 cm, Cirrus Research Optimus CR: 162C, A - weighted). Participants were told that on some trials they would hear a loud “static noise” sound, and that it was irrelevant to the task and they should continue as instructed. The simple-RT task served as a startle screening tool to determine whether a SAS consistently elicited a startle response in the sternocleidomastoid (see *Data reduction* section below for startle response criteria), and was completed first to ensure that previous performance of the stop-signal task would not influence simple-RT task performance. For a participant to be included in the analyses of the stop-signal task (where currently the response to a SAS is unknown), a startle response must have been observed in 60% or more of the simple-RT task startle trials independent of any movement (see Carlsen et al., 2011 for a review).

Following completion of the simple-RT task participants then completed a stop-signal task. Similar to the simple-RT task, participants were instructed to react as quickly and accurately as possible to the visual go-signal. However, on a subset of trials (stop-trials), the visual stimulus changed to red at a variable delay following the onset of the green go-signal (i.e., stop-signal delay: SSD). On these stop trials, participants were instructed to attempt to withhold the action and not initiate the response. On

go-trials the message “Correct” and the corresponding reaction time was displayed following a completed response, and “incorrect” was displayed when a response failed to be initiated. On stop-trials the message “You should have stopped” was displayed following a failed stop (i.e., if a response was initiated), and “correct” was displayed following a successful stop (see *Data reduction* section for response criteria). Participants received 25 points for responding correctly (i.e., responding on go-trials and not responding on stop-trials) and responding quickly (1 point for every 7 ms below 350 ms, up to a maximum of 25 points). Participants were penalized 25 points for an incorrect response or a go-RT exceeding 500 ms.

Participants first completed a baseline block of trials in the stop-signal task consisting of 100 trials, 25 of which were stop trials. For stop trials, the SSD was adjusted individually for each participant using the tracking procedure outlined by (Logan, Schachar, & Tannock, 1997). Specifically, the first SSD was 250 ms, increasing or decreasing by 50 ms with every successful or failed stop respectively. Following the completion of the baseline stop-signal task, a logistic function was fit to the participant’s probability of responding given a stop-signal presented at various delays (i.e., SSD). From this data each participant’s individual SSD that resulted in a probability of responding (*p*-respond) of 0.4 was determined.

Participants then completed a testing block of trials in the stop-signal task consisting of 100 trials, 25 of which were stop trials. Each stop trial was accompanied by a SAS at one of five different times; either with the go-signal (GO+SAS), stop-signal (SS+SAS), or 100 ms (SS+SAS100), 150 ms (SS+SAS150), 200 ms (SS+SAS200) following the stop-signal, with a total of 5 SAS trials per time point. The SAS probe times used were chosen in order to provide an inclusive time course of both the initiation (i.e., SAS probe before and after the stop-signal) and inhibitory (i.e., SAS probe after the stop-signal) processes, as the average time needed for the inhibitory process to stop/prevent the movement (SSRT)

is approximately 200 ms (see Logan, 2015 for a review). Unlike the baseline stop trials, the stop-signal in the testing block was always presented at the individually pre-determined SSD corresponding to *p*-respond of 0.4. Each participant's voluntary probability of responding (i.e., *p*-respond) given a certain SSD was expected to remain relatively stable between and within blocks as computer simulations have shown that the assumption of a constant stopping speed (stop-signal RT) does not bias the estimation substantially (Band, van der Molen, & Logan, 2003; Dejong, Coles, Logan, & Gratton, 1990). A *p*-respond of 0.4 was chosen as to slightly favour the inhibition process reaching threshold before the initiation process, thus increasing the probability of the SAS triggering the inhibitory command following the presentation of the stop-signal and observing changes in response output (e.g., decrease in *p*-respond). The SAS (i.e., stop-signal trials) was presented pseudo-randomly, such that no two consecutive trials included a SAS, and SAS probe time was randomized across trials.

3.3.4. Recording equipment

Surface electromyographic (EMG) data were collected from the muscle bellies of the following muscles: right extensor carpi radialis (ECR), and left sternocleidomastoid (SCM), using bipolar pre-amplified (gain=10) surface electrodes (Delsys Bagnoli DE-2.1) connected via shielded cabling to an external amplifier system (Delsys Bagnoli-8). The EMG recording sites were prepared and cleansed in order to decrease electrical impedance. The electrodes were placed parallel to the muscle fibers, and attached using double sided adhesive strips. The reference electrode was placed on the participant's right lateral epicondyle.

Isometric wrist extension force data were collected using a force transducer (Nano25, ATI Industrial Automation, Inc., Apex, North Carolina USA) placed between the dorsal side of the hand and the plate. On each trial, unfiltered EMG and force data were digitally sampled at 4 kHz (National

Instruments PCIe-6321 via BNC-2090) for 3 seconds using a customized program written with LabVIEW software (National Instruments Inc.) and stored for offline analysis.

3.3.5. Data reduction

Force onset was defined as the point at which force output exceeded two standard deviations above baseline levels (mean force 100 ms prior to the go-signal onset). Reaction time was defined as the interval of time between the go-signal and force onset and was only used to provide feedback and points to the participant during task performance. Peak force was defined as the highest observed value on a given trial. Although the target force was 40% of maximum voluntary isometric force (MVF), any generated force exceeding 5% of MVF was classified as an initiated go-response. If force did not exceed 5% of MVF it was classified as a full-stop response.

EMG data collected on all trials from the ECR (agonist) and SCM were analyzed for muscle burst onset, offset, and peak amplitude. EMG burst onsets were defined as the point in time at which the rectified and filtered (25 Hz low pass 2^{nd} order elliptic filter) EMG signal first began a sustained (>20 ms) rise that exceeded two standard deviations above baseline levels (mean EMG activity 100 ms prior to go-signal onset). Premotor reaction time (RT) was defined as the time from the stimulus of interest (go-signal or SAS) to ECR burst onset. EMG offsets were defined as the point at which the EMG fell below the baseline threshold. Peak EMG amplitudes were defined as the largest amplitude of the rectified and filtered EMG signal recorded within an interval of 100 ms following EMG burst onset.

To distinguish startle response related SCM activity from other SCM activity when the SAS was presented coincident with the go-signal (GO+SAS) in the simple-RT task, SCM onset had to occur within a time window between 30-120 ms following SAS onset (Carlsen et al., 2011). For a participant to be included in the analyses of the stop-signal task (where currently the response to a SAS is unknown), a startle response must have been observed in 60% or more of the simple-RT task startle trials

independent of any movement (resulting in the removal of two participants). This startle response criterion was not applied to SAS trials during the stop-signal task, and thus all SAS trials were included in the analysis (i.e., 5 trials per SAS probe time), as presenting a stimulus prior to a SAS can cause a reduction of the reflexive startle response (i.e., prepulse inhibition), while response triggering effects remain (Maslovat, Kennedy, Forgaard, Chua, & Franks, 2012; Valls-Solé, Kofler, Kumru, Castellote, & Sanegré, 2005). The delay at which the SAS was presented following the stop-signal allowed for voluntary responses to potentially be initiated prior to the presentation of the SAS. Thus responses during SAS trials where ECR EMG onset occurred prior to the SAS were classified as "voluntary responses," whereas responses with EMG onset following the SAS were classified as "SAS responses."

To determine the average time needed for the inhibitory process to stop/prevent the movement from being initiated, the stop-signal RT (SSRT) was estimated using the mean method (Logan et al., 1984). The SSRT was determined for each participants based on their performance in the base block of trials as the SSD was not intentionally set in this block of trials and varied using the tracing procedure. Specifically, a logistic curve was fit to each individual's probability of responding data given a stop-signal as various delays. Individual SSRT was calculated by subtracting the SSD corresponding to a p – respond of .5 (mean of the inhibition function) from the mean RT of go-trials. Given the estimate of the speed of the stop process (i.e., SSRT), a prediction can be made based on the relative timing of go RT vs. the point of no return (SSRT+SSD) whether the response will be successfully inhibited (i.e., go RT > SSRT + SSD) or response inhibition will be unsuccessful and the response incorrectly initiated (i.e., go RT < SSRT + SSRT) (Verbruggen & Logan, 2009). This reliable difference between voluntary incorrectly initiated RT and mean go-RT, which has been demonstrated in several groups, situations, and conditions (see Verbruggen & Logan, 2009 for a review), allowed us to quantitatively distinguish between voluntary and involuntary responses during stop-signal SAS trials.

3.3.6. Statistical analyses

To verify that participants didn't change their response strategy between the baseline and testing block, a paired t-test was used to compare control go-trial premotor RT in the baseline stop-signal task block versus the testing stop-signal task block. Then to determine whether the presentation of a SAS manipulated response outcome in the testing stop-signal block, a Friedman test was used to analyze the change in the probability of responding as a function of SAS probe time, with pre-planned Wilcoxon signed-ranked tests to determine the locus of any differences. The state of the motor-response over time was investigated by analyzing premotor RT relative to SAS presentation time of SAS responses using a one-way repeated measures ANOVA with the repeated factor of time (5 levels: GO+SAS, SS+SAS, SS+SAS100, SS+SAS150, SS+SAS200). Peak force was compared between control and SAS responses using a one-way repeated measures ANOVA with the repeated factor of stimulus (6 levels: GO, GO+SAS, SS+SAS, SS+SAS100, SS+SAS150, SS+SAS200) to determine if response output was affected by the SAS or stop-signal.

In order to examine differences between the simple-RT task and the testing stop-signal task block, premotor RT was analyzed using a 2 task (simple-RT vs. stop-signal) x 2 stimulus (control vs. GO+SAS) repeated measures ANOVA.

Lastly, to quantitatively differentiate voluntary responses from SAS responses observed during the testing stop-signal task block, the difference in premotor RT relative to the expected speed of the stop-process following the stop-signal (premotor RT- [SSD + SSRT]) was analyzed using a 2 response (voluntary vs. SAS) x 2 time (SS+SAS150 vs. SS+SAS200) repeated measures ANOVA.

In cases where sphericity was violated, Greenhouse-Geisser corrected *p* values are reported. Differences with a probability of less than .05 were considered significant. Unless otherwise noted, Tukey's Honestly Significant Differences post-hoc analyses were performed to determine the locus of

any significant differences. For the 2 task x 2 stimulus analyses, comparisons of interest were analysed post-hoc using a Holm-Bonferonni procedure to determine the locus of any significant differences found between i) control go RT and GO+SAS RT in the simple-RT, ii) control go RT and GO+SAS RT in the testing stop-signal task block, iii) control go RT between tasks, and iv) GO+SAS between tasks. All analyses were performed using the statistical software package IBM SPSS 19.0 for Windows (SPSS Inc. Chicago, IL, USA).

3.4. Results

3.4.1. Baseline stop-signal task block

Results from the baseline stop-signal task revealed that mean SSRT was 166.56 ms (*SD* = 56.88), the mean SSD resulting in a *p*-respond of 0.5 was 115.35 ms (63.90), and the mean SSD resulting in a *p*-respond of 0.4 was 95.08 ms (*SD* = 60.88).

3.4.2. Testing stop-signal task block

A paired samples t-test comparing control go-trial premotor RT between the baseline (*M* = 281.91, *SD* = 51.65) and testing (*M* = 271.16, *SD* = 37.14) stop-signal task blocks revealed no significant difference, t(10)= .951, *p* =.364, *d* = -.239, verifying that participants did not alter their response initiation strategy between blocks.

Analysis of the probability of responding revealed that there was a statistically significant difference in the *p*-responding depending on the SAS probe time (Fig 1), χ^2_F (5) = 22.08, *p* = .001, *W* = .401. Pre-planned Wilcoxon signed-ranked tests revealed no statistically significant difference between the predicted probability of observing a response (*p*-respond) of 0.4 and *p*-respond when the SAS was presented concurrent with the go-signal (GO+SAS: *Z* = -.105, *p* = .917, r = .03). However, as seen in Figure 3.1 there was a significant increase in *p*-respond compared to the predicted probability of 0.4 when the

SAS occurred coincident with the stop-signal (SS+SAS: Z = -2.059, p = .04, r = .62), SS+SAS100 (Z = -2.401, p = .016, r = .72), SS+SAS150 (Z = -2.701, p = .007, r = .81), and SS+SAS200 (Z = -2.642, p = .008, r = .80).

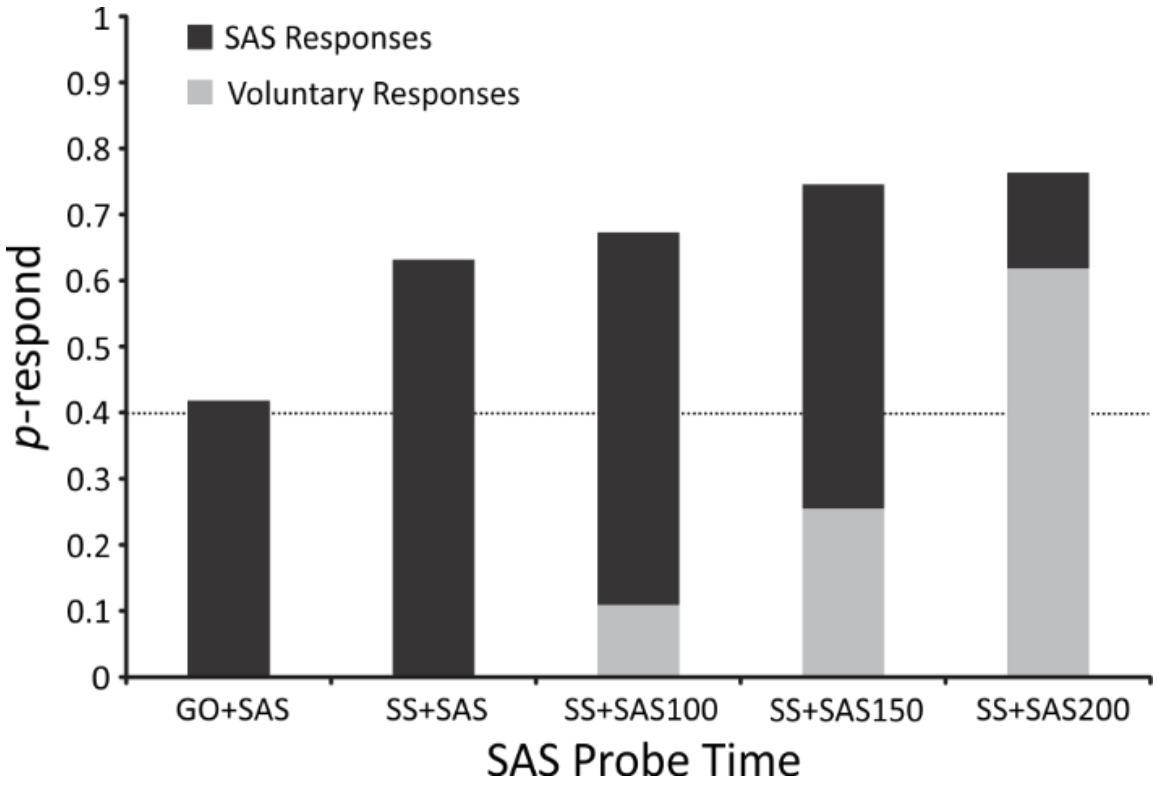

Figure 3.1. Probability of responding (*p*-respond) as a function of startling acoustic stimulus (SAS) probe time. The horizontal dashed line indicates the predicted probability of responding of 0.4. The SAS was presented with the go-signal (GO+SAS); concurrent with the stop-signal (SS+SAS); 100 ms following the stop-signal (SS+SAS100); 150 ms following the stop-signal (SS+SAS150); or 200 ms following the stop-signal (SS+SAS200). The black filled portion of the bars represents the proportion of responses initiated after the presentation of the SAS (SAS Responses) and the light grey filled portion represents the proportion of responses initiated prior to the presentation of the SAS (Voluntary Responses).

When analysing SAS responses from the testing stop-signal task block, values for missing cells were filled using a linear regression-based multiple imputation procedure in SPSS (IBM Inc., Armonk, NY; imputed values per analysis = 12/55), in order to perform a factorial analysis. Analysis of premotor RT relative to the SAS during the stop-signal task revealed a significant main effect of time (Figure 3.2), $F(4,40) = 28.512$, $p < .001$, $\eta^2_p = .740$. Post-hoc tests revealed that GO+SAS premotor RT (M = 120.50 ms, SD = 36.56) was significantly ($p < .001$) slower than SS+SAS premotor RT (M = 82.01 ms, SD = 23.73), SS+SAS100 (M = 59.14 ms, SD = 25.58), SS+SAS150 (M = 42.00 ms, SD = 15.37), and SS+SAS200 premotor RT (M = 40.68 ms, SD = 13.47). Premotor RT did not significantly differ between SAS presentation time of +100, +150 and +200 ms ($p > .05$). Analysis of peak force revealed no significant main effect of stimulus, $F(2.414, 24.137) = 2.414$, $p = .353$, $\eta^2_p = .100$, indicating that responses initiated by the SAS were no different than voluntarily initiated control responses.

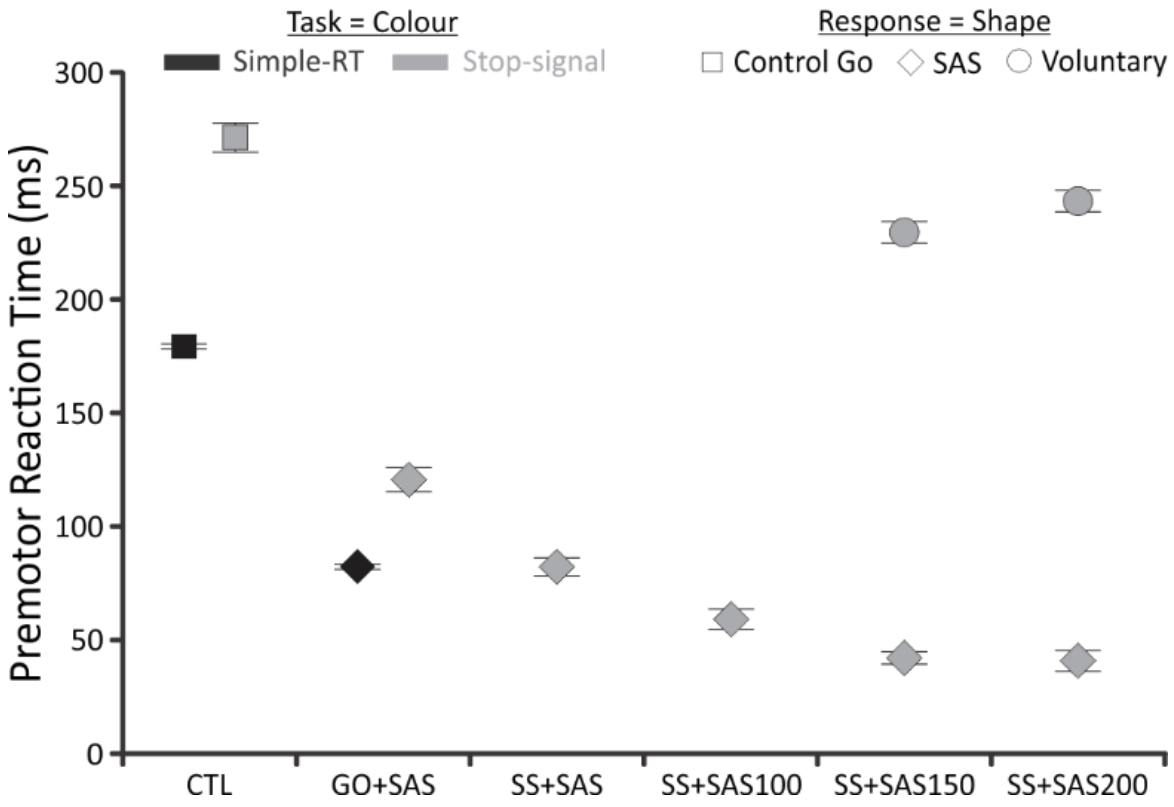

Figure 3.2. Premotor reaction time (ms) during the simple-RT and stop-signal task relative to the stimulus for control go responses (relative to go-signal) and startle probe trials for both SAS responses (relative to SAS onset) and voluntary responses (relative to go-signal). CTL indicates control go trials (visual go-signal only). The SAS was presented with the go-signal (GO+SAS); concurrent with the stop-signal (SS+SAS); 100 ms following the stop-signal (SS+SAS100); 150 ms following the stop-signal (SS+SAS150); or 200 ms following the stop-signal (SS+SAS200). The black fill represents data from the simple-RT task and the light grey fill represents data from the testing stop-signal task block. The squares represent control go responses, diamonds represent responses initiated after the presentation of the SAS (SAS Responses) and the circles represent responses initiated prior to the presentation of the SAS (Voluntary Responses). Error bars denote within-subject 95% confidence intervals computed between control go responses and SAS responses and between voluntary responses.

Analysis of premotor RT as a function of task and stimulus revealed a significant main effect of task, $F(1,10) = 41.416$, $p < .001$, $\eta^2_p = .806$, and significant main effect of stimulus, $F(1,10) = 360.511$, $p < .001$, $\eta^2_p = .973$, which was superseded by a significant interaction, $F(1,10) = 13.228$, $p = .005$, $\eta^2_p = .569$. Post-hoc analyses of interest revealed that a SAS presented coincident with the go-signal (GO+SAS), resulted in a significant decrease in premotor RT compared to control go RT in both the simple-RT task (control: $M = 179.45$, $SD = 11.25$ / SAS: $M = 82.55$, $SD = 12.66$, $t(10) = 26.984$, $p < .01$) and stop-signal task (control: $M = 271.16$, $SD = 37.14$ / SAS: $M = 120.48$, $SD = 36.56$, $t(10) = 20.835$, $p < .01$). Control go RT during the stop-signal task was significantly slower compared to the simple-RT task, $t(10) = 7.406$, $p < .01$, and GO+SAS RT was also significantly delayed during the stop-signal task compared to the simple-RT task, $t(10) = 3.728$, $p < .01$.

To further characterize voluntary responses and SAS responses, the mean within-subject difference in premotor RT relative to the expected speed of the stop-process following the stop-signal was calculated (premotor RT- [SSD + SSRT]) (Figure 3.3). Positive values are RTs from trials which would be expected to be successfully stopped, whereas negative values are RTs from trials which would be expected to result in a failed stop. The analysis revealed a significant main effect of response, $F(1,10) = 126.558$, $p < .001$, $\eta^2_p = .927$, no main effect of time, $F(1,10) = 1.450$, $p = .256$, $\eta^2_p = .127$, and no significant interaction, $F(1,10) = .021$, $p = .889$, $\eta^2_p = .002$. The negative RT difference for voluntary responses observed at SS+SAS150 (-32 ms) and SS+SAS200 (-18 ms) confirms the prediction that these were voluntary responses which were initiated too fast to be inhibited. In contrast, the positive RT difference for SAS responses observed at SS+SAS150 (+26 ms) and SS+SAS200 (+37 ms) suggest that the SAS involuntarily triggered the initiation of these responses past the point in time at which the response would have been expected to be stopped voluntarily provided no SAS was presented.

