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***STIMULUS AND RESPONSE INTERFERENCE:
BEHAVIOUR AND NEUROPHYSIOLOGY
OF THE FLANKER EFFECT***

School of Psychology

Nunzia Valentina Di Chiaro

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ABSTRACT

Constantly, we are influenced by irrelevant information which is fundamental to ignore to result in effective behaviours. In this research project, we used a colour version of the Flanker task to study interference control - the ability to overcome the influence of irrelevant stimuli and competing responses. In Chapter 1, we will present the dual process model used to understand cognitive processes involved in conflict situations. In the Flanker task, the indirect route (controlled) drives the activation of the correct response, whereas the direct route (automatic) activates the incorrect response triggered by flankers. The brain needs to resolve the conflict between the two competing responses before choosing the appropriate one, resulting in slower and less accurate performance. Some previous studies showed that interference effects may differ depending on a person's age, however the literature is still controversial. In our behavioural experiment, presented in Chapter 2, we investigated the lifespan development of stimulus and response interference in children, and in young and older adults. We found that performance at all ages was significantly influenced by competing stimuli and responses. Most importantly, the size of interference effects decreased with age. In the literature, electromyographic (EMG) studies have underlined the importance to investigate, across time, motor processes in both hands. In our experiment (Chapter 3), we used dynamometers and EMG to investigate the motor processes in both responding (chosen) and non-responding (unchosen) hands,

continuously across time. As expected, we found activation of the chosen hand. This activation was preceded (~150 ms) by a deactivation of the unchosen hand. These hand motor processes did not differ between the flanker interference conditions. In the next experiment (Chapter 4), we used transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) to investigate corticospinal excitability related to chosen and unchosen hands. We were interested in motor processes occurring before the reaction time (RT). In the chosen hand, motor-evoked potential (MEP) amplitudes started increasing 156 ms before the RT. This process did not differ across flanker interference conditions. In the unchosen hand, MEP amplitude was not different from baseline. We therefore partially replicated the findings of the EMG experiment. In our last experiment (Chapter 5), we used TMS over the supplementary motor area (SMA) to study the timing and the role of this brain area in preparation of hand motor responses. Surprisingly, we did not find any effects of TMS on the preparation process of responses. We were cautious in interpreting these results considering the exploratory nature. Taking these findings together, we hypothesise a constructive process between hands rather than a response competition as hypothesised in the dual process model. However, further studies are required aimed to explore the functional and temporal aspects of the motor mechanisms.

CONTENTS

CHAPTER 1 - GENERAL INTRODUCTION.....	1
1.1 Interference control	1
1.2 Models of interference control.....	7
1.3 How does interference control develop across the lifespan?	12
1.4 How to measure the interference effect?	16
1.5 Continuous measure of interference control	18
1.6 EMG studies	20
1.7 TMS studies	23
1.7.1 TMS over primary motor cortex (M1).....	25
1.7.2 TMS over supplementary motor area (SMA).....	28
1.8 Aims.....	30
CHAPTER 2 - FLANKER INTERFERENCE AT BOTH STIMULUS AND RESPONSE LEVELS DECREASES WITH AGE	32
2.1 INTRODUCTION	32
2.2 METHOD	38
2.2.1 Participants.....	38
2.2.2 Stimuli and apparatus.....	40

2.2.3	Task description.....	40
2.2.4	Procedure.....	41
2.2.5	Statistical analysis.....	44
2.3	RESULTS.....	47
2.3.1	Measures of interference effect.....	47
2.3.2	Stimulus-balanced versus response-balanced.....	48
2.3.4	Comparing the current data with Cragg (2016) and Hirst et al. (2019).....	54
2.3.5	Results using the same number of trials across groups.....	55
2.4	DISCUSSION.....	57
CHAPTER 3 - TEMPORAL DYNAMICS OF THE MOTOR RESPONSE AT PERIPHERAL LEVEL IN THE FLANKER INTERFERENCE TASK.		63
3.1	INTRODUCTION.....	63
3.2	METHOD.....	66
3.2.1	Participants.....	67
3.2.2	Stimuli, task and apparatus.....	67
3.2.3	Procedure.....	68
3.2.4	Dynamometer and EMG apparatus and processing.....	69
3.2.5	Data and statistical analysis.....	70

3.3	RESULTS.....	74
3.3.1	Stimulus locked analysis in chosen and unchosen hands (correct trials)	74
3.3.2	Stimulus locked analysis in chosen and unchosen hands (incorrect trials)	79
3.3.3	Response locked analysis in chosen and unchosen hands.....	84
3.3.4	Replication of interference effects	89
3.4	DISCUSSION.....	93
3.4.1	Stimulus locked analysis in chosen and unchosen hands	94
3.4.2	Response locked analysis in chosen and unchosen hands.....	97
3.4.3	Replication of interference effects	100
CHAPTER 4 – STUDYING THE TIME COURSE OF MOTOR RESPONSES OVER THE PRIMARY MOTOR CORTEX (M1) USING TRANSCRANIAL MAGNETIC STIMULATION (TMS)..... 102		
4.1	INTRODUCTION	102
4.2	METHOD	107
4.2.1	Participants.....	107
4.2.2	Stimuli, task and procedure	107
4.2.3	TMS apparatus.....	108
4.2.4	Localisation of M1.....	109

4.2.5	TMS parameters.....	110
4.2.6	MEP recording and processing.....	112
4.2.7	Data and statistical analysis	113
4.3	RESULTS.....	118
4.3.1	Slope parameter 'c'.....	118
4.3.2	Kneepoint parameter 'a'	122
4.4	DISCUSSION.....	127
 CHAPTER 5 - TIMING AND ROLE OF THE SUPPLEMENTARY