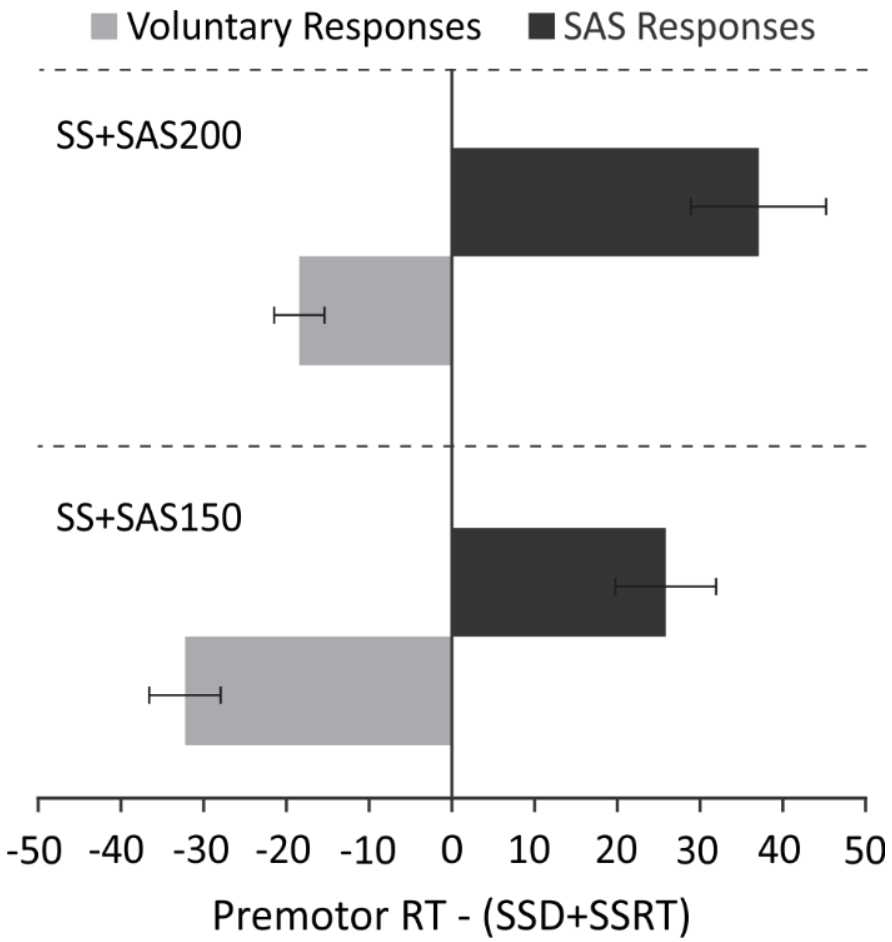

Figure 3.3. Difference in premotor reaction time (RT) relative to the expected speed of the stop-process for both voluntary and SAS responses initiated on trials when the SAS was presented 150 ms (SS+SAS150) and 200 ms (SS+SAS200) following the stop-signal. A Positive value represents a response which was initiated after the expected time needed to successfully stop the response, whereas a negative value represents a response which was initiated prior to the expected time needed to successfully stop the response. Premotor reaction time – (stop-signal delay [SSD] + stop-signal reaction time [SSRT]). The light grey filled bars represents responses initiated prior to the presentation of the SAS (Voluntary Responses) and the black filled bars represent responses initiated after the presentation of the SAS (SAS Responses). Error bars denote within-subject 95% confidence intervals (Morey, 2008).

3.5. Discussion

The present experiment was designed to investigate the activation related to go and stop processes during a stop-signal task. This was done by using a SAS to probe activation related to these responses at varying times with respect to the go-signal and stop-signal. The SAS probe times used in the present experiment provided an inclusive time course of both the initiation (all SAS times, including with the go-signal) and inhibitory (SAS times following the stop-signal) processes, as the average time needed for the inhibitory process to stop/prevent the movement (SSRT) was 166.6 ms. It was hypothesized that if performance during a stop-signal task is controlled via two independent processes racing toward response threshold, then presenting a SAS on or before the stop-signal would increase the probability of responding by providing additional activation to existing voluntary go-activation, pushing initiation activation past response threshold. In contrast, given the potent and rapid accumulation of voluntary inhibitory activation following the stop-signal, it was hypothesized that a SAS presented after the stop-signal would *decrease* the probability of responding by providing additional activation to push inhibition-related activation past response threshold. Results revealed that the use of a SAS during a stop-signal task manipulated response outcome for all SAS probe times, except when presented coincident with the go-signal, by increasing the probability of responding (Figure 3.1). Thus, in contrast to our hypothesis, even when the SAS was presented well after the stop-signal (+150 ms, +200 ms) the probability of responding increased. The latency of SAS responses compared to voluntary responses at the SS+SAS150 and SS+SAS200 time points are quantitatively different and on opposite sides of the predicted finishing time of the stop-process (Figure 3.3). This positive change in RT suggests that SAS responses observed at SS+SAS150 and SS+SAS200 were involuntarily initiated by the SAS. The increased probability of responding when the SAS was presented following the stop-signal (i.e., SS+SAS100, SS+SAS150 and SS+SAS200) provides novel insight into the activation related to the initiation and

inhibitory processes during a stop-signal task, suggesting performance is not determined by a winner take all race between independent go- and stop-activations.

One unexpected result was an increased probability of voluntary responses observed at the SS+SAS200 probe time (>0.4). While the specific reason for the increase in voluntary responses is unknown, it may be interpreted as a change in task performance between the baseline and testing blocks due to the presentation of the SAS on every stop-trial. Yet, other evidence suggest participants did not change how they performed the task between blocks. First, there was no change in control go-trial RT between the baseline and testing blocks, indicating that participants did not change their response strategy to favour speed over stopping success. Second, it does not appear that participants were simply ignoring the stop-signal as the probability of responding was not elevated at the GO+SAS probe time beyond 0.4. Lastly, the failed stop voluntary responses observed during SAS trials were shorter than control go RTs suggesting the SSRT was not increased. In addition, previous findings have revealed that the speed of the stop-process (i.e., SSRT) is functionally independent and unaffected by such factors as task switching (Verbruggen, Liefooghe, Szmalec, & Vandierendonck, 2005), difficulty of perceptual discrimination (Middlebrooks & Schall, 2014), and response compatibility (Logan, 1981). Thus, taken together with the additional behavioural data presented here, we are confident that the increased proportion of “voluntary” responses observed at the last SAS probe time does not affect our interpretation of the data. Our data suggests that performance during a stop-signal task is not determined by a winner take all race between independent go- and stop-activations, and that go-activation endures even 200 ms following the presentation of a stop-signal.

Although the probability of responding when the SAS was presented concurrent with the go-signal (GO+SAS) did not differ from what was predicted (i.e., *p*-respond of .4), the *speed* at which these

responses were initiated was significantly different. The significant shortening in RT observed during GO+SAS trials (120 ms) suggests that these responses were involuntarily initiated by the SAS (i.e., StartReact effect), yet with the same low probability as would be expected if they were initiated voluntarily. In addition, a significant delay in GO+SAS RT was observed during the stop-signal task compared to the simple-RT task (+ 37 ms), which replicates recent findings from our group (Drummond, Cressman, & Carlsen, 2016). Since the additional activation that the SAS provides to the motor system is thought to be relatively consistent across SAS trials (Maslovat et al., 2014; Maslovat et al., 2015), the delayed latency and reduced probability which responses were involuntarily triggered in the current study provides additional support for a reduced level of initiation related preparatory activation during a stop-signal task compared to a simple-RT task (Drummond et al., 2016). Together, our results suggest that during the stop-signal task, the level of preparatory activation related to the go-response was not held as close to response threshold compared to a simple-RT task as a way to deal with the potential of having to inhibit the response. In this way, the time needed for go-activation to reach response threshold would be increased in order to allow inhibitory processes sufficient time to inhibit response output if necessary. This provides additional support for the suggestion that the ability to inhibit motor output not only depends on the speed and strength of the inhibitory processes, but also depends on the amount of voluntary preparatory activation related to the go-response (Drummond et al., 2016; Ko, Cheng, & Juan, 2015).

In contrast to when a SAS was presented coincident with the go-signal during the stop-signal task (GO+SAS), we found an increased probability of responding at all subsequent SAS probe times. Given the increased probability of responding (particularly at the later SAS probe times), it was important to dissociate voluntary response initiation from SAS triggered response initiation, since the

delay at which the SAS was presented following the stop-signal allowed for voluntary responses to be initiated prior to the presentation of the SAS. To quantitatively dissociate response types we calculated the difference in RT relative to the expected speed of the stop-process (premotor RT – [SSD+SSRT]) for voluntary responses (i.e., EMG onset prior to SAS presentation) and SAS responses (EMG onset following SAS presentation). Positive values (seen in Figure 3.3) reflect RTs which would have been successfully stopped (i.e., inhibited) whereas negative values reflect RTs which would have resulted in a failed stop (i.e., initiation). Consistent with previous literature on stop-signal task performance (see Verbruggen & Logan, 2009 for a review), voluntary responses (collapsed across SS+SAS150 & SS+SAS200) were initiated prior to the completion of the stop-process with a difference of -28 ms (see Figure 3.3), indicating that the go-process reached threshold before the stop process. In contrast, SAS responses (collapsed across SS+SAS150 & SS+SAS200) were initiated after the completion of the stop-process with a difference of +32 ms, indicating that despite the stop-process finishing before the go-process, presenting a SAS still resulted in the initiation of the go-response (see Figure 3.3). This suggests that even 200 ms following the stop-signal there was sufficient residual go- activation to allow the additional activation provided by SAS to cause the involuntary triggering of the go-response. The ability of SAS to trigger the go-response well after the stop-response had “won” provides evidence against a winner take all race between independent go- and stop- activations.

Assuming that following the presentation of a stop-signal two independent go- and stop-activations race towards a single response threshold for control over movement outcome (i.e., initiation or inhibition), it was hypothesized that a SAS would be able to independently interact with each process. Just as it has been shown that a SAS can boost voluntary go-activation and initiate the movement when presented before or after a go-signal (Maslovat et al., 2014; Maslovat et al., 2015), we sought to

determine whether presenting a SAS following a stop-signal may provide a boost to voluntary stop-activation thus aiding response inhibition. In contrast to our hypothesis, when the SAS was presented following the stop-signal it increased the probability responding compared to that predicted under voluntary control even when the SAS was presented 200 ms following the stop-signal. As opposed to a winner take all race between independent go- and stop-activations, the results of the present study provide novel insight in support of two possible interactive/dependent neural mechanisms governing behavioural control during a stop-signal task. However before discussing how our results fit into these models, we first indicate how the horse-race model needs to be modified to account for the current findings.

One possible mechanism is that there are two independent sets of activation related to the go- and stop-responses respectively, however the response which reaches threshold first is not necessarily the winner. The latency of SAS responses at SS+SAS150 and SS+SAS200 suggested that the SAS was able to cause the initiation of the response past the point in time where the stop-response reached response threshold and inhibited the response. In contrast to our hypothesis, presenting the SAS following the stop-signal may have only provided additional activation to the go-response resulting in the initiation of the movement after the time point at which it would have normally been inhibited. This interpretation of the results does not preclude the independent race model, however, it does imply a modification to the model. Specifically, it requires the elimination the winner take all finish line, replacing it with a window of time in which initiation can influence inhibition and vice versa. This interpretation is consistent with the proposed phantom point of no return by McGarry & Franks (1997), which suggests that response production processes can be modified right up to the point of muscle activation. EMG data revealed that the response production processes could be interrupted before and after their initial

execution as indicated by partial and interrupted responses respectively (McGarry & Franks, 1997). Ko and colleagues (2012) have also demonstrated reductions in force production on failed-stop trials, providing further evidence for an inhibitory effect on response output past the threshold of response initiation. These results provide evidence that the stopping process is able to directly affect the production of the response after the point at which the response is initiated, indicating that response production does not mark the onset of a final ballistic process. Similarly, it appears that the additional activation provided by the SAS when presented following a stop-signal was able to affect the initiation of the response after the point at which the response would have been expected to be inhibited. As opposed to an irrevocable commitment to action once response threshold is crossed (i.e., after the point of no return), it appears that input signals, either initiation or inhibitory in nature, can produce an effect on behavioural output.

The present results can also be considered within the context of two conceptually similar models; the interactive race model (Boucher et al., 2007) and dependent process model (Dunovan, Lynch, Molesworth, & Verstynen, 2015), which are based on data from saccadic eye movements and limbs movements respectively. Rather than behavioural outcome being determined by a parallel race between go- and stop-activations, as predicted by the independent race model, these models suggest that the initiation or inhibition of a response during a stop-signal task is determined by the ability of the stop process to interact with the go-activation prior to the initiation threshold being reached. A single go-activation that can be diminished via stopping-related inhibitory processes provides an explanation for the increase in probability of responding that we see compared to that predicted under voluntary control when the SAS was presented following the go-signal, as the SAS would only be adding activation to the initiation of the go-response. In response to the stop-signal, inhibition is applied to go-activation

driving it down and away from threshold. This decreasing (yet enduring) go-activation, which remains well after inhibition is applied, explains the involuntary triggering of the go-response even at the latest SAS probe times (i.e., SS+SAS150 & SS+SAS200). SAS response latency as a function of probe time can be used to map the time course of go-activation relative to threshold as a reduction in SAS response latency is proposed to reflect an increased amount of voluntary initiation-related activation (Maslovat et al., 2014; Maslovat et al., 2015). We found that SAS response latency decreased with increasing delay from the go, with short response latencies observed at SS+SAS150 (42 ms) & SS+SAS 200 (40 ms) similar to those found in previous SAS experiments when presented late in the RT interval (Maslovat et al., 2014; Maslovat et al., 2015).

While both the independent and interactive models can be used to account for the decrease in RT from GO+SAS to SS+SAS150 with increasing go-activation, the two models predict very different SAS RTs late in the stop-process (i.e., SS+SAS150 & SS+SAS 200). As opposed to the continued increase of the independent go-activation and thus continued decrease in SAS RT, the cessation of RT reduction observed between SS+SAS150 & SS+SAS 200 is in line with a decrease in go-activation caused by the interacting stop-process between these two SAS probe times (i.e., SSRT= 166.6 ms). The short and similar SAS response latencies found at the last two probe times suggests that voluntary go-activation is very close to response initiation threshold when inhibition is proposed to have a potent effect on go-activation late in the RT interval. The concept of stop-signal inhibition decreasing initiation-related activation is quite compatible with the increase in *p* – respond and SAS response latencies observed in the present study and provides further evidence in support of models of inhibitory control other than the independent race model. The present findings extend and provide new information regarding the

competition between initiation and inhibitory processes, which may be used to modify the models to best mimic the behavioural and physiological data.

3.6. Conclusion

In summary, the use of a startling acoustic stimulus (SAS) has provided novel insight regarding the time course of motor related activation during a stop-signal task. Results from SAS trials indicate that the go-response was prepared in advance of the go-signal and this go-activation increases following the onset of the go-signal. The increase in probability of responding for all SAS probe times following the stop-signal suggests that go-activation endures even 200 ms following a stop-signal. The latency of SAS responses at SS+SAS150 and SS+SAS 200 suggest that these responses would have been voluntarily inhibited but instead were involuntarily triggered by the SAS. Thus, go-activation remains accessible and modifiable well after the response has been voluntarily inhibited. The present results provide evidence against an irrevocable commitment to initiation or inhibition once response threshold is crossed in the context of an independent neural accumulator race model. Moreover, the SAS results yield further understanding into the end of the race between go- and stop-processes, which provides further support for alternative models and mechanisms of inhibitory control.

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4. Chapter 4

Increased response preparation overshadows neurophysiological evidence of proactive selective inhibition.

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4.1. Abstract

Humans are able to proactively inhibit a single limb when provided a precue in a bimanual selective stopping task (e.g., maybe stop right hand). We investigated how advance preparation affects proactive selective inhibition, as previous experiments have been performed in a choice reaction time paradigm where the ability to prepare responses ahead of the go-signal was limited. One group (simple selective stopping task) was instructed to initiate a bilateral wrist extension upon the appearance of two squares, while the other group (choice selective stopping task) was instructed to initiate either a bilateral wrist extension or flexion depending upon the location of two squares. Occasionally, the go-signal was followed by a stop-signal which instructed participants to stop one limb while still initiating the other response. Prior to the go-signal, a precue (e.g., maybe stop right/left) provided participants with information indicating which limb might have to be stopped. To probe the state of the stop-cued and non-cued responses, suprathreshold TMS was delivered during the foreperiod (between precue and go-signal), over the motor representation for the wrist extensor. In addition, a startling acoustic stimulus was delivered concurrent with the go-signal (simple selective stopping task group only). Consistent with previous findings, corticospinal excitability (CE) during the choice selective stopping task was reduced in the stop-cued limb compared to the non-cued limb. In contrast, the simple selective stopping task revealed an overall increase in CE compared to when at rest but no difference between the stop-cued and non-cued limbs. In line with the neurophysiological results, inspection of startle trials revealed that when a startle response was elicited, both responses (non-cued and stop-cued) were triggered early and in synchrony. The results suggest that the increased go-related preparatory activation of the stop-cued response overshadows the small selective inhibitory effect typically seen in choice selective stopping tasks with limited advance preparation.

4.2. Introduction

A selective stopping task requires participants to initiate a bimanual limb movement as fast as possible in response to a go-signal, but to inhibit a specific movement/limb in response to an infrequent stop-signal (Aron & Verbruggen, 2008; Claffey, Sheldon, Stinear, Verbruggen, & Aron, 2010). Essential to this selective stopping paradigm is that advance information (i.e., a precue) is given about which specific response/limb needs to be stopped, if and when a stop stimulus is presented, while the other response/limb should nevertheless continue to be initiated (De Jong, Coles, & Logan, 1995; Jahfari, Stinear, Claffey, Verbruggen, & Aron, 2010). Unlike traditional precue studies which indicate features of the response that will have to be initiated (Goodman & Kelso, 1980; Rosenbaum, 1980), the precue in a selective stopping task provides information about what response might have to be inhibited (e.g., "maybe stop right hand"). Providing this precue information has consistently shown to allow for a proactive and selective mode of inhibition, such that only the limb that is cued to maybe stop demonstrates inhibition (Cai, Oldenkamp, & Aron, 2011; Majid, Cai, Corey-Bloom, & Aron, 2013). For instance, neurophysiological evidence from single pulse TMS delivered following the precue demonstrates a decrease in corticospinal excitability in the hemisphere contralateral to the maybe stop cued hand, while no change in corticospinal excitability (CE) is observed in the hemisphere ipsilateral to the maybe stop cued hand (Cai et al., 2011; Majid, Cai, George, Verbruggen, & Aron, 2012; Majid et al., 2013). This proactive selective inhibitory control is in contrast to traditional stop-signal tasks which demonstrate a reactive and global mode of inhibition, such that all limbs (right and left hand in a bilateral response task), including those not involved in the task (e.g., leg), are inhibited only after the presentation of the stop-signal (Badry et al., 2009; Greenhouse, Oldenkamp, & Aron, 2012; Majid et al., 2012). Additional evidence of selective inhibition has been shown in behavioural data, as indicated by a small difference in reaction time (RT) in the non-cued limb between trials with and without a stop-signal

(referred to as the stopping interference effect). This is in contrast to the large delay in RT during stop-trials observed in the non-cued limb when a non-informative ambiguous cue is presented (Aron & Verbruggen, 2008; Claffey et al., 2010; Majid et al., 2012; Majid et al., 2013).

Previous selective stopping experiments demonstrating a proactive selective decrease in corticospinal excitability had participants complete the task in a choice-RT paradigm, in which the response required for each limb was unknown prior to the go-signal. Because go-related response preparation and initiation can only occur after the go-signal, the selective decrease in CE revealed during the foreperiod is tied to the limb as opposed to a particular response since it has not yet been selected or prepared. Thus, the purpose of the present experiment was to determine how advance preparation related to the initiation of known responses (i.e., task completed in a simple-RT paradigm) affects proactive selective inhibition.

Similar to previous experiments examining the neurophysiological effect of proactive selective inhibition (Cai et al., 2011; Majid et al., 2013), we delivered single pulse TMS during a trial's foreperiod to examine response specific changes in corticospinal excitability as a consequence of the precue. In addition, a loud startling acoustic stimulus (SAS: 120 dB) was delivered coincident with the go-signal to probe the preparatory state of the stop-cued and non-cued response. It has been demonstrated that the presentation of a SAS not only causes a reflexive startle response (Brown et al., 1991; Landis, Hunt, & Strauss, 1939), but if a motor response is sufficiently prepared, it can also involuntarily trigger the prepared action at short latencies (Carlsen, Maslovat, & Franks, 2012; Castellote & Valls-Sole, 2015; Siegmund, Inglis, & Sanderson, 2001; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999). This phenomenon, known as the "StartReact" effect, is suggested to arise as a result of the SAS increasing the activation in the neural circuits related to the motor response beyond the required threshold for

motor output (Carlsen et al., 2012). In addition to triggering a single response, it has been shown that the presentation of a SAS can simultaneously trigger two independently prepared movements (e.g., right wrist flexion and left wrist extension) (Carlsen et al., 2009). Given this, startle was used to provide further insight into the activation related to both the stop-cued and non-cued responses during a bimanual simple selective stopping task.

In contrast to a choice selective stopping task, we hypothesized that the TMS results during the performance of a simple selective stopping task would reveal an increase in CE related to the advance preparation of both responses. However, the TMS results were still expected to replicate the selective decrease in CE in the stop-cued response compared to the non-cued response. In accordance with this selective inhibition of the stop-cued response, we hypothesized that the presentation of a SAS concurrent with the go-signal during the simple selective stopping task would only result in the involuntary triggering of the non-cued response. These results would indicate that proactive inhibition is selective to a specific motor response and does not interfere with preparatory activation and initiation of the response that must always be initiated.

In contrast to our hypotheses, TMS results from the simple selective stopping task (Experiment 3.1) did not replicate the selective decrease in the stop-cued response CE compared to the non-cued response. To account for differences in protocol, a control experiment (Experiment 3.2) was conducted to determine whether our protocol could replicate previous neurophysiological findings when performed in the typical choice selective stopping task.

4.3. Materials and Methods

4.3.1. Experiment 3.1: Simple selective stopping task

4.3.1.1. Participants

Data were collected from eleven healthy participants (M = 7, F = 4; *M*age = 25, *SD* = 6) with normal or corrected to normal vision, and no history of neurological, sensory, or motor disorders. However, two participants failed to show a reflexive startle response (see data reduction section for details) in the majority of the startle trials in the simple-RT task (<60%) and thus their data were excluded from the analyses (Carlsen, Maslovat, Lam, Chua, & Franks, 2011). Data are presented from the remaining 9 participants (M = 6, F = 3; *M*age = 26, *SD* = 7). All participants provided written informed consent, and passed a TMS safety-screening questionnaire (Rossi, Hallett, Rossini, Pascual-Leone, & Safety of, 2009) prior to beginning the experiment. The study was approved and conducted in accordance with the ethical guidelines set by the University of Ottawa's Research Ethics Board and conformed to the latest revision of the declaration of Helsinki.

4.3.1.2. Experimental Set-up

Participants sat facing a 24-inch LCD computer monitor with their right and left arms placed in custom made force measurement devices. Their torso was restrained using a harness to limit the contribution of force generation solely to the left and right wrist. Each arm was supported and secured in a neutral position (semi-pronated with the palm perpendicular to the floor) such that the dorsal surface of the hand was in contact with a solid plate, restricting movement to concentric wrist flexion and isometric wrist extension. The plate was attached to a force transducer (Nano25 FT, ATI Industrial Automation, NC, USA) allowing measurement of force produced during an isometric wrist extension.

4.3.1.3. Experiment 3.1: Simple Selective Stopping task

The selective stopping task used in the present study (see Figure 4.1) was designed to be as similar as possible to that used in previous studies (Majid et al., 2012; Majid et al., 2013), with the major exception being that only a single known bimanual response was required (i.e., 40% isometric bimanual wrist extension). To start the trial, a cue was presented for 500 ms, “Maybe Stop Right” (MSR), “Maybe Stop Left” (MSL), “Maybe Stop XXX” (MSX), or “Null”. Each cue had an equal likelihood of appearing, and was presented on the computer monitor in white text on a black background. Participants were instructed to use the cue to prepare for the upcoming response. The MSR and MSL were selective stopping cues, indicating to participants that they should prepare to stop that particular hand as a stop-signal may appear later in the trial. The MSX cue was ambiguous as to which hand they might have to stop, serving as a non-informative stopping cue which should prevent proactive selective inhibition and result in a reactive mode of inhibition. On Null trials, participants were instructed that no response was necessary and to remain relaxed. Null trials served as a baseline for TMS measures in order to determine a potential modulation in CE relative to null. Following cue offset, there was a variable foreperiod of 1500 ms - 3000 ms prior to the go-signal. 1000 ms into the foreperiod, single pulse TMS was delivered (see TMS section below for details) over the left or right cortical representation of the extensor carpi radialis (ECR) in M1. TMS was used to probe CE in order to examine proactive selective inhibition during a simple selective stopping task.

On trials in which a MSR, MSL, or MSX cue was presented, the go-signal was indicated by the appearance of two separate blue squares (6.5 cm x 6.5 cm each), one to the right of center representing a right response and one to the left of center representing a left response, which remained visible for 2000 ms. Participants were instructed to initiate a bimanual isometric wrist extension with a target force

of 40% of their maximum voluntary force (MVF, see below) as fast and accurately as possible in response to the go-signal. On a subset of trials (stop-trials), a red stop-signal (either the letter “W” or the letter “M”) was presented centrally on the monitor at a variable delay following the go-signal [stop-signal delay (SSD)]. The presentation of “M” as a stop-signal indicated that the participant was to stop the right response and continue with the left, whereas presentation of “W” meant to stop the left response and continue with the right. M and W were chosen as stop-signals in order to promote the use of the pre-cue and thus a proactive mode of inhibition to correctly stop the response. Directional arrows or the go-signal changing colour were not used as stop-signals as they may have promoted a reactive mode of inhibition due to stimulus saliency and direct mapping (Goodman & Kelso, 1980). Participants were instructed that when a stop-signal occurred, they were to withhold the response corresponding to the stop-signal and initiate the other response. Participants were informed that the stop-signal was always congruent with the MSR and MSL cue, and that the stop-signal (M or W) following a MSX cue indicated whether to stop the right or left response respectively. Following the response period, feedback was displayed for 3.5 s, which included performance feedback (reaction time and peak force relative to the target force), paired with a message indicating whether their response was “Correct” or “Incorrect.” Participants received 25 points for responding correctly (i.e., responding on go-trials and not responding on stop-trials) and responding quickly (1 point for every 7 ms below 350 ms, up to a maximum of 25 points). Participants were penalized 25 points for an incorrect response or a go-RT exceeding 500 ms. In addition, real time force feedback was provided so that the home position could be achieved for the subsequent trial. Failure to respond with both hands simultaneously (defined as >70 ms difference in RT between hands) on a go-trial resulted in a “decoupled” warning, discouraging participants from cheating and not responding as fast as possible with the “Maybe Stop” hand. A correct

response was when force onset occurred between 50 ms and 500 ms following the go-signal, peak force of the responding limb exceeded 5% MVF and peak force of stop limb was below 5% MVF.

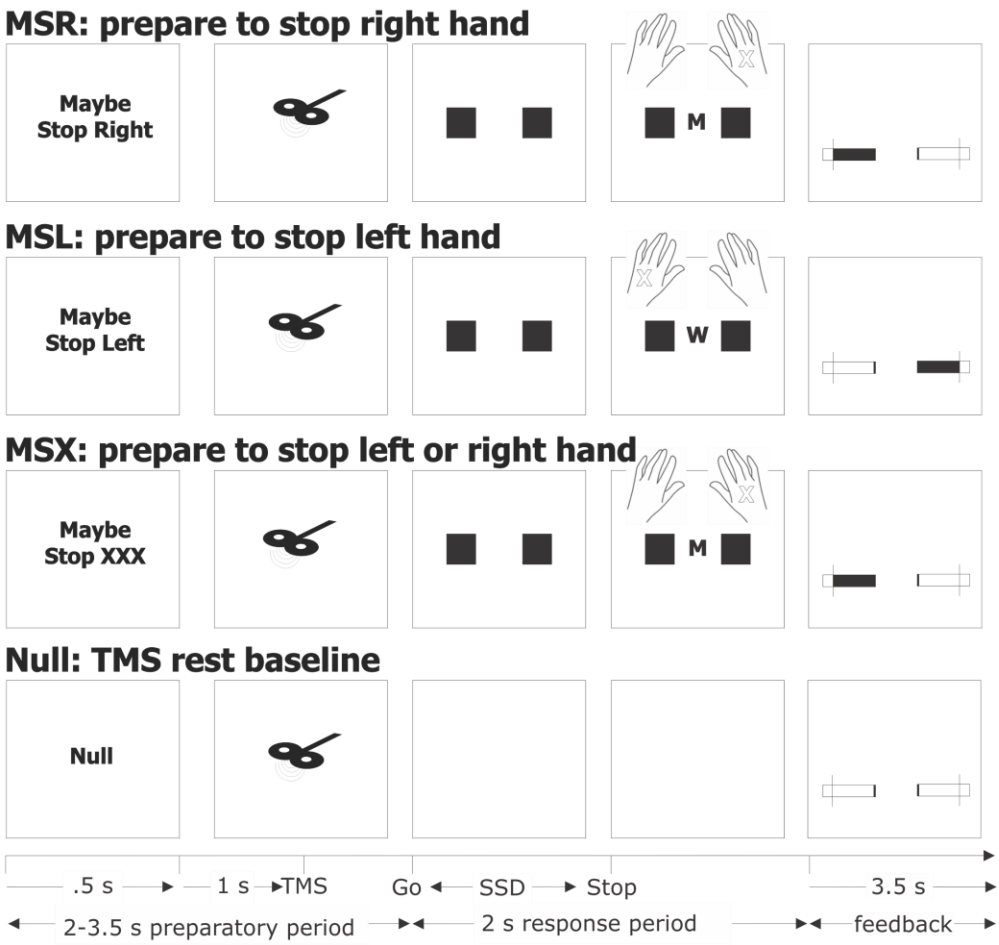

Figure 4.1. Schematic representation of the time course of the simple selective stopping task (Experiment 3.1). Each row is an example of a trial in which a stop-signal is presented (i.e., stop-trial) following each cue type [Maybe stop right (MSR), Maybe stop left (MSL), Maybe stop unknown (MSX), and Null (TMS baseline)]. The first column is the precue (e.g., Maybe Stop Right), the second is the foreperiod where the TMS pulse was delivered 1 s after cue offset, the third is the go-signal (illumination of squares), the fourth is the stop-signal (M or W) following the stop-signal delay (SSD), and the fifth is visual performance feedback following the correct response output for each cue type [dashed line is the target (40% MVF), and the filled bar represented peak force achieved]. Explicit feedback related to accuracy and reaction time is not shown.

4.3.1.4. Procedure

Prior to behavioural testing, each participant's hotspot for their ECR in M1 and resting motor threshold were determined (see Transcranial Magnetic Stimulation section below for details). Participants then completed a bilateral MVF test, which consisted of 3 MVF trials per hand, with 1 minute of recovery between trials (for a detailed protocol see Sahaly, Vandewalle, Driss, & Monod, 2001). MVF for each hand was then determined by calculating the average peak force from the three trials.

Participants next performed a simple-RT task, consisting of 10 practice trials followed by 20 testing trials. Simple-RT task trials followed a similar time course as the simple selective stopping task, with the exception of the following; The cue to start a trial was "Get Ready" (Go Only), and participants were instructed to simply respond as fast as possible to a visual go-signal because all trials were go-trials (i.e., there were no stop-signal trials). In addition, TMS was not delivered during the simple-RT task as no differences in CE between limbs were expected. A SAS was presented pseudo-randomly on five of the 20 simple-RT task testing trials concurrent with the go-signal (see Startling Acoustic Stimulus section below for stimulus details). Participants were told that on some trials they would hear a loud "static noise" sound. They were instructed that this was irrelevant to the task and they should continue to initiate their movement as fast as possible. The simple-RT task served as a startle screening tool to determine whether a SAS consistently elicited a startle response in the sternocleidomastoid (see *Data reduction* section below for startle response criteria), and was completed first to ensure that previous performance of the selective stopping task would not influence simple-RT task performance. For a participants data to be included in the analyses of the selective stopping task (where currently responses to a SAS are unknown), a startle response must have been observed in 60% or more of the

simple-RT task startle trials independent of any movement which was analyzed offline following completion of the entire experiment (see Carlsen et al., 2011 for a review).

Participants then performed the simple selective stopping task. Each participant first completed a practice block without TMS or SAS, which consisted of 20 randomized trials; 5 trials per cue type (i.e., MSR, MSL, MSX, Null). For each cue type, a stop-signal was presented in two out of each of the five trials, one with a SSD of 100 ms (higher probability of stopping) and the other with a 300 ms SSD (lower probability of stopping). Following completion of the practice block, participants performed 6 testing blocks consisting of 24 trials each (144 total trials). Each block contained 6 trials per cue type (i.e., MSR, MSL, MSX, Null). The breakdown for the MSR, MSL, and MSX cue trials was as follows: 3/6 = go-trial (50%), 2/6 = stop-trial (33%), and 1/6 = go-trial + SAS (17%). Trials were presented pseudo-randomly, such that a SAS did not occur on two consecutive trials nor did a stop-signal occur on two consecutive trials. For stop-trials, the SSD was adjusted individually for each participant using a tracking procedure to produce approximately 50% successful stops (Logan, Schachar, & Tannock, 1997). Specifically, the stop-signal was first presented at 250 ms following the go-signal (i.e., SSD = 250 ms), then increased or decreased by 50 ms on the subsequent stop-trial with every successful or failed stop respectively. This tracking procedure was chosen to discourage participants from slowing down (a strategy which makes inhibition easier).

4.3.1.5. Starling Acoustic Stimulus (SAS)

A SAS was presented concurrent with the go-signal during simple- RT and selective stopping task testing trials. The SAS was a 120 dB, 25 ms duration, white noise waveform (with equal power from 1Hz to 22 kHz), which was delivered via a loudspeaker (MG Electronics M58-H, rise time < 1 ms) located behind the participant's head. Stimulus intensity and frequency content was confirmed using a precision

sound level meter located at the same distance from the loudspeaker to the ears (30 cm, Cirrus Research Optimus CR: 162C, A - weighted).

4.3.1.6. Transcranial Magnetic Stimulation (TMS)

TMS was delivered in the selective stopping task using a MagStim 200^2 system (MagStim Inc.) via a 70 mm figure of eight coil. Stimulation side (left or right ECR motor representation) was counterbalanced across participants. In the nine participants who were included in the full analyses of Experiment 1, five participants received stimulation over their right ECR motor representation; the other four participants received stimulation over their left ECR motor representation. The approximate location of the ECR was first determined by finding Cz (based on the international 10-20 system for EEG electrode placement), then measuring 4.7 cm lateral and 1.1 cm anterior. From this initial position, suprathreshold TMS was systematically delivered to the surrounding area to determine the location that elicited the largest MEP in the ECR (i.e., the "hotspot"). This location was tracked and saved using an image guided TMS NeuroNavigation system (ANT visor2), which allowed precise, reliable and consistent TMS targeting. Once the location of the ECR representation was found, RMT was determined by finding the stimulator output that produced a MEP of at least 100 μV in five out of ten pulses (Rossini et al., 1994). Similar to previous studies (Cai et al., 2011), TMS intensity during testing blocks was delivered at 112% of RMT, resulting in a mean stimulator output of 44% across participants (*SD* = 7).

4.3.1.7. Recording Equipment

Surface electromyographic (EMG) data were collected bilaterally from the muscle bellies of the following muscles: extensor carpi radialis (ECR) and sternocleidomastoid (SCM), using bipolar pre-amplified (gain=10) surface electrodes (Delsys Bagnoli DE-3.1) connected via shielded cabling to an external amplifier system (Delsys Bagnoli-8). The EMG recording sites were prepared using an abrasive

gel (Nuprep, Weaver & Company) and cleansed with an alcohol wipe in order to decrease electrical impedance. The electrodes were placed parallel to the muscle fibers, and attached using double sided adhesive strips. The reference electrode (Dermatrode) was placed on the participant's right lateral epicondyle.

Isometric wrist extension force data were collected using a force transducer (Nano25, ATI Industrial Automation, NC, USA) placed between the dorsal side of each hand and the plate. On each trial, unfiltered EMG and force data were digitally sampled at 4 kHz (National Instruments PCIe-6321 via BNC-2090) for 5 seconds using a customized program written with LabVIEW software (National Instruments Inc.) and stored for offline analysis.

4.3.1.8. Data Reduction

Force onset was defined as the point at which force output exceeded two standard deviations above baseline levels (mean force for 100 ms prior to the go-signal onset). Reaction time was defined as the interval of time between the go-signal and force onset. If reaction time in the responding limb was less than 50 ms it was considered an error due to anticipation and discarded (0.9% of total trials). Force offset was defined as the first point at which the force dropped below the threshold for onset. Peak force was defined as the highest observed value on a given trial as a percentage of MVF. A force exceeding 5% of MVF was classified as an initiated go-response. If force did not exceed 5% of MVF it was classified as a full-stop response. If the difference in force onset times for the two limbs during go-trials was greater than 70 ms it was considered an error and discarded from further analyses (6% of total go-trials).

EMG data were collected on all trials from the ECR (agonist) and SCM and were analyzed for muscle burst onset, offset, and peak amplitude. EMG burst onsets (premotor RT) were defined as the point in time at which the rectified and filtered (25 Hz low pass 2nd order elliptic filter) EMG signal first began a sustained (>20 ms) rise exceeding two standard deviations above baseline levels (mean EMG activity for 100 ms prior to go-signal onset). Premotor RT for go-trials was defined as the mean premotor RT of the right and left limb response. EMG offsets were defined as the point at which the EMG fell below the baseline threshold. Peak EMG amplitudes were defined as the largest amplitude of the rectified and filtered EMG signal recorded within an interval of 100 ms following EMG burst onset.

To distinguish startle response related SCM activity from other SCM activity, bilateral SCM onset had to occur within 30-120 ms following SAS onset (Carlsen et al., 2011). The magnitude of the EMG startle response was calculated as the integrated area of the rectified and filtered EMG signal (iEMG) recorded within an interval of 30 ms (Q30) and 100 ms (Q100) following EMG burst onset. To examine differences in the startle response between the simple-RT task and the simple selective stopping task within subjects, percent modulation in SCM magnitude (Q30 & Q100) was calculated using the following formula: (stop-signal task - simple RT task)/ simple RT task X 100%. Thus, positive and negative numbers reflect a larger or smaller startle response magnitude respectively during the selective stopping task compared to the simple-RT task (0%). Stop-cued SCM activation refers to the SCM on the same side as the stop-cue (e.g., right SCM - MSR), non-cued SCM activation is the SCM opposite to the cued stop side (e.g., right SCM - MSL), and Ambiguous SCM activation is collapsed across left and right SCM during the MSX cue. The proportion of startle responses observed as a function of cue type was calculated by dividing the total number of observed startle responses during SAS trials by the total number of SAS trials. Only SAS trials where a bilateral startle response was observed in the SCM at short latency were

included in the analyses of the remaining SAS dependent variables (e.g., premotor RT, peak force). Stop-cued peak force is the response on the same side which was cued to stop (e.g., right response - MSR), non-cued peak force is the response opposite to the cued stop side (e.g., right response - MSL), and Ambiguous is collapsed across left and right responses during the MSX cue.

MEPs elicited by TMS were identified in the EMG data using custom LabVIEW software (National Instruments Inc.). Consistent with previous studies examining selective inhibition, MEP trials were excluded if the root mean square of EMG activity in the 100 ms prior to TMS delivery was >10 μV to ensure no preliminary extensor activation (Cai et al., 2011). The exclusion criteria for MEPs resulted in two of the nine participants being excluded from the MEP analyses due to an insufficient number of trials. Peak-to-peak MEP amplitude was determined by finding the difference between the maximum and minimum value of the MEP. The top and bottom 10% of MEP amplitudes across participants were trimmed for each condition (MSR, MSL, MSX, or Null) to ensure distribution normality (Stinear & Byblow, 2004; Wilcox, 2010). Percent wrist extensor modulation, a measure of motor excitability changes compared with the Null baseline condition was determined for all non-Null conditions for each participant (i.e., MSR, MSL & MSX) using the following formula with mean MEP amplitude values: (Condition MEP-Null MEP)/Null MEP X 100% (Majid et al., 2013). The results of percent wrist extensor modulation served as a neurophysiological check for proactive selective inhibition. The stopping interference effect was calculated for the three cue conditions (MSR, MSL, MSX) for each participant with data from successful go-trials and stop-trials using the following formula: [e.g., MSL: mean RT of right hand (i.e., non-cued) during go-trials – mean RT of right hand (i.e., non-cued) during stop-trial]. The results of the stopping interference effect served as a behavioural check for proactive selective inhibition.

4.3.1.9. Statistical Analyses

In order to establish behavioural evidence of selective inhibition during the simple selective stopping task, the stopping interference effect was analyzed with a one-way repeated measures ANOVA with the repeated factors of cue condition (3 levels: MSR, MSL, MSX). To confirm that participants were preparing the responses in advance, a non-parametric Freidman test was performed to assess the change in wrist extensor excitability relative to null (0%), with one-tailed Wilcoxon signed ranks test used to determine whether each cue condition had a significant increase in excitability relative to null (0%). In order to determine whether there was neurophysiological evidence of proactive selective inhibition, a paired samples t-test was used to compare the percent wrist extensor modulation of MEPs elicited in the stop-cued response (e.g., MSR: right ECR) vs. the non-cued response (e.g., MSR: left ECR).

To determine the effect of cue condition on voluntary response initiation, premotor RT during correct control go-trials was analyzed using a one-way repeated measures ANOVA with the repeated factors of cue condition (4 levels: Go Only, MSR, MSL, MSX). To determine the effect of cue condition on the response to a SAS, the proportion of SAS trials that elicited a startle response (SCM activity) were analyzed using a one-way repeated measures ANOVA with the factor of cue condition (4 levels: Go Only, MSR, MSL, MSX). Proportion values were subjected to an arcsine square root transformation prior to analysis (Osborne, 2010).

To determine the presence of the StartReact effect in participants, a paired samples t-test was used to compare premotor RT during control (i.e., no SAS) and SAS trials during the simple-RT task. Statistical significance was at set an alpha level less than 0.05, and the effect size is reported for all tests.

4.3.2. Experiment 3.2: Choice selective stopping task

A new group of participants completed a similar task as that used in Experiment 3.1 but now in a 2-choice paradigm requiring either bimanual wrist flexion or bimanual wrist extension. The control experiment was designed to closely match the choice selective stopping task used by Majid and colleagues (2013), as well as our simple selective stopping task. The materials and methods were used were consistent with the simple selective stopping task unless otherwise noted below.

Six participants (M = 1, F = 5; *M*age = 22, *SD* = 1.0) completed 90 practice trials, followed by 8 testing blocks consisting of 30 trials. The trial breakdown for each block was as follows, with an equal number of flexion and extension trials; MSR (4 go & 2 stop trials), MSL (4 go & 2 stop trials), MSX (8 go & 4 stop trials) and Null (6 resting baseline trials). Participant's arms were secured in a semi-pronated position to custom made manipulandum which restricted movement to wrist flexion and extension. In contrast to the simple selective stopping task, the location of the go-signal (two blues squares) indicated the response to be made, either a bimanual wrist flexion or extension movement with a target distance of 40° relative to the neutral home position (0°). To distinguish flexion (inward) and extension (outward) movement's two lines were always visible on the monitor, the lines extended vertically from the middle (0° = home position) of the right and left displacement feedback bars. To indicate whether the response was bimanual flexion or extension the two blue squares appeared either inside or outside of the two vertical lines respectively. The time course of a trial was identical to that in the simple selective stopping task (see Figure 4.1), with single pulse TMS delivered at 112% of RMT (stimulator output; *M* = 46 %, *SD* = 5) 1000 ms into the foreperiod of every testing trial. In addition, the presentation of the stop-signals (either the letter "W" or the letter "M") and proportion of stop-signal trials (33%) were identical to the simple selective stopping task. Instructions, and performance feedback were similar to the simple

selective stopping task. A correct response was when displacement onset in the proper direction occurred between 50 ms and 500 ms following the go-signal, peak displacement of the responding limb exceeded 5° and peak force of stop limb was below 5°. If reaction time in the responding limb was less than 50 ms is was considered an error due to anticipation and discarded (3% of total trials).

Similar to the simple selective stopping task used in Experiment 3.1, the stopping interference effect was analyzed using a one-way repeated measures ANOVA with the repeated factors of cue condition (3 levels: MSR, MSL, MSX). A non-parametric Freidman test was performed to assess the change in wrist extensor excitability relative to null (0%), and paired samples t-tests were used to compare CE relative to null when the MEP was elicited in the stop-cued limb vs. the non-cued limb (see Data Reduction section above for details).

4.4. Results

4.4.1. Experiment 3.1: Simple selective stopping task

4.4.1.1. Behavioural evidence

In stop-trials, analyses of the stopping interference effect as a function of cue condition revealed a significant main effect of condition (see Figure 4.2 dark grey bars), $F(2,16) = 10.655$, p .001, $\eta^2_p = .571$. Post-hoc analyses revealed a significantly larger interference effect in the MSX condition (M = 217.29 ms, SD = 148.89) compared to both the MSR (M = 36.46, SD = 77.16) and MSL conditions (M = 36.00 ms, SD = 69.18) (all p *values* <.01), with no difference between MSR and MSL conditions (p >.05). The small stopping interference effect - only 36 ms - when provided a selective stop-cue (i.e., MSR & MSL) compared to a non-selective stop-cue (i.e., MSX) replicates previous findings and provides behavioural evidence of selective inhibition during the performance of the current simple selective stopping task (Aron & Verbruggen, 2008; Claffey et al., 2010; Majid et al., 2012).

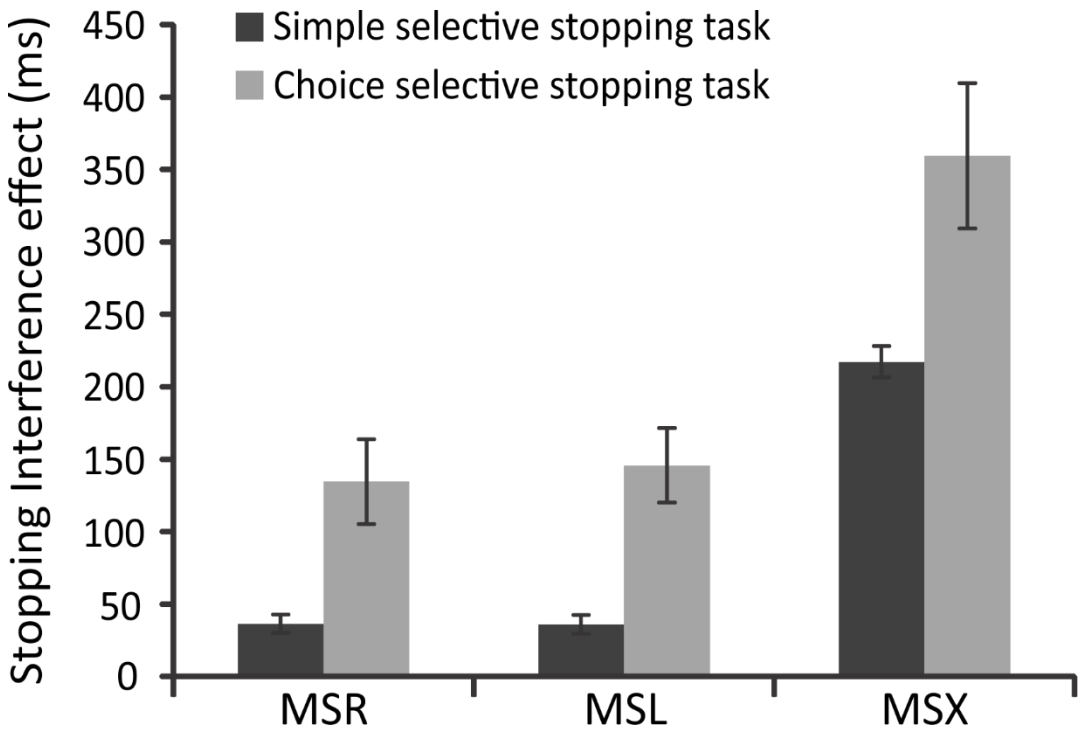

Figure 4.2. Group mean stopping interference effect as a function of cue condition during the simple (black bars) and choice (light grey bars) selective stopping task. The stopping interference effect indicates the delay in reaction time during stop-trials relative to go-trials in the continuing hand. Error bars represent within-subject 95% confidence intervals. Maybe stop right (MSR), Maybe stop left (MSL), Maybe stop unknown (MSX).

4.4.1.2. Neurophysiological evidence

As hypothesized, knowing the responses in advance (simple selective stopping task) of the go-signal allowed advance preparation related to the initiation of the response which was demonstrated by a significantly elevated amount of corticospinal excitability relative to null (0%) across all cue conditions (see Figure 4.3 black bars), χ^2 (3) = 8.675, *p* = .034, *W* = 0.412. Post-hoc analysis revealed that each cue condition was significantly elevated relative to null (stop-cued: Z= -2.366; non-cued: -1.690; ambiguous: -1.859, all *p values* <.05). However, analyses of percent wrist extensor modulation relative to null (i.e., 0%) revealed no significant difference between stop-cued (*M* = 29.92 %, *SD* = 35.04) and non-cued (*M* = 28.05 %, *SD* = 43.57) responses, t(6) = .2075, *p* = .843, r = .08. The present neurophysiological results do not provide evidence of proactive selective inhibition during the simple selective stopping task as the CE related to the stop-cued response was no different than the non-cued response. These findings are incongruent to the behavioural results which provided strong evidence of proactive selective inhibition during the simple selective stopping task.

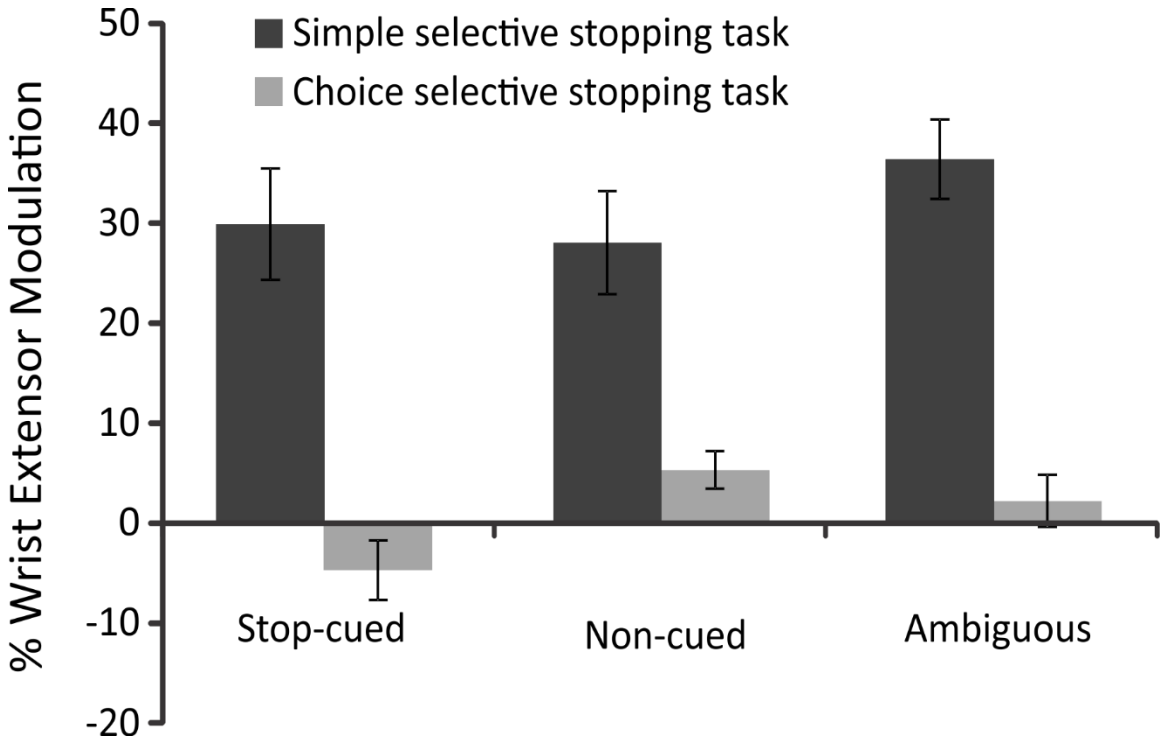

Figure 4.3. Group mean modulation of MEP amplitude relative to null trials for each selective stopping task cue type [(cue-null)/null *100]. Positive and negative values represent an increased or decreased state of corticospinal excitability respectively relative to when a rest (0%). The black bars are data from the Experiment 3.1, in which participants completed a simple selective stopping task, which had a single known bimanual response, and the light grey bars are data from Experiment 3.2, in which participants completed the choice selective stopping task which had a 2-choice bimanual response. Note the elevated and similar level of excitability for stop-cued and non-cued hands in the simple version, whereas the choice version demonstrated a significant reduction in excitability in the stop-cued hand compared to the non-cued hand. Error bars represent within-subject 95% confidence intervals.

4.4.1.3. Startle response

Analyses of the proportion of startle responses as a function of cue condition revealed a significant main effect of condition (see Figure 4.4A), $F(3,24) = 96.232$, $p < .001$, $\eta^2_p = .923$. Post-hoc analyses revealed that the proportion of startle responses elicited in the simple-RT task (Go Only: *M* = .96, *SD* = .09) was significantly higher than all selective stopping task conditions (MSR: *M* = .13, *SD* = .26; MSL: *M* = .07, *SD* = .15; MSX: *M* = .06, *SD* = .12, all *p values* <.01). The proportion of startle responses did not differ between selective stopping task cue conditions (all *p values* >.05).

This dramatic decrease in startle responses between tasks was unexpected, with a complete absence of a startle response in 6/9 participants. The proportion of startle responses for each selective stopping task cue condition only consists of data from 2 participants (MSR: participants 3 & 5 / MSL: participants 3 & 5 / MSX: participants 5 & 9). Due to the severely limited amount of startle trials that elicited a startle response, further analysis of the selective stopping task SAS trials is purely descriptive.

Examination of the startle responses elicited in these participants indicates between task differences such that there appears to be a large within-subject reduction in the size of the startle response relative to the simple-RT task (0%) in the first 30 ms (stop-cued: *M* = -60.55 %, *SD* = 39.77; non-cued: *M* = -60.11 %, *SD* = 23.97; ambiguous: *M* = -77.55, *SD* = 11.04) and 100 ms (stop-cued: *M* = -42.77 %, *SD* = 37.45; non-cued: *M* = -41.63 %, *SD* = 27.26; ambiguous: *M* = -66.77, *SD* = 26.29) across all three cue types (Figure 4.4B & C).

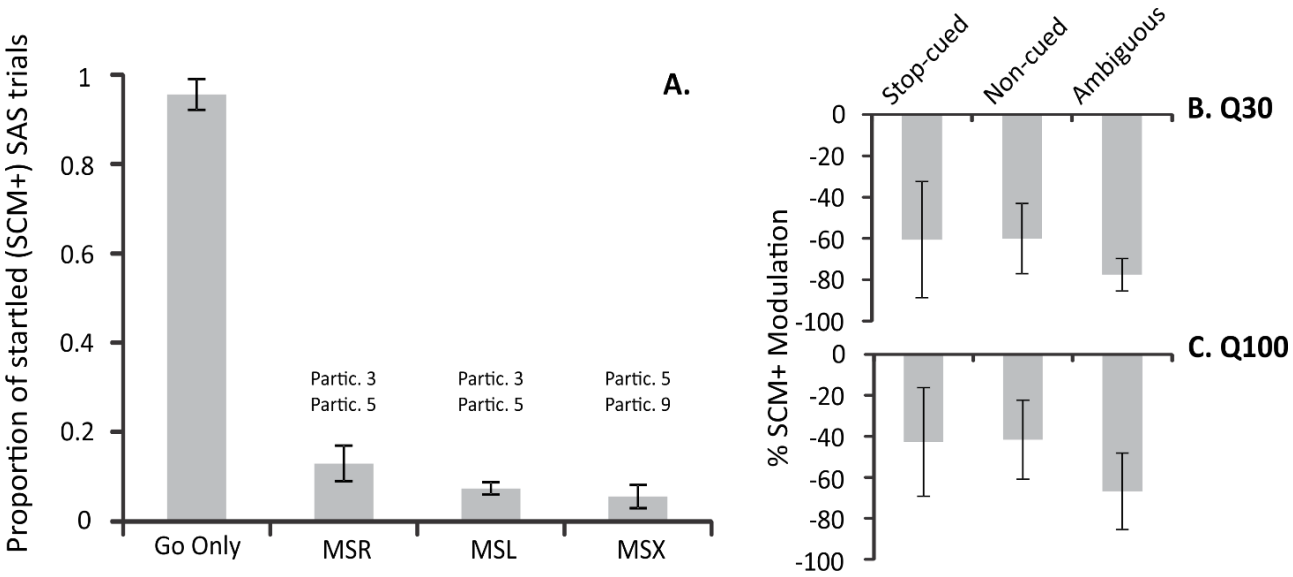

Figure 4.4 A. Group mean proportion of startle responses (SCM+) elicited during SAS trials as a function of cue condition [Go Only (Get Ready), Maybe stop right (MSR), Maybe stop left (MSL), Maybe stop unknown (MSX)]. Participants who had a proportion of SCM+ SAS trials > 0 during simple selective stopping task cue conditions are indicated above. Error bars represent within-subject 95% confidence intervals. **B. & C.** Within-subject % modulation of startle response (SCM+) Q30 (top) and Q100 (bottom) as a function of cue condition during the simple selective stopping task relative to the simple-RT task [(stop-signal-simple)/simple*100]. The data presented are means (SE) of participants who had a SCM+ during the simple selective stopping task. Stop-cued is SCM on the same side which was cued to stop (e.g., right SCM - maybe stop right), Non-cued is the SCM opposite to the cued stop side (e.g., right SCM-maybe stop left), and Ambiguous is collapsed across both SCM during the MSX cue.

4.4.1.4. Premotor reaction time

Analyses of premotor RT during control (i.e., no SAS) go-trials as a function of cue condition revealed a significant main effect of condition (Figure 4.5), $F(3,24) = 88.514$, $p < .001$, $\eta^2_p = .917$. Post-hoc analyses revealed that premotor RT during the simple-RT task (Go Only: *M* = 189.01 ms, *SD* = 16.86) was significantly faster than the MSR (*M* = 463.48 ms, *SD* = 76.16), the MSL (*M* = 462.88 ms, *SD* = 67.10), and the MSX (*M* = 483.74 ms, *SD* = 77.20) selective stopping cue conditions (all *p values* <.01). Premotor reaction time did not differ between selective stopping task cue conditions (all *p values* >.05). Analyses of premotor reaction time during the simple-RT task revealed a significant speeding of RT when the SAS was presented (*M* = 79.09 ms, *SD* = 15.94) compared to control trials (*M* = 189.01 ms, *SD* = 16.86), $t(8) = 19.822$, $p < .001$, $r = .99$.

To gain further insight into the discrepancy between the behavioural and neurophysiological data regarding proactive selective inhibition we examined premotor reaction time in the three participants who had a startle response during the simple selective stopping task. To distinguish voluntary responses during SAS trials vs. involuntary SAS triggered responses, trials were broken down into 3 types; control, startle trial with no SCM (SCM-) and startle trial with SCM (SCM+). Looking at figure 4.5, there is a clear distinction between SCM+ responses (*M* = 135.17 ms, *SD* = 76.86) and control and SCM- responses (CTL: *M* = 413.76 ms, *SD* = 100.24/ SCM-: *M* = 340.25 ms, *SD* = 109.24), while SCM- responses appear to have a similar premotor RT to control responses. Differences in premotor RT between trial types suggests that even though it occurred infrequently, when a startle response was elicited (SCM+), it resulted in the early triggering of both responses as the reduction in RT was not simply reflective of a stimulus intensity effect (SCM-).

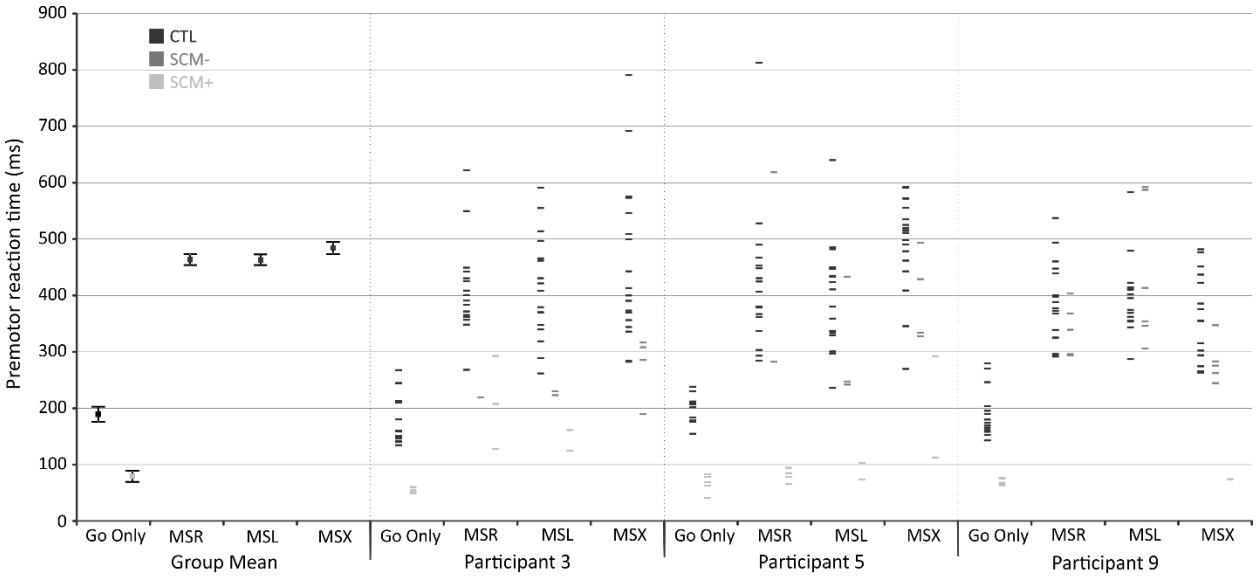

Figure 4.5. Premotor reaction time (ms) as a function of cue condition during control (CTL) and startle (SCM+/-) trials. Group mean as well as individual trial data from Participant 3, 5, and 9 are presented. These 3 participants were the only ones in which a SAS elicited a startle response (SCM+). Control trials are represented by the black shade, startle trials which did not result in a startle response (SCM-) are represented by the dark grey shade and startle trials which did result in a startle response (SCM+) are represented in by the light grey shade. Simple RT = Go Only, Maybe stop right = MSR, Maybe stop left = MSL, Maybe stop unknown = MSX. Error bars on group mean data represent within-subject 95% confidence intervals.

Premotor RTs during SCM+ trials were further broken to examine any effect of proactive selective inhibition, investigating the onset of each individual response (right & left limb) as a function of cue (MSR, MSL, and MSX). The between hand difference in premotor RT was calculated, with a positive value indicating that the left hand responded first and a negative value indicating that the right hand responded first. The mean difference in premotor RT between response hands during MSR (*M* = 5.57 ms) and MSL SCM+ trials (*M* = -10.88 ms) appears to be reduced compared to that typically observed during participants control trial performance (MSR: *M* = 25.22 ms / MSL: *M* = -29.97 ms), revealing minimal asynchrony in response initiation between limbs irrespective of cue condition. In addition, peak force between cue conditions provided no indication of a difference in stop-cued (*M* = 52.93 %, *SE* = 9.43) and non-cued (*M* = 60.01, *SE* = 2.34) SCM+ responses, with peak force collapsed across stop-cued and non-cued responses greater during participants SCM+ trials (*M* = 56.42 %) compared to their control trials (*M* = 43.6 %). While the findings are based on a limited data set, the results from the SCM+ startle data is in line with the neurophysiological findings revealing no evidence of proactive selective inhibition of the stop-cued response.

The neurophysiological results seen in Experiment 3.1 in the context of a simple selective stopping task did not replicate previous findings. In particular, we found no difference in the percent wrist extensor modulation between stop-cued and non-cued responses as opposed to the selective decrease in the stop-cued limb seen previously (Cai et al., 2011; Majid et al., 2012; Majid et al., 2013). The null effect of proactive inhibition in the present MEP data contradicts the behavioural evidence supporting proactive selective inhibition of the stop-cued limb. While the two lines of evidence should be congruent, there is a major difference between the task in Experiment 3.1 and those previously reporting evidence of selective inhibition at the corticospinal level, which may account for the

incongruence. Specifically, the participants performing Experiment 3.1 knew prior to the go-signal which response they were required to initiate (i.e., simple RT paradigm: bimanual wrist extension), whereas participants in previous studies did not know until the go-signal (i.e., 2-choice RT paradigm: bimanual wrist extension or flexion). A control experiment was conducted to determine whether a similar testing protocol to the simple selective stopping task could replicate behavioural and neurophysiological findings of proactive selective inhibition during a choice selective stopping task.

4.4.2. Experiment 3.2: Choice selective stopping task

4.4.2.1. Behavioural evidence

In stop-trials, analyses of the stopping interference effect as a function of selective stop-signal cue condition revealed a significant main effect (see Figure 4.2 light grey bars), $F(2,10) = 11.529$, $p = .003$, $\eta^2_p = .697$. Post-hoc analyses revealed a significantly larger interference effect in the MSX condition ($M = 359.53$ ms, $SD = 132.92$) compared to both the MSR ($M = 134.62$, $SD = 114.00$) and MSL conditions ($M = 145.78$ ms, $SD = 99.47$) (all *p values* <.05), with no difference between MSR and MSL conditions ($p > .05$). Note that these differences in stopping responses between informative (MSR & MSL) and non-informative (MSX) cues were not due to any difference in initiating responses as go trial RT was similar between cues, $F(2,10) = 2.451$, $p = .175$, $\eta^2_p = .329$. Similar to the simple selective stopping task, the behavioural results again replicate previous findings of less interference in the continuing hand when provided a selective stop-cue (i.e., MSR & MSL) compared to a non-selective stop-cue (i.e., MSX) which provides evidence of selective inhibition during the performance of the current choice selective stopping task.

4.4.2.2. Neurophysiological evidence

Analyses of corticospinal excitability relative to null (0%) revealed no significant difference for all cue conditions (see Figure 4.3 light grey bars), χ^2 (3) = 1.800, p = .615, W = 0.10. Unlike the simple selective stopping task, not knowing the responses in advance during the performance of the choice selective stopping task did not significantly elevate CE in advance of the go-signal.

Analyses of the percent wrist extensor modulation during the choice selective stopping task revealed a significant difference between excitability in the stop-cued (M = -4.72 %, SD = 12.36) and non-cued limbs (M = 5.32 %, SD = 13.99), t(5) = 2.818, p = .037, r = .78. Unlike the simple selective stopping task, performing the task in a 2-choice paradigm resulted in the replication of previous neurophysiological findings, with results now reflecting proactive selective inhibition of the stop-cued hand (see Figure 4.3 light grey bars).

4.5. Discussion

Experiment 3.1 was designed to investigate the effect of go-related preparatory activation of a bimanual response on proactive selective inhibition. This was done using TMS and a SAS to probe the state of stop-cued and non-cued responses during the foreperiod and coincident with the go-signal in a simple selective stopping task. It was hypothesized that when provided with a relevant cue (i.e., MSR or MSL), there would be evidence of proactive inhibitory control in the form of a selective decrease in corticospinal excitability only in the stop-cued response limb, thus resulting in the early triggering only of the non-cued response by a SAS. While the simple selective stopping task revealed an overall increase in CE relative to a resting state (i.e., Null), in contrast to our hypothesis there was no difference in CE between stop-cued and non-cued responses. Despite this lack of neurophysiological evidence of proactive selective inhibition there was behavioural evidence of selective inhibition as indicated by the

small stopping interference effect observed during MSR and MSL cues compared to the MSX cue. In line with the neurophysiological results, inspection of the limited startle trials revealed that when a startle response was elicited, both responses (non-cued and stop-cued) were triggered early and in synchrony. Together the neurophysiological and SAS results indicate that the amount of preparatory activation for each response was similar.

To determine whether the incongruence between the neurophysiological and behavioural evidence for proactive selective inhibition observed in Experiment 3.1 (simple selective stopping task) was due to the use of a simple-RT paradigm, we conducted a control experiment (Experiment 3.2) that employed the typical *choice* selective stopping task. Similar to previous findings (Cai et al., 2011; Majid et al., 2012; Majid et al., 2013), behavioural and neurophysiological results from the choice selective stopping task were congruent and provided evidence of proactive selective inhibition, indicating that the null effect of the stop-cue on CE during the simple selective stopping task can likely be attributed to task differences related to advance preparation. Together the results suggest increased preparatory activation of the stop-cued and non-cued response overshadows proactive selective inhibition in the foreperiod of a simple selective stopping task despite retaining the ability to selectively inhibit the stop-cued response.

4.5.1. Inhibition during the selective stopping task

The current selective stopping task allowed us to examine the effect of advance preparation on both proactive selective inhibition (MSR & MSL) and reactive global inhibition (MSX). Proactive selective inhibition is thought to involve the basal ganglia and occur via the indirect pathway (striatum to globus pallidus pars externa [GPe] to globus pallidus pars interna [GPi] to thalamus) (Aron, 2011), whereas reactive global inhibition is thought to occur via the hyperdirect pathway (subthalamic nucleus to GPi to

thalamus). Unlike the ambiguous cue (MSX), the informative cues (MSR & MSL) provide advance information about which response might have to be stopped, allowing inhibition of that response limb to be specified in advance. Similar to previous selective stopping tasks performed in a choice-RT paradigm (Cai et al., 2011; Majid et al., 2012; Majid et al., 2013), our control experiment using a choice selective stopping task revealed neurophysiological and behavioural evidence of proactive selective inhibition. This was demonstrated by a decrease in CE only in the stop-cued limb and a small stopping interference effect during informative cues (Figure 4.3). In contrast, the neurophysiological and behavioural results from the simple selective stopping task were in disagreement. Similar to the choice selective stopping task and a previous simple selective stopping task (Aron & Verbruggen, 2008; Majid et al., 2012; Majid et al., 2013), informative cues during our simple selective stopping resulted in a small stopping interference effect providing behavioural evidence of proactive selective inhibition (Figure 4.2). Yet, despite the behavioural evidence for proactive selective inhibition we found no neurophysiological evidence of proactive selective inhibition during the simple selective stopping task. The results revealed increased but similar levels CE related to both the stop-cued and non-cued responses even though selective inhibition was preserved in behavioural results (see Figures 4.2 and 4.3). Given that the responses were known in advance of the go-signal in the simple selective stopping task, the increase in excitability relative to null in the foreperiod was expected (Kennefick, Maslovat, & Carlsen, 2014; Leuthold, Sommer, & Ulrich, 2004); however the lack of stop-cued response CE inhibition was not expected. The discrepancy in neurophysiological findings between the simple and choice selective stopping tasks provides novel insight into the interaction between initiation and inhibitory neural activation and their contributions to CE.

Analysis of CE modulation relative to Null during the performance of the choice selective stopping task revealed that CE was similar to the resting state (see Figure 4.3). This provides evidence that in preparation for a choice selective stopping trial there was a limited amount of excitability within the corticospinal tract. Given the limited amount of excitatory activation during the foreperiod, neurophysiological evidence of proactive selective inhibition is detectable as a small difference in MEP modulation between the stop-cued and non-cued responses. In contrast, analysis of MEP modulation relative to Null indicates that the corticospinal tract was in an elevated excitatory state during the foreperiod of the simple selective stopping task (Figure 4.3). Specifically, MEPs elicited during the simple selective stopping task were approximately 6x the size of those observed during the choice selective stopping task. As the only major difference between tasks was knowledge (or not) of the required response, this suggests that the increase in excitability is related to the ability to undertake advance response preparation. Together, the results suggest that the abundance of go-related preparatory activation contributing to CE during the simple selective stopping task led to an overshadowing of the relatively smaller effect of inhibitory activity on CE.

One major difference between the simple and choice versions of the selective stopping task is the capability for advance preparation of the response. That is, while a response can be readied in advance of the imperative stimulus in a simple-RT task, it is often not possible to do so in a choice RT task. The current data suggest that the task not only affects the preparatory state of the response, but also has implications related to proactive inhibition and what is being selectively inhibited. During the choice selective stopping task used in Experiment 3.2, evidence for proactive inhibition in the wrist extensor can only be described as selective inhibition of the stop-cued “limb” since the response direction (extension or flexion) was unknown prior to the go-signal. In contrast, during the simple

selective stopping task we found no evidence of proactive inhibition in the wrist extensor which is specific to the known "response."

While the CE data appear to indicate that participants were preparing both responses in advance of the go-signal during the simple selective stopping task, the SAS rarely elicited a startle reflex (8%) across all cue conditions, and those observed were smaller in size compared to those elicited during the simple-RT task. Of the 9 participants who had a reliable startle response in the simple RT Go Only task (i.e. SCM activation observed in >90% of SAS trials), a startle response was completely absent in 6 participants and the remaining 3 participants rarely were startled during the subsequent simple selective stopping task (8.6 % of SAS trials). A "simple" version of the selective stopping task was chosen to enable participants to prepare the single known response(s) in advance. Increased excitability within the motor system associated with response preparation has been shown to lead to both an increase in the probability of eliciting a startle reflex as well as an increase in startle magnitude (Carlsen, Chua, Inglis, Sanderson, & Franks, 2003; Carlsen et al., 2012; Valls-Solé, Valldeoriola, Tolosa, & Nobbe, 1997). Thus, the low proportion of SAS trials where a startle reflex was observed in Experiment 3.1 would suggest that participants were not preparing the response in advance. However, this conclusion is in contrast to TMS data which revealed an increase in excitability relative to Null during all cue conditions prior to the go-signal, suggesting that preparatory activation was indeed elevated.

The data indicating that participants were preparing the responses in advance during the simple selective stopping task suggests that the mechanism underlying the nonspecific suppression of the startle reflex is related to inhibitory control associated with the task. Nonspecific suppression of the startle reflex across all cue conditions indicates that activation of both the proactive and reactive inhibitory pathways in response to the cue also inhibits lower level brain areas related to the auditory

startle reflex. The auditory startle reflex pathway is a short serial circuit; neurons from the cochlear nucleus send their axons to the caudal reticular formation, with the giant neurons of the nucleus reticularis pontis caudalis (nRPC) acting as control neurons for the startle reflex with conductions to various levels of the spinal cord via the reticulo-spinal tract (Koch, 1999; Yeomans & Frankland, 1996). Given the simplicity of the startle reflex pathway, suppression of the startle reflex likely arises from inhibition of the reticular formation stemming from inhibitory control related to the performance of the simple selective stopping task. Acoustically evoked action potentials of the nRPC have been shown to be blocked by the inhibitory transmitter y-amino-butyric acid [GABA (Kungel, Ebert, Herbert, & Ostwald, 1994)], indicating that GABA exerts an inhibitory effect on the startle response. It is thus plausible that under both reactive and proactive inhibitory control, the release of GABA from the GPi not only inhibits the thalamus but also inhibits the reticular formation causing the suppression of the startle response. That said, it is hard to tell whether the inhibition of the startle response is a result of proactive and reactive inhibitory activation in general, or specific to the advance preparation that the simple version of the selective stopping task affords. Since the choice version of the selective stopping task limits preparation, the startle response would naturally habituate similar to traditional choice-RT task (Valls-Solé et al., 1997), thus limiting the contrast in the effect on the startle responses to the simple-RT task. Further investigation is warranted to determine the cause of startle response suppression.

Combining the neurophysiological, behavioural, and startle results together provides insight into the inhibitory control during the performance of a simple selective stopping task. The startle results suggest the presence of nonspecific inhibition prior to the go-signal regardless of mode of inhibition (proactive or reactive) due to the increase in preparation related to both responses. Once this nonspecific inhibition is lifted due to the voluntary initiation of the movements in response to the go-

signal, proactive or reactive inhibition remains to stop movements in response to the stop-signal. Because proactive inhibition only applies inhibition selectively to a single response the other can continue its initiation rather unaffected, resulting in the minimal stopping interference effect observed during the MSR and MSL cue conditions. The MSX cue likely employs a reactive mode of inhibition which would apply inhibition to both responses in reaction to the stop-signal then re-initiate the single continuing response resulting in the large stopping interference effect observed.

4.6. Conclusion

While there was a minimal stopping interference effect when using directional cues (MSR & MSL), proactive selective inhibition did not significantly supress CE in the stop-cued response during the foreperiod when responses were able to be prepared in advance. Despite the suppression of the startle response, the limited SAS triggered responses supported the neurophysiological data and also revealed no differences between stop-cued and non-cued responses. Results from a control experiment demonstrated the typical behavioural and neurophysiological markers of proactive selective inhibition when the task was completed in choice-RT type paradigm which limited advance preparation. We argue that these findings are most congruent with the explanation that the similar level of CE in the stop-cued and non-cued response during the simple selective stopping task is a result of the abundance of go-related preparatory activation overshadowing the minimal contribution of proactive selective inhibition on CE. Together, the results indicate that when performing a selective stopping task in a simple-RT paradigm both responses are prepared in advance to a similar degree, this preparatory activation washes out neurophysiological evidence of proactive selective inhibition but behavioural evidence of selective inhibition remains.

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5. Chapter 5

Offline cTBS over rIFG and preSMA impairs inhibition during a go/no-go task

A version of this chapter is currently under revision (stage: revise re-submit):

Drummond, N.M., Cressman, E.K., & Carlsen, A.N. (2016) Offline theta burst stimulation over right inferior frontal gyrus and pre-supplementary motor area impairs inhibition during a go/no-go task.

5.1. Abstract

In a typical go/no go task a single imperative stimulus is presented each trial, either a go or no-go stimulus. Participants are instructed to initiate a known response upon appearance of the go-signal and withhold the response if the no-go signal is presented. It is unclear whether the go-response is prepared in advance of the imperative stimulus in a go/no-go task. Moreover, it is unclear if inhibitory control processes suppress preparatory go-activation. The purpose of the present experiment was to 1) to determine whether the go-response is prepared in advance of stimulus identification with the use of a startling acoustic stimulus (SAS), and 2) investigate the inhibitory role of the rIFG and preSMA during the performance of a go/no-go task with the use of continuous theta burst stimulation (cTBS). The experiment consisted of three phases; a pre-cTBS phase in which participants completed a go/no-go and simple RT task, followed by offline cTBS to temporarily deactivate either rIFG or preSMA (with a sham control), then a post-cTBS phase which was identical to the pre-cTBS phase. Results revealed that stimulation to both cortical sites impaired participants' ability to withhold movements during no-go trials. Notably, rIFG or preSMA stimulation did not affect the latency of voluntary go responses and did not enable the SAS to involuntarily trigger responses. These findings suggest that preparation and initiation of the go-response occurs after the imperative stimulus, with the rIFG and preSMA involved in inhibiting the go-response once the stimulus is identified as a no-go signal.

5.2. Introduction

Donders' seminal paper (1969) showed that reaction time (RT) in a go/no-go task was faster than in a choice RT task but nevertheless slower than in a simple RT task. From these findings he concluded that during a go/no-go task the motor response was selected and prepared in advance, with the increase in RT from a simple RT task accounted for by the time required to identify the imperative stimulus and decide whether to go or not. Since the work of Donders, several other studies have confirmed the proposal that a response is prepared in advance of the imperative stimulus in go/no-go tasks (Danek & Mordkoff, 2011; Miller & Low, 2001).

A unique test of this hypothesis used a startling acoustic stimulus (SAS) to probe the preparatory state of the response (Carlsen et al., 2008). The presentation of a SAS has been shown to trigger the early release of the movement during simple RT tasks but not during choice RT tasks (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004), suggesting that a SAS has the ability to trigger movements involuntarily so long as they are sufficiently prepared. As such, it was hypothesized that startle would trigger the prepared movement during the go/no-go task. Contrary to the hypothesis, presentation of the SAS concurrent with the imperative stimulus had no effect on RT (i.e., did not lead to the early release of the movement), suggesting that the movement was in fact *not* prepared in advance during a go/no-go task, and thus response programming occurred after the go-signal similar to a choice RT task (Carlsen et al., 2008). However, an alternative interpretation of these results is that the movement was indeed prepared in advance, but the response could not be triggered by startle due to interference from inhibitory control processes put in place to ensure that the movement was not initiated before the stimulus was identified during a no-go trial.

Evidence of inhibitory control on "go" response-related preparatory activation during a go/no-go task has been shown using EEG and its associated measure known as the lateralized readiness potential (LRP) (Danek & Mordkoff, 2011). In brief, as movement onset approaches, the readiness potential becomes lateralized such that the electrode placed over the motor cortex contralateral to the response side becomes more negative than the electrode over the ipsilateral motor area. It has been suggested that the LRP can measure limb specific motor activation and can therefore be used to infer the amount of activation related to processes underlying movement preparation and initiation (Leuthold, Sommer, & Ulrich, 2004; Miller & Hackley, 1992). Interestingly, similar mean LRP amplitudes have been found between simple RT and go/no-go RT tasks prior to go-signal onset, indicating similar levels of limb specific advance preparation in the two tasks (Danek & Mordkoff, 2011). More importantly, for the question of interest, the mean LRP amplitude at the time of response initiation has consistently been shown to be greater for go/no-go task than the simple RT task (Mordkoff & Grosjean, 2001; Mordkoff, Miller, & Roch, 1996). To account for these findings, it has been suggested that there is a high state of motor preparedness in both the simple RT and go/no-go tasks during the foreperiod. The elevated initiation threshold seen in the go/no-go task is due to inhibitory control processes. These same inhibitory processes may be responsible for preventing the SAS from triggering the prepared response in the work by Carlsen and colleagues (2008).

Studies investigating the inhibitory control network in the human brain have repeatedly shown that both the right inferior frontal gyrus (rIFG) and pre-supplementary motor area (preSMA) are critical nodes for inhibitory control during stop-signal and go/no-go tasks (reviewed by Aron, 2011; Chambers, Garavan, & Bellgrove, 2009; Chikazoe, 2010; Levy & Wagner, 2011; Nachev, Kennard, & Husain, 2008). For example, neuroimaging studies consistently report activation within the rIFG, preSMA, and

subcortical circuitries involving thalamic regions and the striatum during successful response inhibition (for reviews see Chambers et al., 2009; Swick, Ashley, & Turken, 2011). Complementary to the imaging data, functional contributions from rIFG and preSMA to inhibitory control has been demonstrated with the use of non-invasive brain stimulation techniques, specifically transcranial magnetic stimulation (TMS). For instance, single pulse TMS over preSMA has been shown to impair inhibitory processing during the performance of a stop-signal task (Chen, Muggleton, Tzeng, Hung, & Juan, 2009; Obeso, Robles, Marron, & Redolar-Ripoll, 2013) and inhibitory repetitive TMS and inhibitory continuous theta burst stimulation (cTBS) over the rIFG have been shown to reduce inhibitory control during the performance of a stop-signal task (Chambers et al., 2007; Chambers et al., 2006; Obeso et al., 2013; Verbruggen, Aron, Stevens, & Chambers, 2010) and go/no-go task (Dambacher, Sack, Lobbestael, Arntz, Brugmann, et al., 2014). In addition, excitatory and inhibitory repetitive TMS of preSMA was able to improve and impair inhibitory performance respectively during the performance of a stop-signal task (Watanabe et al., 2015). Furthermore, transcranial direct current stimulation (tDCS) studies have shown that increasing rIFG or SMA/preSMA excitability (anodal stimulation) improved stopping performance, whereas decreasing preSMA excitability (cathodal stimulation) impaired stopping performance (Hsu et al., 2011; Jacobson, Javitt, & Lavidor, 2011).

While there is converging evidence that both rIFG and preSMA contribute to inhibitory control, the causal evidence for the involvement of both rIFG and preSMA in inhibition appears to be inconsistent between experiments and tasks. Therefore, the purpose of the current study was twofold: 1) to determine whether the go-response is prepared in advance in a go/no-go task and inhibited prior to stimulus identification with the use of a SAS, and 2) to investigate the inhibitory role of the rIFG and preSMA during the performance of a go/no-go task with the use of cTBS. The combination of cTBS and

SAS allows us to determine which cortical area(s) are responsible for inhibiting go-activation and preventing overt movement during the performance of a go/no-go task. If the response is prepared in advance, but unable to be involuntarily triggered by SAS due to inhibition influencing the initiation processes, then impairing this inhibition through cTBS may allow for the involuntary release of the response by SAS.

The experiment consisted of three phases; a pre-cTBS phase where participants completed a go/no-go and simple RT task, followed by offline continuous theta burst stimulation (cTBS) to temporarily deactivate either rIFG or preSMA (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005) (with a sham control), then a post-cTBS phase which was identical to the pre-cTBS. It was hypothesized that in the pre-cTBS phase, the presentation of a SAS concurrent with the stimulus would not result in the early triggering of the prepared movement. However, if rIFG and/or preSMA is involved in inhibiting the motor system and consequently preventing the SAS from triggering the prepared response, then their deactivation should increase the proportion of failed-stop responses (go during a no-go trial) and enable the SAS to involuntarily trigger the response in the post-cTBS phase.

5.3. Materials and Methods

5.3.1. Participants

Data were collected from 13 healthy participants (6M, 7F; *M age* = 26, *SD* = 3) with normal or corrected to normal vision, and no history of neurological, sensory, or motor disorders. All participants completed three separate sessions separated by a minimum of 24 hrs to allow for complete washout of the effects of active stimulation (Huang et al., 2005). All participants provided written informed consent, passed a transcranial magnetic stimulation (TMS) safety-screening questionnaire, and verbally reported no drug contraindications for repetitive TMS prior to testing. The study was conducted in accordance

with the ethical guidelines set by the University of Ottawa Research Ethics Board and conformed to the most recent version of the Declaration of Helsinki.

5.3.2. Experimental set-up

Participants sat facing a 24-inch LCD computer monitor with their right arm placed in a custom made force measurement device. The arm was supported and secured in a neutral position (semi-pronated with the palm perpendicular to the floor) such that the dorsal surface of the hand was in contact with a solid plate, restricting movement to concentric wrist flexion and isometric wrist extension. The plate was attached to a force transducer (Nano25 FT, ATI Industrial Automation, NC, USA) allowing measurement of force produced during an isometric wrist extension.

5.3.3. Tasks

In the pre-cTBS and post-cTBS phases, participants completed a go/no-go task followed by a simple RT task. To start a go/no-go trial, a red cue was presented (a red plus-sign in the middle of a black screen) indicating to participants to get ready, while not exerting any force against the force transducer. Following a variable foreperiod (1.5-3 s), the imperative stimulus, white O (go) or white X (no-go), replaced the warning cue. Participants were instructed to respond by performing an isometric right wrist extension movement [target force = 40% of maximum voluntary force (MVF)] as fast as possible if a go-signal (O) appeared, or to refrain from making the movement if a no-go signal (X) appeared (see Figure 5.1A). Participants received 25 points for responding correctly (i.e., responding to the “O” and not responding to the “X”) and responding quickly (1 point for every 7 ms below 350 ms, up to a maximum of 25 points). Participants were penalized 25 points for an incorrect response or a go-RT exceeding 500 ms.

Unlike the go/no-go task, the simple RT task started with the presentation of a white cue (instead of red) and consisted only of go trials. Participants were instructed that the cue would always be followed by a white O (go) and that they were to respond as fast as possible when the go-signal appeared. Similar to the go/no-go task, participants received 25 points for responding correctly (i.e., responding to the "O") and responding quickly (1 point for every 1 ms below 200 ms, up to a maximum of 25 points). All other task procedures were identical to the go/no-go task.

Similar to Carlsen and colleagues (2008), an acoustic stimulus was presented on all trials concurrently with the imperative stimulus via a loudspeaker placed behind the participants head with an intensity of either 82 dB (Control trial: 1000 Hz, 25 ms) or 120 dB (SAS trial: white noise, 25 ms)(see the startling acoustic section below for details). Participants were told that on some trials they would hear a loud "static noise" sound instead of the usual tone, but should continue as instructed. After each trial peak force, constant error with respect to % MVF, and reaction time feedback was displayed on the monitor for 3.5 seconds.

5.3.4. Procedure

Prior to behavioural testing in each session, participants' ECR hotspot, maximum voluntary isometric extension force, and active motor threshold were determined (see TMS and offline cTBS section below for details).

Participants completed a brief practice block of the go/no-go task to familiarize them with the task and timeline of a trial. Following this, the 3 main phases of the experiment were completed in a serial order: pre-cTBS, offline cTBS, and post-cTBS (see Figure 5.1B). In the pre-cTBS phase, participants completed 80 trials of the go/no-go task (60 go & 20 no-go trials) followed by 20 trials of the simple RT task. Upon completion of the pre-cTBS phase, participants received cTBS applied either to the rIFG or

preSMA, or they received sham stimulation (see Offline cTBS section below for details). 10 mins following the completion of the cTBS (to allow time for maximum suppression (Huang et al., 2005)) participants performed the post-cTBS phase which was identical to the pre-cTBS phase. The pre-cTBS and post-cTBS phases took approximately 25 mins each to complete.

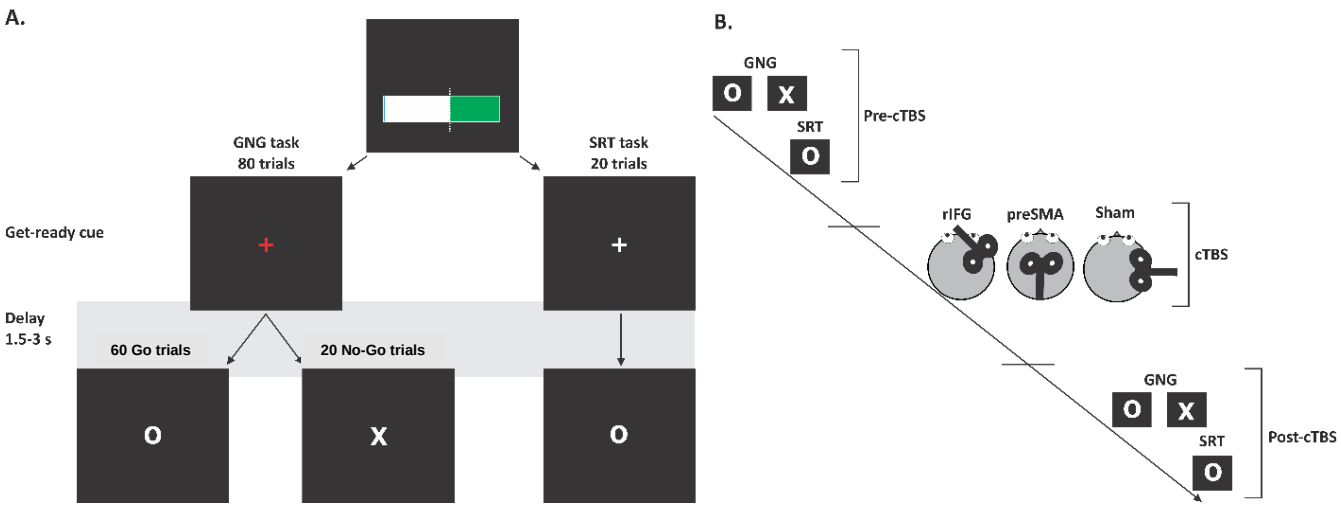

Figure 5.1 A. Schematic representation of the time course of the go/no-go (GNG) and simple reaction time (SRT) tasks. Top panel depicts pre-trial real-time force (blue) feedback (0 % = far left no force) and indicates the target 40% MVF force (dashed line at the beginning of the green area). The get-ready cue was either a red (go/no-go task) or white (simple reaction time task) cross and was presented centrally on the monitor. Following a delay of 1.5-3 s, a white imperative stimulus replaced the get-ready cue indicating either a go (O) or no-go (X) trial. A control (82 dB) or startle (120 dB) auditory stimulus was presented concurrently with the imperative stimulus every trial. **B.** Schematic representation of the experimental procedure depicting the time course of the three experimental testing phases (pre-cTBS, cTBS, & post-cTBS). During the pre & post-cTBS phases participants completed the GNG task followed by the SRT task. Each cTBS session consisted of stimulating one of three stimulation sites with continuous theta burst stimulation [right inferior frontal gyrus (rIFG), pre-supplementary motor area (preSMA) or no neural stimulation (Sham)].

5.3.5. Transcranial Magnetic Stimulation (TMS) & Offline Continuous Theta Burst Stimulation (cTBS)

To determine the location of participant's motor cortex hotspot and active motor threshold (AMT), single pulse TMS was delivered using a MagStim 200^2 system (MagStim Inc.) via a 70 mm figure of eight coil. The approximate location of the ECR was first determined by finding Cz (based on the international 10-20 system for EEG electrode placement), then measuring 4.7 cm lateral and 1.1 cm anterior. From this initial position, suprathreshold TMS was systematically delivered to the surrounding area to determine the location that elicited the largest MEP in the ECR (i.e., the "hotspot"). This location was tracked and saved using an image guided TMS NeuroNavigation system (ANT visor2), which allowed precise, reliable and consistent TMS targeting. Once the ECR hotspot was found, participants then completed a maximal voluntary force (MVF) test, which consisted of 3 MVF trials, with 1 minute of recovery between trials (Sahaly, Vandewalle, Driss, & Monod, 2001). On each trial participants were instructed and encouraged to extend their wrist and push as forcefully as possible for 3 seconds against the plate with the back of their hand. MVF was then determined by calculating the average peak force from the three trials. Participants' active motor threshold (AMT) was then determined in order to establish the stimulator output for cTBS (80% of AMT). AMT was defined as the minimum stimulator output that produced a MEP greater than 200 μV on more than five out of ten pulses while the participant maintained a voluntary contraction of 20% of their MVF (Huang et al., 2005).

Within each of the three sessions, participants received offline cTBS to one of three locations; right inferior frontal gyrus (rIFG), pre-supplementary motor area (preSMA), or Sham (see Figure 5.1B). cTBS was delivered using the Magstim Super Rapid^2 system (Magstim Inc.) with a 70mm air film figure of eight coil. Each train of cTBS consisted of three pulses at 50 Hz, with every cTBS burst repeated at a 5 Hz

rate resulting in a total of 600 individual pulses over 40 seconds at 80% of the participant's AMT (Wassermann et al., 1996). These stimulation parameters have been shown to result in a long term depression (LTP) like decrease in excitability for a period of up to 40 minutes in the stimulated neurons (Huang et al., 2005). The mean stimulator output across cTBS sessions was 22% (*SD* = 4), ranging from 16% - 30% across participants but only varied an average of 1% between sessions for a particular participant. For rIFG stimulation the coil was centered on F8 (localized according to the international 10-20 system), with the handle in an upward vertical orientation (Asahi, Okamoto, Okada, Yamawaki, & Yokota, 2004; Dambacher, Sack, Lobbestael, Arntz, Brugman, et al., 2014). For preSMA stimulation the coil was centered 4 cm anterior to Cz, with the handle pointing caudally (Mars et al., 2009; Rushworth, Hadland, Paus, & Sipila, 2002). For sham stimulation, the coil was tilted 90° over the right motor cortex to mimic the sound of pulses with no neural stimulation. Stimulation order was counterbalanced across participants. Upon completion of the last session participants completed a debriefing form notifying them of the deception regarding the sham stimulation session, with participants verbally reporting not being aware of which session consisted of "real" or "fake" stimulation. No adverse events or effects from either TMS or cTBS were reported.

5.3.6. Starling Acoustic Stimulus (SAS)

A SAS was sometimes presented concurrent with the imperative stimulus during the pre-cTBS and post-cTBS phases. The SAS was a 120 dB, 25 ms duration, white noise waveform (with equal power from 1Hz to 22 kHz), which was delivered via a loudspeaker (MG Electronics M58-H, rise time < 1 ms) located behind the participant's head. Stimulus intensity and frequency content was confirmed using a precision sound level meter located at the same distance from the loudspeaker to the ears (30 cm, Cirrus Research Optimus CR: 162C, A - weighted). In each phase of the go/no-go task the SAS was

presented on 16 out of the 80 trials, equally distributed between go and no-go trials (SAS: 8 go & 8 no-go). During the simple RT task, a SAS was presented in 5 of the 20 trials. The SAS was pseudorandomly presented, such that a SAS did not occur in the first two trials of a task and 2 SAS trials were never presented consecutively.

5.3.7. Recording Equipment/ Data Reduction

Surface electromyographic (EMG) data were collected from the muscle bellies of the right extensor carpi radialis (ECR), and left sternocleidomastoid (SCM), using bipolar pre-amplified (gain=10) surface electrodes (Delsys Bagnoli DE-3.1) connected via shielded cabling to an external amplifier system (Delsys Bagnoli-8). The EMG recording sites were prepared and cleansed in order to decrease electrical impedance. The electrodes were placed parallel to the muscle fibers, and attached using double sided adhesive strips. The reference electrode was placed on the participant's left lateral epicondyle.

Isometric wrist extension force data were collected using a force transducer (Nano25, ATI Industrial Automation, Inc., Apex, North Carolina USA) placed between the dorsal side of the hand and the plate. On each trial, unfiltered EMG and force data were digitally sampled at 4 kHz (National Instruments PCIe-6321 via BNC-2090A) for 3 seconds using a customized program written with LabVIEW software (National Instruments Inc.) and stored for offline analysis.

5.3.8. Data reduction

Force onset was defined as the point at which force output exceeded two standard deviations above baseline levels (mean force 100 ms prior to the go-signal onset). Reaction time was defined as the interval of time between the go-signal and force onset. If reaction time was less than 50 ms it was considered an error due to anticipation and discarded (3% of total go-trials). Peak force was defined as the highest observed value on a given trial. A force exceeding 5% of maximum voluntary isometric force

(MVF) was classified as an initiated go-response. If force did not exceed 5% of MVF it was classified as a full-stop response.

EMG data collected on all trials from the ECR (agonist) and SCM were analyzed for muscle burst onset. EMG burst onsets were defined as the point in time at which the rectified and filtered (25 Hz low pass 2nd order elliptic filter) EMG signal first began a sustained (>20 ms) rise that exceeded two standard deviations above baseline levels (mean EMG activity 100 ms prior to go-signal onset).

To distinguish startle response related SCM activity from other SCM activity when the SAS was presented coincident with the go-signal, SCM onset had to occur within a time window between 20-150 ms following SAS onset (Kumru et al., 2006). For a participant's startle data from the go/no-go task to be included in the analysis of SAS trial performance, they had to consistently exhibit a startle response (>50%) in the pre-cTBS simple RT task across all three sessions. This resulted in the exclusion of three participants. Thus analyses of SAS results consisted of data from ten participants, whereas all other analyses included data from all thirteen participants. Similar to Carlsen et al (Carlsen et al., 2008), only SAS trials which elicited a startle response were analyzed since the presence of an early SCM response is able to distinguish between stimulus intensity and startle triggered responses (total SAS trials discarded: rIFG = 139/320, preSMA = 128/320, Sham = 139/320).

5.3.9. Statistical Analyses

5.3.9.1. Control trial performance

Data from control trials which did not include the presentation of a SAS were first analyzed. Variables of interest were premotor RT and the number of failed-stop responses. Data was computed to reflect a change in performance in the post-cTBS phase relative to the pre-cTBS phase to normalize

performance across days and isolate the effect of cTBS stimulation. To determine whether stimulation altered the latency of voluntary responses, Δ premotor RT during control go trials was analyzed using a 2 task (simple-RT, go/no-go) x 3 stimulation location (rIFG, preSMA, Sham) repeated measures analysis of variance (RM ANOVA). To investigate whether stimulation had an effect on inhibitory control, Δ in the number failed-stop responses during control no-go trials was analyzed using a 3 stimulation location (rIFG, preSMA, Sham) RM ANOVA.

5.3.9.2. SAS trial performance

Analysis of SAS trial performance consisted of data from the subset of ten participants who consistently exhibited a startle response. In order to perform a factorial analysis, values for missing cells were filled using a linear regression-based multiple imputation procedure in SPSS. The number of imputed values per analysis is indicated below.

To determine whether stimulation altered RT or any response speeding effect of the SAS during the performance of a simple-RT task, go trial premotor RT was analyzed using a 2 tone (control, SAS) x 3 stimulation location (rIFG, preSMA, Sham) x 2 time (pre-cTBS, post-cTBS) RM ANOVA (imputed values = 1/120). To investigate whether cTBS stimulation led to a change in the proportion of trials in which a SAS was able to involuntary trigger the response, premotor RT was analyzed using a 2 tone (control, SAS) x 3 stimulation location (rIFG, preSMA, Sham) x 2 time (pre-cTBS, post-cTBS) RM ANOVA (imputed values = 8/120). In addition, the change in the number of failed-stop responses pre- to post-cTBS (Δ failed-stop response) during SAS no-go trials was analyzed using a 3 stimulation location (rIFG, preSMA, Sham) RM ANOVA (imputed values = 8/60).

All analyses were performed using the statistical software package IBM SPSS 21.0 for Windows (IBM Inc., Armonk, NY). Statistical significance was set at an alpha less than or equal to .05. To determine to locus of significant main effects, Bonferroni corrected post-hoc tests were conducted where appropriate.

5.4. Results

5.4.1. Control trial performance

Analyses of Δ in premotor RT revealed a significant main effect of task, $F(1,12) = 10.022$, $p = .008$, $\eta^2_p = .455$, but no main effect of stimulation, $F(2,24) = .067$, $p = .935$, $\eta^2_p = .006$, or significant interaction, $F(2,24) = .203$, $p = .818$, $\eta^2_p = .017$. The results indicate that the decrease in premotor RT post-cTBS relative to pre-cTBS was larger during the go/no-go task (*M* = 14.5 ms, *SD* = 19.7) compared to the simple RT task (*M* = 2.9 ms, *SD* = 12.5), however there was no difference between stimulation conditions indicating that cTBS did not affect the latency of voluntary responses.

Analyses of the Δ in failed-stop responses revealed a significant main effect of stimulation condition (see Figure 5.2), $F(2,24) = 8.604$, $p = .002$, $\eta^2_p = .418$. Post-hoc tests revealed a significant increase in the number of failed-stop responses following rIFG stimulation (*M* = +.69 failed-stop responses, *SD* = 1.70) compared to Sham stimulation (*M* = -.69 failed-stop responses, *SD* = 1.8), $t(12) = 2.840$, $p = .015$, and a significant increase in the number of failed-stop responses following preSMA stimulation (+1.62 failed-stop responses, *SD* = 1.71) and Sham stimulation, $t(12) = 4.308$, $p = .001$, but no difference between rIFG and preSMA stimulation, $t(12) = 1.431$, $p = .178$. The change in failed-stop responses represents an increase of 19% and 65% following rIFG and preSMA cTBS respectively, and a decrease of 16% following Sham cTBS [(Δ/pre-cTBS)*100].

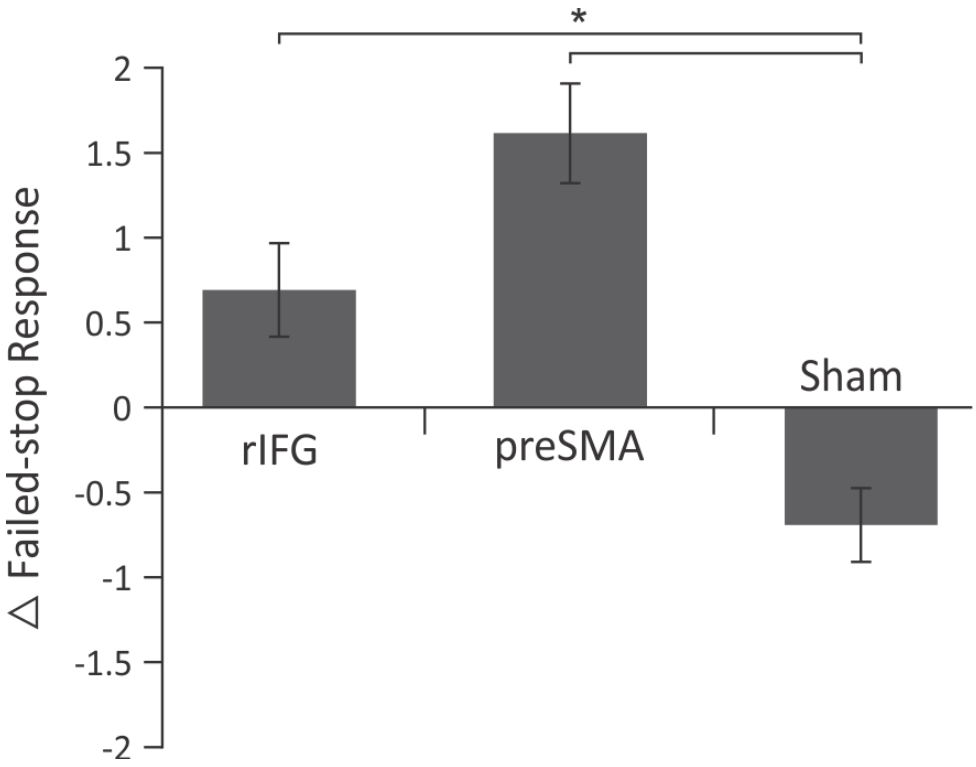

Figure 5.2. Mean change in the number of failed-stop responses (Δ failed-stop response) during control no-go trials as a function of stimulation location. A positive change indicates an increase in the number of failed-stop responses post-cTBS, and a negative change indicates a reduction in the number of failed-stop responses post-cTBS. Right inferior frontal gyrus = rIFG, pre-supplementary motor area = preSMA, no neural stimulation = Sham. Error bars on group mean data represent within-subject 95% confidence intervals.

5.4.2. SAS trial performance

In the simple RT task, analysis of premotor RT revealed a significant main effect of tone, $F(1,9) = 100.901$, $p < .001$, $\eta^2_p = .918$, no main effect of stimulation location, $F(2,18) = 3.016$, $p = .074$, $\eta^2_p = .251$, no main effect of time, $F(1,9) = .604$, $p = .457$, $\eta^2_p = .063$, and no significant interactions (all *p values* >.27). Specifically, results show that premotor RT during SAS trials (*M* = 92.70 ms, *SD* = 14.30) was significantly faster than premotor RT during control tone trials (*M* = 146.3 ms, *SD* = 23.8), but that these differences were not affected by stimulation condition or time (see Figure 5.3, squares).

For the go/no-go task, analysis of premotor RT revealed no significant main effect of tone, $F(1,9) = .446$, $p = .521$, $\eta^2_p = .047$, stimulation location, $F(2,18) = 1.329$, $p = .289$, $\eta^2_p = .129$, or time, $F(1,9) = 1.427$, $p = .263$, $\eta^2_p = .137$, nor any significant interactions (all *p values* >.4). Thus, the latency of responses initiated during SAS trials (*M* = 271.70 ms, *SD* = 155.80) was no different than during control trials (*M* = 256.30 ms, *SD* = 39.70), regardless of stimulation (see Figure 5.3, circles).

Analyses of Δ in failed-stop responses during SAS no-go trials revealed no significant main effect of stimulation location (see Figure 5.4), $F(2,18) = .938$, $p = .410$, $\eta^2_p = .094$. Thus, results indicate that stimulation did not have an effect on the ability to withhold a response when a SAS was presented (rIFG: *M* = .1, *SD* = 1.6; preSMA: *M* = -1.10, *SD* = 1.79; sham: *M* = -.80, *SD* = 2.35).

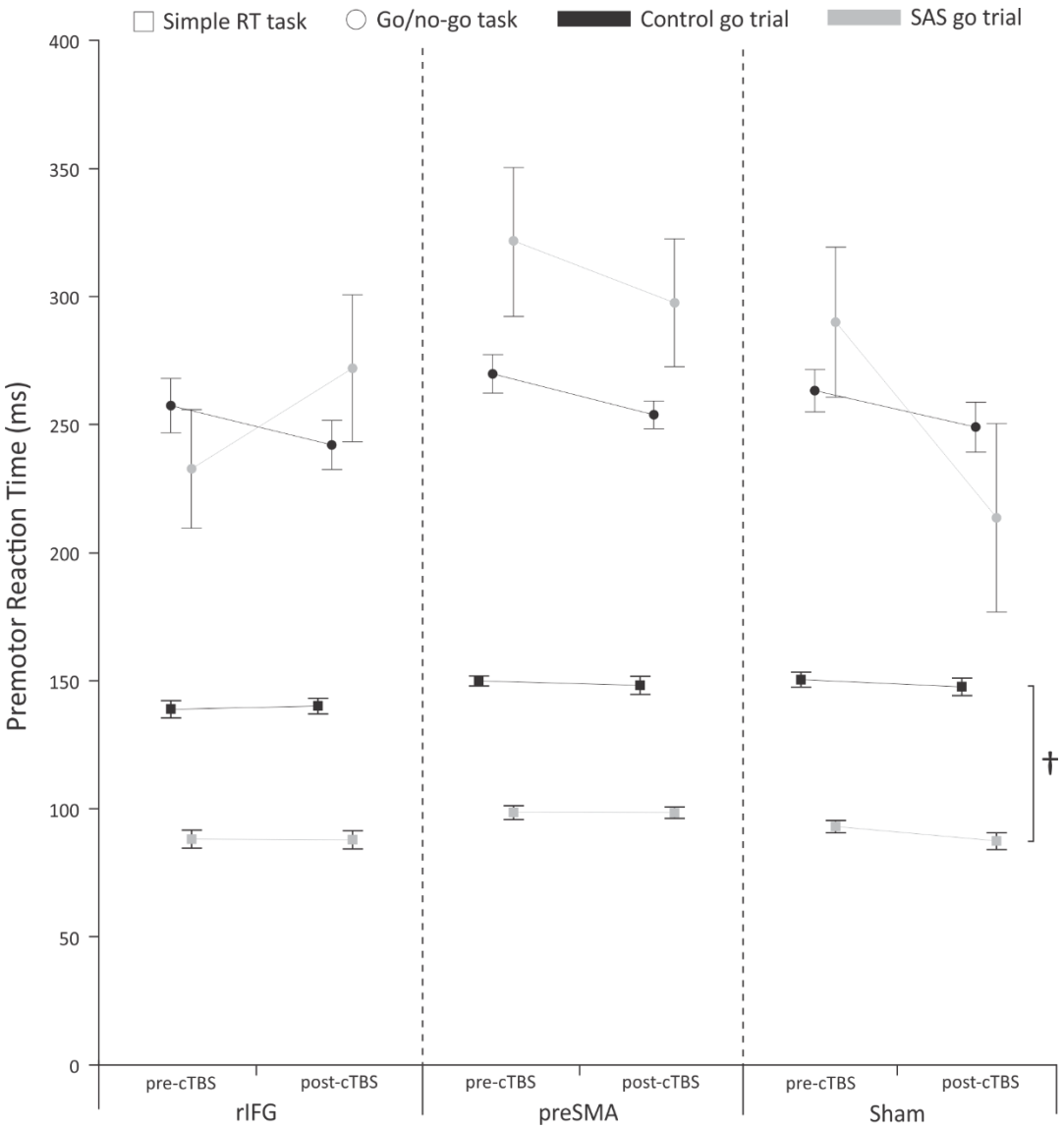

Figure 5.3. Mean control and SAS go trial premotor reaction time during the simple- RT task and go/no-go task as a function of stimulation location. Shapes denote task (simple-RT = square, go/no-go = circle) and shades denote tone (control = black, SAS = grey). Right inferior frontal gyrus = rIFG, pre-supplementary motor area = preSMA, no neural stimulation = Sham. Error bars on group mean data represent within-subject 95% confidence intervals calculated for each task. † = significant main effect of tone (control vs. SAS) only in the simple RT task.

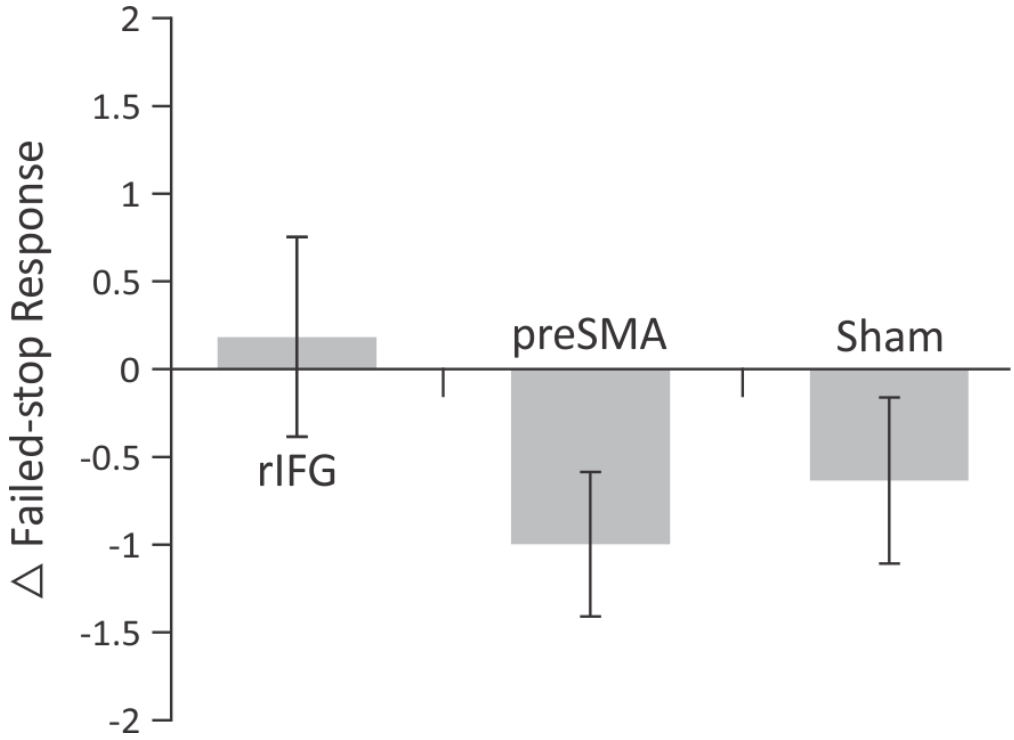

Figure 5.4. Mean change in the number of failed-stop responses (Δ failed-stop response) during SAS no-go trials as a function of stimulation location. A positive change indicates an increase in the number of failed-stop SAS responses post-cTBS, and a negative change indicates a reduction in the number of failed-stop SAS responses post-cTBS. Right inferior frontal gyrus = rIFG, pre-supplementary motor area = preSMA, no neural stimulation = Sham. Error bars on group mean data represent within-subject 95% confidence intervals.

5.5. Discussion

The present experiment systematically investigated the role of rIFG and preSMA in response inhibition during a go/no-go task. In addition, a SAS was presented prior to and after the temporary (~40 min) decrease in rIFG and preSMA activation via cTBS to investigate the preparatory state of the go-response. Based on converging evidence demonstrating the role of rIFG and preSMA in response inhibition (Aron, 2011), it was hypothesized that applying cTBS over the rIFG and/or preSMA would disrupt inhibitory control and impair participants' ability to withhold responses during no-go trials. Furthermore, if inhibition applied to go-activation in advance of the imperative stimulus prevents the involuntary triggering of the prepared go-response by SAS, then disrupting or reducing inhibition was expected to lead to the release of the go-response by SAS. Analysis of Δ in failed-stop responses during control no-go trials revealed that cTBS applied over the rIFG and preSMA decreased participants' ability to voluntarily stop their responses (i.e. not respond) during no-go trials, whereas following Sham stimulation, participants were better at not responding during no-go trials. Furthermore, the Δ in premotor RT did not differ between Sham, rIFG, and preSMA cTBS conditions. Thus, the observed impairment in participant's ability to withhold movements during control no-go trials following cTBS over rIFG and preSMA cannot be attributed to a speeding in response initiation. Together, results during control trial performance suggest that cTBS applied over rIFG or preSMA was effective at disrupting inhibitory control during no-go trials while having no effect on the latency of voluntary response initiation during go trials.

Having established the effect of rIFG and preSMA cTBS on behaviour, we next looked to determine whether the disruption or impairment in response inhibition would enable the release of the go-response by SAS. In line with the results reported by Carlsen and colleagues (2008), pre-cTBS, SAS go

trial premotor RT in the go/no-go task was no different than control go trial premotor RT, indicating that responses were not involuntarily triggered early by the SAS. In contrast to our hypothesis, SAS go trial premotor RT post-cTBS was no different than control go trial premotor RT, regardless of cTBS locations. Furthermore, the Δ in failed-stop responses during SAS no-go trials revealed no difference between Sham, rIFG, or preSMA cTBS. Together, results from SAS trial performance suggest that the SAS was not able to trigger the involuntary release of the go-response even after cTBS to the rIFG and preSMA. In contrast to the go/no-go task, the results from the simple RT task revealed that SAS responses were significantly faster than control responses. However, the latency of control and SAS responses in the simple RT task were also unaffected by cTBS. This suggests that cTBS applied over rIFG and preSMA did not affect the ability of SAS to involuntarily trigger the prepared response within a given task. Together, the results indicate that rIFG and preSMA contribute to inhibitory control during the performance of the go/no-go task, and suggest that the go-response is not prepared in advance during a go/no-go task. These findings provide novel insight into the neural mechanism for behavioural control during the go/no-go task.

5.5.1. Inhibitory role of rIFG and preSMA during a go/no-go task

Offline cTBS to rIFG and preSMA was found to increase the amount of failed-stop responses during no-go trials compared to Sham stimulation, which decreased the amount of failed-stop responses (Figure 5.2). This result is in line with a vast body of neuroimaging literature highlighting the important role of both rIFG and preSMA in response inhibition (for reviews see Aron, 2011; Stuphorn & Emeric, 2012; Swick et al., 2011). Having found the predicted change in response inhibition induced by cTBS over rIFG and preSMA suggests that the present study was successful in disruption of neural activity in these two cortical regions. The activation of these two brain areas has been observed during the performance

of several different inhibitory tasks, including stop-signal paradigms (Chambers et al., 2009; Swick et al., 2011), antisaccade tasks (Chikazoe, Konishi, Asari, Jimura, & Miyashita, 2007) and go/no-go tasks (Dambacher, Sack, Lobbestael, Arntz, Brugman, et al., 2014; Simmonds, Pekar, & Mostofsky, 2008; Swick et al., 2011). Complementary to the findings from neuroimaging, non-invasive brain stimulation techniques such as TMS (Chambers et al., 2007; Chambers et al., 2006; Chen et al., 2009), tDCS (Hsu et al., 2011; Jacobson et al., 2011), and cTBS (Dambacher, Sack, Lobbestael, Arntz, Brugmann, et al., 2014; Obeso et al., 2013; Verbruggen et al., 2010) have shown that altering neural activation within the rIFG or preSMA can affect inhibitory control in a positive or negative manner. Despite the abundant evidence for the involvement of preSMA in the successful inhibition of motor responses, a previous study employing cTBS to non-invasively decrease activation in preSMA during a go/no-go task failed to modulate inhibition (Dambacher, Sack, Lobbestael, Arntz, Brugmann, et al., 2014). Specifically, Dambacher et al. (2014) showed that cTBS delivered to the right inferior frontal cortex (specifically the anterior insula) decreased the ability for participants to restrain responses during a go/no-go task, but cTBS delivered to the preSMA did not have any effect on inhibition. The authors attributed the null effect of preSMA stimulation to the fact that the individual target site for stimulation localized with fMRI were significantly deeper than the TMS could actually reach. Thus, the present study is the first cTBS study to show the involvement of both rIFG and preSMA for the inhibition of movements during the performance of a go/no-go task.

5.5.2. Preparation and initiation during a go/no-go task

In a previous study by Carlsen and colleagues (2008), the presentation of a SAS concurrent with the imperative stimulus of a go/no-go task did not result in involuntarily and early triggering of the response, leading the authors to suggest that the go-response was not prepared in advance during the

go/no-go task. However, EEG data has shown a similar level of motor activation preparation prior to the imperative stimulus in both simple RT and go/no-go tasks, providing evidence for advance motor preparation during a go/no-go task. Thus, as opposed to an absence of preparation in the go/no-go task, it is possible that the go-response is prepared in advance, but inhibitory control supresses the initiation process until the stimulus is identified (go stimulus = release brake; no-go stimulus = keep brakes on), thus preventing the involuntary triggering of the response by SAS.

Similar to Carlsen et al (2008), presentation of the SAS in the pre-cTBS phase did not significantly reduce RT compared to control trials (Figure 5.3), suggesting that the response was not reliably triggered involuntarily by the SAS. Despite cTBS applied to rIFG and preSMA decreasing participants' ability to inhibit response initiation during no-go trials (Figure 5.2), presentation of the SAS post-cTBS again resulted in RTs akin to voluntary control RTs. Specifically, there was no change in RT post-cTBS relative to pre-cTBS, or the number of failed-stop responses during SAS trials (Figures 5.3 & 5.4). Therefore, contrary to the proposal that inhibition suppresses go-activation, preventing the involuntary release of the prepared response with SAS, the lack of early response triggering observed in the current experiment following the disruption of inhibition control by cTBS provides further evidence that the go-response is not prepared in advance in a go/no-go task.

Based on the present findings, it appears that go-activation only increases following the imperative stimulus, irrespective of the stimulus type (i.e., go or no-go) for future response output. Evidence of go-activation increasing following the imperative stimulus during a go/no-go task has previously been shown in a study which recorded local field potentials from cortical regions of monkeys (Ledberg, Bressler, Ding, Coppola, & Nakamura, 2007). Results demonstrated that potentials began to increase in cortical motor areas approximately 50 ms following the onset of the imperative stimulus in

both go and no-go trials. In addition, activity only began to predict response output (go or no-go) approximately 150 ms following stimulus onset in a distributed network that included parietal and frontal regions (Ledberg et al., 2007). In humans, a similar time for making a "decision" in a visual categorization task has been reported (Thorpe & Fabre-Thorpe, 2001; VanRullen & Thorpe, 2001). Together the results suggest that following the imperative stimulus, stimulus identification and response execution (including response preparation and initiation) processes occur in parallel. Following identification of a no-go signal the inhibitory control network is activated, likely propagating through preSMA, rIFG, and BG to stop the inappropriate initiation of the response. As such, a disruption to inhibitory control would only affect the ability to prevent the initiation of the response during no-go trials, a finding supported by results in the current study showing an increase in failed-stop responses following cTBS delivered to rIFG and preSMA. Our results further suggest that once the no-go stimulus is identified, a successful no-go response may be achieved by inhibition supressing and potently driving go-activation down and away from response threshold resulting the response being successfully withheld.

Given that the latency of simple RT SAS responses and control go-trial responses during both tasks were unaffected by cTBS applied to rIFG and preSMA, our results suggest that preparatory and initiation processes were unaltered. Therefore, the decreased ability to prevent responses during no-go trials following stimulation can be attributed to an impairment in the ability of inhibition to prevent go-activation from reaching initiation threshold. This impairment could be achieved in two ways; 1) delay in the onset of inhibition which would reduce or have no effect on the latency of failed-stop responses post-cTBS, or 2) reduction in the strength of inhibition which would delay the latency of failed-stop responses post-cTBS. Inspection of the data revealed that the latency of failed-stop responses following active cTBS was reduced (Δ -29 ms), however this reduction was not significantly difference (p >.05) than

that observed following Sham cTBS (Δ -14 ms). While the present study was not designed to address the neural mechanism governing the cTBS induced impairment in inhibition, the results suggest that the decrease in inhibitory control induced by cTBS to rIFG and preSMA may be a result of a delay in the onset of inhibition on go-activation.

The results of the current study provide further support for neural models proposing that response outcome is governed by the interaction between initiation and inhibitory processes as opposed to a parallel race between the two processes (Boucher et al., 2007; Dunovan et al., 2015). Although these interactive neural models are based upon stop-signal task data, given the current results and the high degree of overlap in the neural networks active during the performance of the go/no-go task and stop-signal tasks, the neural processes underlying inhibitory control during the stop-signal task can be applied to the processes underlying performance in a go/no-go task. It is important to note that the effects observed in the present study were achieved without the use of MRI guided stimulation which is often used when targeting cortical inhibitory network sites (e.g., Dambacher, Sack, Lobbestael, Arntz, Brugman, et al., 2014; Obeso et al., 2013). Regardless of this limitation, the present findings suggest that the surface locations used to target rIFG and preSMA were appropriate and able to induce the predicted changes in inhibitory control.

5.6. Conclusion

Offline cTBS to rIFG and preSMA were found to increase the amount of failed-stop responses during no-go trials compared to a Sham condition, which decreased the amount of failed-stop responses. No change in response latency (RT) was found across all stimulation conditions. These findings add to the literature implicating the rIFG and preSMA as important cortical nodes for response inhibition but not initiation. We propose that the decrease in inhibitory control induced by cTBS to rIFG

and preSMA may be a result of a delay in the onset of inhibition on go-activation. Despite a decrease in stopping ability, stimulation had no effect on voluntary initiation process as indicated by the unaltered latency of control go responses, and no effect on the ability of SAS to involuntary trigger responses. The results indicate that the go-response is not prepared in advance of the imperative stimulus and that rIFG and preSMA appear to only contribute to the inhibitory control process. Together, the findings suggest that following the imperative stimulus, response execution (including response preparation and initiation) and stimulus identification processes occur in parallel, with the identification of a no-go signal recruiting the inhibitory control network likely propagating through preSMA and rIFG to stop the inappropriate initiation of the response.

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6. Chapter 6 General Discussion

Humans must be able to adapt their movements to interact with a constantly changing environment. Thus, the ability to stop an ongoing movement or prevent unwanted movements from being initiated is undoubtedly an important aspect of behavioural control. With the use of laboratory paradigms such as the stop-signal task and the go/no-go task, researchers have been able to identify inhibitory control pathways and important neural structures involved in the ability to reactively inhibit a movement and the ability to proactively inhibit a movement (Aron, 2011; Stuphorn & Emeric, 2012). Reactive inhibition has been described as a late correction mechanism, relying upon the detection of a stop-signal which results a quick but non-focal method of inhibiting motor output (Braver, 2012). In contrast, proactive inhibition uses task-relevant information to bias the motor system prior to performing the movement, resulting in a slow but focal method of inhibiting a specific motor output (Braver, 2012). Although many advances have been made in understanding the neural pathways and circuitry related to both forms of inhibitory control, it remains unclear how inhibitory processes compete or interact with initiation processes to determine movement outcome (i.e., determine if the movement is initiated or inhibited). Specifically, previous investigations into stopping behaviour have seldom considered the influence of preparation and initiation on inhibitory control. The studies included in this thesis were designed to investigate the processes underlying movement inhibition when humans are preparing to initiate a motor response with the possibility of having to inhibit that response. Unique to the studies presented in the thesis is the use of a startling acoustic stimulus (SAS) to investigate inhibitory control. Given the ability of a SAS to involuntarily trigger responses which are sufficiently prepared (i.e., have sufficient go-related activation relative to response threshold) (Carlsen, Maslovat, &

Franks, 2012), combining the SAS with inhibition paradigms provided novel insight into the interaction between initiation and inhibition processes during movement preparation and execution.

In the present thesis, four specific questions were addressed; 1) Does the possibility of having to stop a movement decrease the amount of go-related preparatory activation? 2) Are go- and stop-processes independent or do they interact to determine response outcome? 3) Does preparing to stop selectively decrease the amount of preparatory activation related to a particular response? and 4) Does the rIFG and preSMA contribute to inhibitory control and supress preparatory go-activation because it may need to be withheld? Together, the findings have implications for understanding the behavioural and neural mechanisms involved in movement preparation and inhibition, and the interaction between initiation and inhibition in the control of human movement.

The following sections elaborate on the research implications based on the findings of the four experiments presented in Chapters 2 through 5. The first section discusses the relationship between preparing to go while maybe having to stop. The second section discusses the implications of the current findings on neural models of inhibitory control.

6.1. Preparing to go when faced with the possibility of having to stop

A startling acoustic stimulus (SAS) has been used as a tool to investigate processes related to response preparation and initiation. It has been demonstrated that if sufficient response preparation has occurred, the presentation of a SAS has the unique ability to trigger the prepared response involuntarily at a very short latency by increasing response initiation and activation levels to the extent that response threshold is achieved (Carlsen et al., 2012). One proposed pathway which SAS acts to provide the require input to trigger the cortically prepared movement is from ascending activation travelling from the reticular formation to the thalamus and up to the motor cortex. Given that the reactive and proactive fronto-BG-thalamic-M1 inhibitory pathways share common neural substrates

with that of the SAS, specifically the thalamus, the SAS was used to provide novel insight into the level of preparatory go-activation as well as the influence of inhibition on go-activation during the performance of stop-signal and go/no-go tasks.

The stop-signal task has been used for decades to study inhibitory control (Logan, Cowan, & Davis, 1984; Verbruggen & Logan, 2008). The stop-signal task involves the presentation of a go-signal and initiation of a motor response on the majority of trials, however on a subset of trials a stop-signal is presented following the go-signal which requires the participant to stop/prevent the initiation of the motor response. The ability to prevent a response from being initiated during a stop-trial is suggested to involve a reactive mode of inhibition, which results in the global suppression of relevant and non-relevant limbs in response to the stop-signal (Aron, 2011; Majid, Cai, George, Verbruggen, & Aron, 2012). Despite there only being one known go-response and hence the possibility of preparing the movement in advance similar to a simple RT task, results have consistently shown that RT on stop-signal task go trials (which don't require inhibition) is longer compared with a simple RT task (by ~ 100 – 200 ms). Results presented in Chapter 2 revealed that the level of preparatory activation is reduced during the performance of a stop-signal task compared to a simple RT task. Specifically, we found that the probability of a response being triggered early by SAS was reduced, and those that were elicited early were delayed compared to those in the simple RT task (Figure 2.1., p. 32 & Figure 2.2., p.34). This finding suggests that when humans are faced with the possibility of having to stop their movement, they decrease the amount of go-related preparatory activation. This is a clever strategy, as a reduction in preparatory activation increases the time required for activation associated with the go response to reach response threshold. This not only increases go trial reaction time but also provides more time for inhibitory processes to prevent go-activation from reaching response threshold should a stop-signal be presented.

Chapter 3 built on findings from Chapter 2, and investigated go- and stop-response activation prior to and following the stop-signal. Specifically, the experiment reported in Chapter 3 tested the predictions of an independent and parallel race between go- and stop-activations by adding SAS activation prior to the stop-signal (which should increase the rate of go-activation) and after the stop-signal (which should increase the rate of stop-activation). Results showed that the SAS increased the probability of responding during stop trials even when the SAS was presented 200 ms following the stop-signal (Figure 3.1., p. 67). In addition, the latency of SAS responses when the SAS was presented 150 ms and 200 ms following the stop-signal were initiated after the expected finishing time of the stop-process (Figure 3.3., p. 71), suggesting that these SAS responses were triggered involuntarily even after they had been inhibited at the neural level. These findings are interesting for two reasons, first it suggests that the SAS only increased or added to the rate of go-activation, and second, it reveals that go-activation endures well after a stop-signal is presented. Together, the results from Chapter 3 provide evidence against the predictions of independent go- and stop-activations, and instead suggests there is a single go-activation which is modulated towards or away from response threshold. The findings provide further support for alternative inhibitory control models describing inhibitory control as the interaction of inhibition on initiation as opposed to independent parallel processes racing for control (Boucher, Palmeri, Logan, & Schall, 2007; Dunovan, Lynch, Molesworth, & Verstynen, 2015).

In Chapter 4, I presented two experiments which investigated the effect of response preparation on inhibitory control during a selective stopping task. The selective stopping task is a variant of the traditional stop-signal task used in Chapters 2 and 3, and has been suggested to allow for a proactive mode of inhibition evidenced by the selective decrease in corticospinal excitability (CE) only in the maybe stop-cued limb (Cai, Oldenkamp, & Aron, 2011; Majid et al., 2012). While behavioural evidence of selective inhibition was revealed in both experiments through a small stopping interference effect

(Figure 4.2., p. 103), neurophysiological results revealed contrasting findings between simple (Experiment 3.1) and choice (Experiment 3.2) selective stopping tasks (Figure 4.3., p. 105). Since the only major difference between tasks was knowledge (or not) of the required response, the findings indicate that the ability to prepare the response in advance during the simple selective stopping task increased the amount of CE relative to null, which appears to overshadow the small proactive selective decrease in CE typically observed in the stop-cued response. Furthermore, despite the characteristic increase in CE representative of response preparation during the simple selective stopping task, the presentation of the SAS rarely elicited a startle reflex or the early triggering of the responses (Figure 4.4., p. 107). The nonspecific suppression of the startle reflex was unanticipated, and appears to be specific to the selective stopping task as the startle reflex was consistently observed in the traditional stop-signal task used in Chapters 2 and 3. Based on the current results, it is unclear if the inhibition of the startle reflex during the selective stopping task is related to processes associated with preparing to stop or processes associated with preparing to go. To further characterize the neural mechanisms involved, future studies could examine populations which have degeneration to the basal ganglia mediated inhibitory pathways and determine whether presenting a SAS during the performance of a selective stopping task elicits a startle reflex, as a previous study has shown that individuals with pre-manifest Huntington's disease (striatal and pallidal volume reduction) were unable to proactively inhibit the stop-cued response and showed an impaired behavioural selectivity when stopping (Majid, Cai, Corey-Bloom, & Aron, 2013). Together the findings suggest that similar to a typical stop-signal task, during the performance of a simple selective stopping task both the stop-cued and non-cued responses are prepared in advance. However, there appears to be an additional nonspecific inhibitory mechanism supressing the motor system which may be associated with having to stop a single response while continuing with another.

Chapter 5 examined preparing to go and maybe having to stop using go/no-go task. The go/no-go task can be conceptualized as a stop-signal task but with a delay between the go- and stop-signal equal to zero, such that the imperative stimulus is either a go or no-go (i.e., stop) signal, to which the response is to be initiated or withheld respectively. While a previous study (Carlsen et al., 2008) showed that the presentation of a SAS during a go/no-go task did not result in the early release of the response, they were unable to determine whether it was due to a lack of response preparation or proactive inhibition suppressing the prepared response. The use of cTBS to non-invasively and temporarily (~ 40 mins) supress neuronal activity in the rIFG and preSMA (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005), in combination with a SAS to probe the preparatory state of the go-response, we were able to provide novel insight into inhibitory and initiation processes during the performance of a go/no-go task. In relation to inhibitory processes, the results revealed that cTBS applied to rIFG and preSMA increased the number of failed-stop responses (Figure 5.2., p. 138). This provides evidence for the important role of rIFG and preSMA for inhibitory control during a go/no-go task. Furthermore, this impairment in inhibition induced by cTBS failed to have any effect on the initiation of voluntary or SAS responses (Figure 5.3., p. 140 & Figure 5.4., p. 141). Together the results suggest that the inability for a SAS to cause the early release of the response is simply due to a lack of response preparation. Thus, the findings from Chapter 5 indicate that the go-response is not prepared and initiated until after the imperative stimulus during a go/no-go task.

One of the main goals of the current thesis was to determine whether preparatory go-activation is modulated during the performance of inhibitory task, presumably as a way to deal with the possibly of stopping. Indeed, the research presented in the current thesis provided evidence that preparatory go-activation is highest in the simple RT task, followed a reduced level of preparatory go-activation during the stop-signal task, and a near absence of preparatory go-activation during a go/no-go task. Not

surprisingly premotor reaction times during the simple RT tasks were the fastest (approximately 180 ms), however, go/no-go RT (256 ms) was found to be faster than stop-signal task RT (approximately 290 ms). If there is more preparatory go-activation during the stop-signal task than the go/no-go task then why is RT slower during the stop-signal task? This is an interesting question and certainly one that requires further investigation, however, it does suggest there may be another mechanism in addition to modulating preparatory activation as a way to aid with inhibitory control. In relation to the go-response, there is evidence supporting the independence of preparation and initiation (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004; Haith, Pakpoor, & Krakauer, 2016; Maslovat, Drummond, Carter, & Carlsen, 2015; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999; Weinberg, 2016), which explains why preparatory activity in the motor cortex is only weakly predictive of variability in RT (Afshar et al., 2011; Michaels, Dann, Intveld, & Scherberger, 2015). Therefore, another plausible mechanism playing a role in inhibitory control between tasks is the rate of initiation-activation accumulation, typically referred to as "drift rate." Indeed, fixed threshold models including non-constant drift rates are best able to account for behavioural data (Dunovan et al., 2015; Hawkins, Forstmann, Wagenmakers, Ratcliff, & Brown, 2015; Ratcliff & Tuerlinckx, 2002; Ratcliff, Van Zandt, & McKoon, 1999), providing a means to account for elements such as variability in RT (Ratcliff & McKoon, 2008). It may suggest that the slower RTs observed during the stop-signal task compared to the go/no-go task is due to differences in drift rate (i.e., rate of initiation-activation accumulation toward threshold). Specifically, a slow drift rate paired with limited preparation during the stop-signal task and a fast drift rate paired with no preparation during the go/no-go task. Further inquiry into the modulation of drift rate between tasks is warranted as it appears preparatory activation alone cannot account for between task differences in RT reported.

Collectively, the four studies included in this thesis suggest that the extent to which humans prepare to go is affected by the possibility of having to stop. During the performance of a traditional

stop-signal task, preparatory go-activation is reduced to prolong the time required for go-activation to reach initiation threshold and increasing the ability of reactive inhibition to prevent response initiation (Chapter 2). Specifically, response inhibition appears to be achieved by inhibition interacting with a single go-activation, driving it down and away from response threshold (Chapter 3). Unlike the stop-signal task, during the performance of a go/no-go task preparation and initiation of the go-response appear to occur after the imperative stimulus, with recruitment of inhibitory processes once the stimulus has been identified as a no-go signal (Chapter 5). During the performance of a selective stopping task a much more complex set of processes appear to be involved. Performing the task in a simple RT paradigm did provide evidence for increased preparatory go-activation compared to the choice RT paradigm, however, there appears to be nonspecific inhibition of the motor system unrelated to proactive inhibition that prevents the release of prepared go-responses (Chapter 4). Further studies that combine methods similar to the ones used in Chapter 5 to target the inhibitory and initiation control networks should be conducted to provide further insight into initiation and inhibitory related processes during a selective stopping task. Together the findings provide evidence that preparatory go-activation is modulated to as a means to deal with the possibility of having to inhibit the response, while providing a future direction of research into possible between task differences in the rate of initiation-related go-activation.

6.2. Implications for neural models of inhibitory control

The findings presented in the present thesis extend and provide new information regarding the competition between initiation and inhibitory processes. Specifically, the findings of Chapter 3 have clear implications on the two competing neural models of inhibitory control, with results most harmonious with interactive neural models of inhibitory control. The independent race model suggests that go-activation and stop-activation increase in parallel toward a fixed response threshold, making the

simple prediction that whichever independent activation (go or stop) reaches threshold first determines response output (initiation or inhibition) (Logan et al., 1984). Or put another way, consider a bucket, with the top edge representing response threshold and the level of water in the bucket related to activation. The independent model suggests the bucket has two chambers, with a go and a stop tap adding water to their respective chamber independently. The side that fills up first and overflows is the winner, thus determining response outcome. It was hypothesized that the SAS would be able to influence both taps independently, increasing the rate of flow in the go and the stop tap at the same time. The response would then be initiated or inhibited depending on which activation level (go or stop) was closer to the top of the bucket when the SAS was presented. In contrast to the prediction that the SAS would preferentially propagate go-activation when presented prior to the stop-signal (increasing the probability of initiating a response) and preferentially propagate independent stop-activation when presented following the stop-signal (decreasing the probability of initiating a response), the results indicate that the SAS was only able to propagate go-activation as revealed by the increase in probability of responding even when the SAS was presented 200 ms following the stop-signal (Figure 3.1., p. 67). While the results presented in Chapter 3 do not discount the independent race model, they do necessitate a modification to the model. The finding that the SAS was triggering the involuntary initiation of the response beyond the time at which the response should have been successfully stopped, provides evidence against the irrevocable commitment to the action corresponding to the process that achieves threshold first. As such, we propose that the winner take all finish line be replaced with a window of time in which initiation can influence inhibition and vice versa. This window would start from the go-signal and given the results last for at least 200 ms after the stop-signal. This interpretation is consistent with the proposed phantom point of no return put forth by McGarry & Franks (1997), which suggests that response production processes can be modified right up to the point

of muscle activation. The findings suggest that as opposed to an irrevocable commitment to action (go or stop) once response threshold is crossed, it appears that input signals, either initiation or inhibitory in nature, can still produce an effect on behavioural output.

In contrast to the independent race model, alternative models of inhibitory control (interactive/dependent model) suggest that a single go-activation increases toward a fixed response threshold, with the ability of the stop-process to interact and diminish go-activation prior to or after response threshold determining response outcome (Boucher et al., 2007; Dunovan et al., 2015). Thus, returning to the bucket analogy, interactive models suggest there is a single (go) tap to fill the bucket, with a (stop) drain that can deplete the amount of water in the bucket and prevent it from reaching the top. The increase in the probability of responding observed even when the SAS was presented 150 ms and 200 ms following the stop-signal is supported by this model, as the SAS would only be propagating go-activation regardless of when it was presented. Specifically, the SAS leading to the involuntarily triggering of the go-response (even at the latest SAS probe times: SS+SAS150 & SS+SAS200) provides evidence of a decreasing yet enduring go-activation following the interaction of inhibitory-related processes. Furthermore, we found that SAS response latency decreased with increasing delay from the go signal, with short latencies observed at SS+SAS150 (42 ms) & SAS+SAS200 (40 ms) (Figure 3.2., p. 69). The latency of SAS responses can be used to map the time course of go-activation relative to threshold as a reduction in SAS response latency is proposed to reflect an increased amount of voluntary initiation-related activation (Maslovat, Carter, Kennefick, & Carlsen, 2014; Maslovat et al., 2015). Therefore, the very short (and similar) SAS response latencies seen at the last two SAS probe times suggest that go-activation is likely very close to response initiation threshold when inhibition has a potent effect on go-activation late in the RT interval.

The findings from Chapter 3 are most harmonious with interactive neural models of inhibition which do not include an independent stop-activation, suggesting behavioural control during the performance of a stop-signal is accomplished via the control of a single go-activation towards or away from a response threshold. That said, given the body of literature rooted in the independent horse-race model, the findings of the present study alone cannot discount the possibility of independent go- and stop-activations competing for control over movement outcome. The present study is however one of an increasing number of studies providing evidence in support of alternative models of inhibitory control (Boucher et al., 2007; Dunovan et al., 2015).

If behavioural control is determined by the modulation of a single go-activation, the findings from Chapters 3 and 5 provide new insight into the neural mechanisms governing behavioural control during the stop-signal and go/no-go tasks. While it has been suggested that inhibition during a stop-signal task involves different processes than those during a go/no-go task (Dambacher et al., 2014; Sebastian et al., 2013; Swick, Ashley, & Turken, 2011), the results reported in Chapter 5 suggest that the neural mechanism underlying inhibition may be similar for both tasks. The results from our combined cTBS and SAS experiment provide evidence that performance during a no-go trial is governed by the ability of inhibitory control processes (controlled in part by rIFG and preSMA) to inhibit go-activation. We suggest that similar to successful inhibition of a response in a stop-signal task, that is, a successful no-go response is achieved by inhibition interacting with initiation processes following stimulus identification, driving go-activation down and preventing it from reaching initiation threshold.

Specifically, in Chapter 5 we found that (1) cTBS applied to rIFG and preSMA led to an increase in failed-stop responses during no-go trials (Figure 5.2., p. 138) and (2) cTBS did not alter the latency of control go responses or the latency of SAS responses triggered during the performance of the simple RT task (Figure 5.3., p. 140). Together, the findings indicate that only the inhibitory processes were affected

by cTBS over rIFG and preSMA, while preparation and initiation of the go-response was unaltered. Having isolated the effect of cTBS as an impairment in inhibition, the inability of SAS to trigger the response early during the go/no-go task suggests that the response is not prepared in advance. Based on the findings reported, it appears that go-activation only increases following the imperative stimulus, irrespective of the stimulus type (i.e., go or no-go) for future response output. As such, a disruption of inhibitory control would only affect the ability to prevent the initiation of the response during no-go trials, a finding supported by the results reported in Chapter 5 with the observed increase in failed-stop responses following cTBS delivered to rIFG and preSMA. Evidence of go-activation increasing following the imperative stimulus during a go/no-go task has previously been shown in a study which recorded local field potentials from cortical regions of monkeys (Ledberg, Bressler, Ding, Coppola, & Nakamura, 2007). The results suggest that following the imperative stimulus, stimulus identification and response execution (including response preparation and initiation) processes occur in parallel. After identification of a no-go signal the inhibitory control network is activated, likely propagating through preSMA, rIFG, and BG to stop the inappropriate initiation of the response. The recruitment of inhibition following stimulus identification is supported by previous research demonstrating the divergence of go and no-go response activity approximately 150 ms following stimulus onset in a distributed network that includes parietal and frontal regions (Ledberg et al., 2007). With an observed go/no-go task premotor RT of 256 ms (Chapter 5) and neural processes associated with deciding and engaging inhibition proposed to take approximately 150 ms, it suggests that inhibition has a potent effect on go-activation which occurs late in the RT interval. These findings consistent with those reported in Chapter 3, with inhibitory processes interacting with increasing go-activation late in the RT interval and well after stop-signal presentation. Indeed, a go/no-go task is effectively a stop-signal task with a delay between the go and stop-signal equal to zero. While the level of advance preparation differs between tasks, both tasks increase go-

related activation towards response threshold upon presentation of the imperative stimulus. Once a stop or no-go signal is identified, the inhibitory control network is activated to interact with go-activation to prevent it from reaching response threshold. The same neural mechanism governing go/no-go and stop-signal task inhibitory control is consistent with the high degree of neural overlap in the networks active during the performance of both tasks (Aron, 2011; Chambers, Garavan, & Bellgrove, 2009). Adding to the growing body of evidence to suggest that inhibitory control is a multifaceted process comprised of separable, but not independent, control mechanisms.

The decreased ability to prevent responses during no-go trials seen in Chapter 5 (Figure 5.2., p. 138.) following cTBS applied to rIFG and preSMA was attributed to an impairment in the ability of inhibition to prevent go-activation from reaching initiation threshold. While the study was designed to induce a temporary impairment in inhibition, it was not intended to answer questions regarding the neural mechanism governing the impairment itself. However, within the framework of an interactive model we can speculate as to how this impairment could be achieved. The impairment may arise from a delay in the onset of inhibition, or a reduction in the strength of inhibition. Interestingly, each account makes distinct predictions regarding the latency of failed-stop responses. A delay would either reduce or have a null effect on the latency of failed-stop responses post-cTBS, while a reduced strength would delay the latency of failed-stop responses post-cTBS. Inspection of the data revealed that the latency of failed-stop responses following active cTBS was reduced (Δ -29 ms), however this reduction was not significantly different (p >.05) than that observed following Sham cTBS (Δ -14 ms). Although speculative, the results suggest that the decrease in inhibitory control induced by cTBS to rIFG and preSMA may be a result of a delay in the onset of inhibition on go-activation. Further research into the neural mechanisms governing this impairment in inhibition following disruption of neural activation in rIFG and preSMA is warranted.

6.3. Limitations and future directions

There are several limitations of the four thesis experiments described in this dissertation that should be considered and that may warrant discussion and further experimentation. For example, a startling acoustic stimulus (SAS) was used as a tool to probe or manipulate motor activation across all experiments. While the use of a SAS to investigate motor processes is novel and useful, a limitation to its use is the critical requirement for the SAS to elicit a startle reflex in order to infer its resultant effect on motor output and cortical processes. While important, this criterion can limit the number of usable data points for analysis of the already limited number trials the SAS is presented on due to auditory exposure safety concerns. Future experiments should therefore take the knowledge gained from the present set of experiments and use a SAS to probe specific time points in order to maximize the number of possible data points per time of interest.

The use of continuous theta burst stimulation (cTBS) delivered over rIFG and preSMA in Experiment 4 (Chapter 5) was not MRI guided. While the use of approximate stimulation sites based on the 10-20 system appears to have resulted in the hypothesized targeted effects, it is important to note that stimulation of these cortical sites was not anatomically confirmed. Despite this limitation, following cTBS over the rIFG and preSMA a significant reduction in participant's ability to withhold responses during no-go trials was observed (Figure 5.2, p. 138). Since the study was not designed to determine the locus of this effect, future studies targeting cortical nodes of the inhibitory pathway should determine the mechanism(s) underlying the decreased ability to withhold responses following cTBS. While the current data provided evidence for a delay in the onset on inhibitory activation on go-activation, a reduction in the strength of the inhibitory activation is also possible.

As a whole, the current series of experiments presented in the thesis provided support for an interactive model of inhibitory control (Boucher et al., 2007; Dunovan et al., 2015) and novel insight into

the role of preparation and initiation of a go-response on the ability to inhibit response output. Future experiments should combine the knowledge regarding the neural pathways and circuitry of the initiation and inhibitory control systems to determine where the two processes converge and interact. Specifically, the thalamus and motor cortex are two areas of interest for this interaction between go- and stop-activation. It may also be plausible that the locus of this interaction changes depending on task constraints. For example, tasks allowing advance response preparation possibly have a cortical - motor cortex - locus for the interaction whereas tasks which limit response preparation possibly have a subcortical - thalamus - locus.

6.4. Concluding remarks

On an everyday basis humans must synthesize sensory input in order to behave in accordance with their environment and goals. Thus, an important aspect of behavioural control is the ability to not only plan and initiate movement but also inhibit movement if necessary. The results from the four studies included in this thesis provided novel insight into inhibitory control during the preparation and initiation of motor responses. Specifically, with regards to inhibitory processes we showed that i) modulation of preparatory go-activation contributes to the ability to inhibit a motor response, ii) motor response inhibition is achieved by initiation activation being prevented from reaching threshold, iii) preparatory go-activation overshadows proactive inhibition, iv) inhibitory control depends on the integrity and recruitment of top-down inhibitory control to supress initiation activation once a no-go stimulus is identified. These findings support the overarching hypotheses that behavioural control is achieved via the interaction of initiation and inhibitory processes.

6.5. References

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7. Appendices

7.1. Appendix A: Schematic representation of experimental set-ups

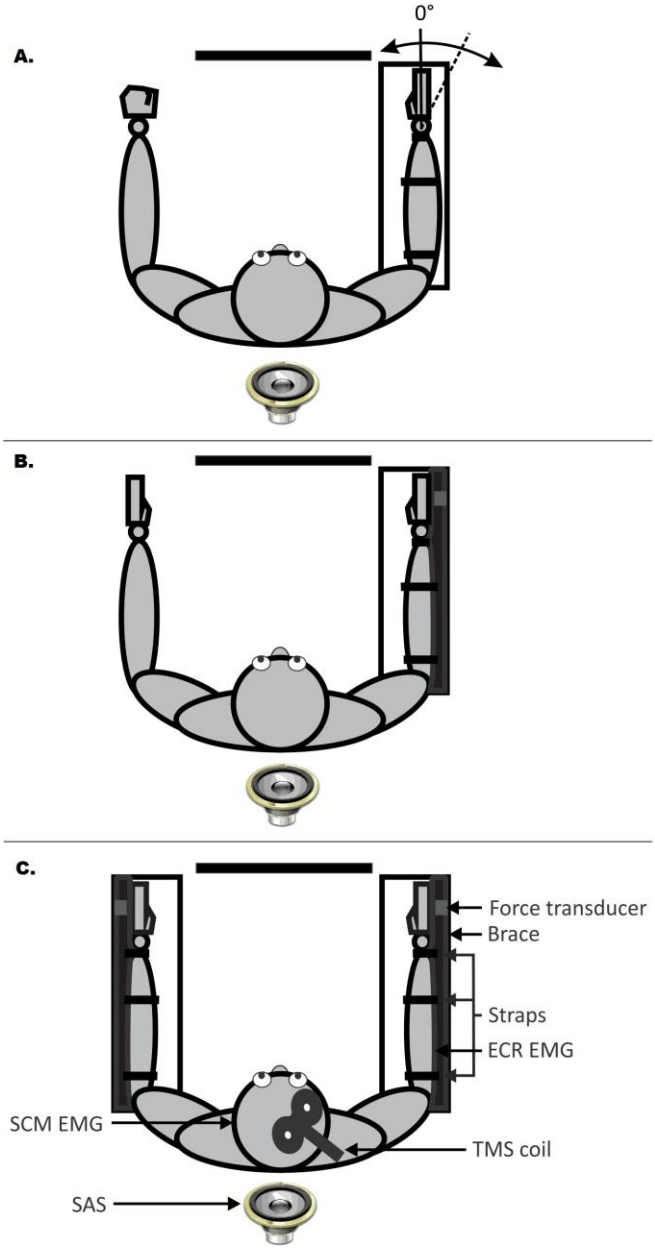

Figure A-1. Schematic representation of experimental set-ups. **A.** Experiment 1 (Chapter 2) unimanual wrist extension **B.** Experiment 2 (Chapter 3) & 4 (Chapter 5) unimanual isometric wrist extension. **C.** Experiment 3.1 (Chapter 4) bilateral isometric wrist extension. A startling acoustic stimulus (SAS) was used in all experiments, electromyography was recorded from the extensor carpi radialis (ECR) and sternocleidomastoid (SCM) in all experiments. Single pulse transcranial magnetic stimulation (TMS) was delivered over the wrist extensor hotspot during the performance of Experiment 3 (Chapter 4).

7.2. Appendix B: Exemplar raw data for Chapter 3

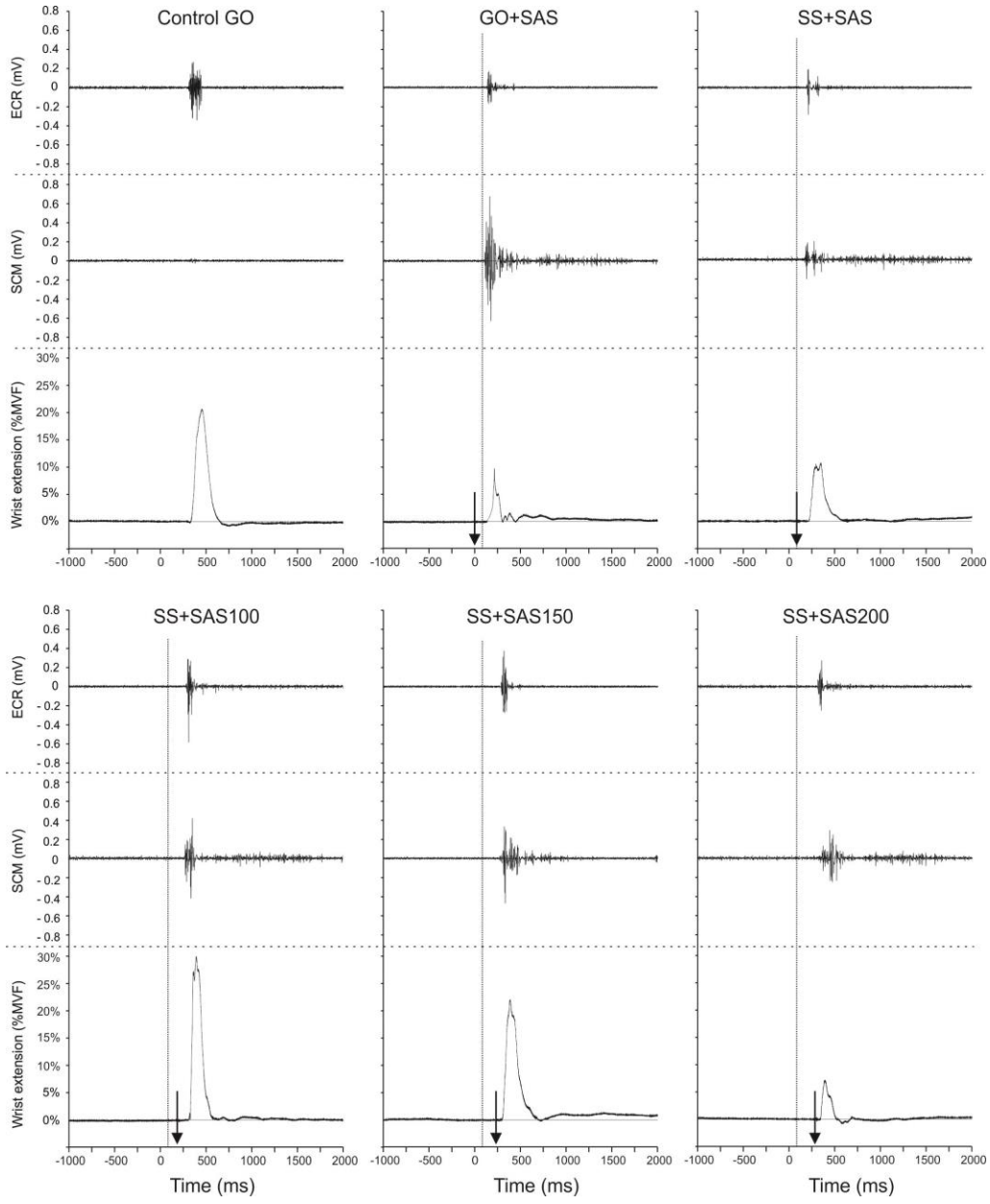

Figure A-2. Exemplar raw data from the testing block of Experiment 2 presented in Chapter 3. Data from a single participant is shown, with raw electromyography from the extensor carpi radialis (ECR) and sternocleidomastoid (SCM = startle reflex indicator) and response force as a percent of maximum voluntary contraction (%MVF) for each trial type. Time zero (0) on the X-axis represents the go-signal, the vertical dashed line represents the stop-signal (SS), and the vertical arrow represents the startling acoustic stimulus (SAS) onset.

7.3. Appendix C: Exemplar raw data for Chapter 4

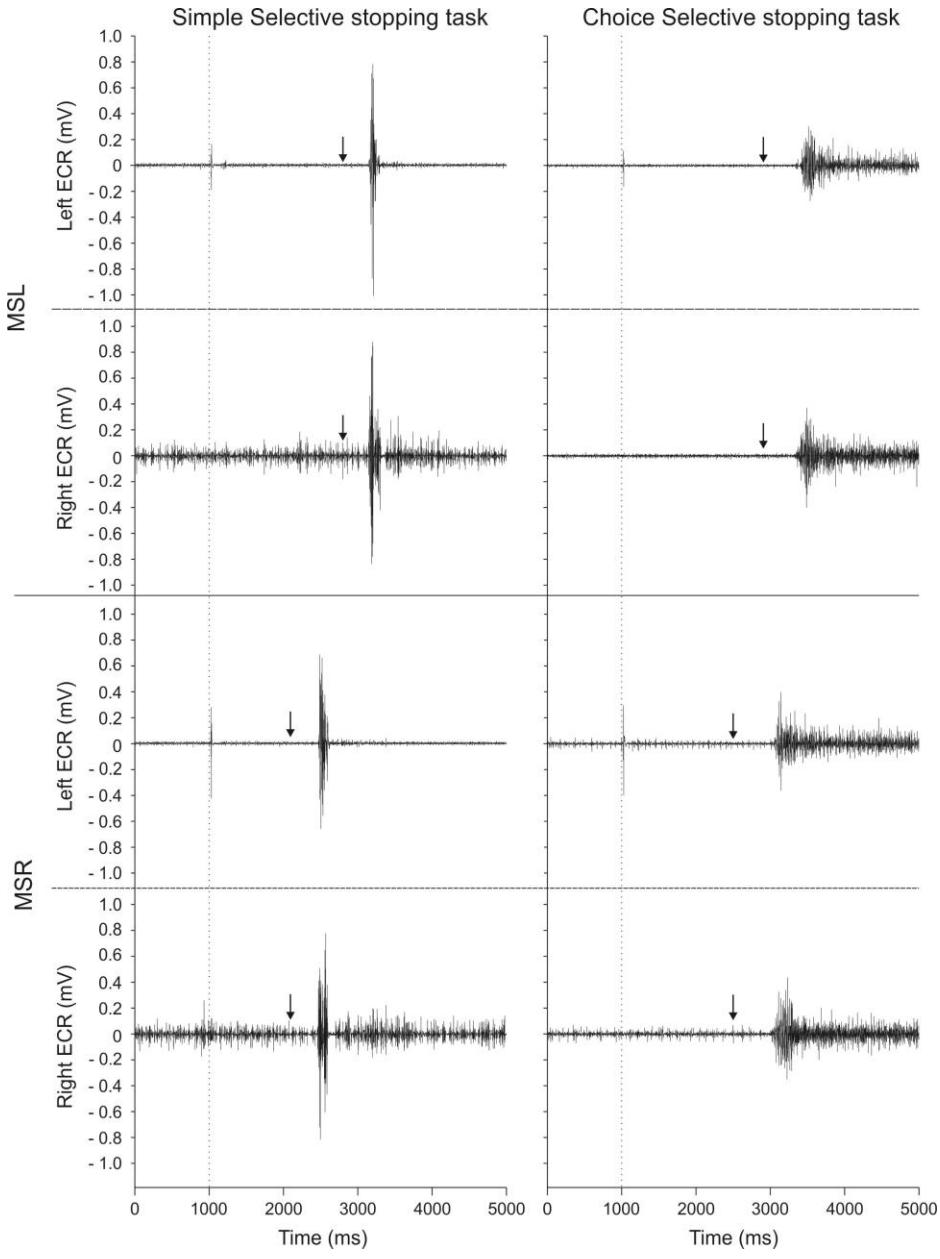

Figure A-3. Exemplar raw data from the right and left extensor carpi radialis (ECR) during the performance of the simple (Experiment 3.1) and choice (Experiment 3.2) selective stopping tasks as a function of maybe stop left (MSL) and maybe stop right (MSR) cue conditions. The dashed vertical line represents the time of single pulse transcranial magnetic stimulation delivery over the right motor cortex. The vertical arrows represent the time of go-signal onset for that particular trial. Data for each task is from separate participants**.**

7.4. Appendix D: Ethics approval for Chapters 2 and 3

File Number: H05-11-06 **Date (mm/dd/yyyy):** 06/18/2014

Université d'Ottawa University of Ottawa
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Ethics Approval Notice

Health Sciences and Science REB

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File Number: H05-11-06

Type of Project: Professor

Title: Investigating Preparatory Processes Underlying Fast, Goal-Directed Actions

Renewal Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
06/13/2014	06/12/2015	Ia

(Ia: Approval, Ib: Approval for initial stage only)

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N/A

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7.5. Appendix E: Ethics approval for Chapter 4

File Number: H04-16-01 **Date (mm/dd/yyyy):** 05/12/2016

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

Ethics Approval Notice

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File Number: H04-16-01

Type of Project: Professor

Title: Assessing neural activation related to preparation and initiation of voluntary actions

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
05/12/2016	05/11/2017	Approved

Special Conditions / Comments:
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7.6. Appendix F: Ethics approval for Chapter 5

File Number: H09-15-06 **Date (mm/dd/yyyy):** 02/29/2016

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Certificate of Ethics Approval

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File Number: H09-15-06

Type of Project: PhD Thesis

Title: Investigating how modulating cortical excitability affects motor performance

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
02/29/2016	02/28/2017	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:
N/A

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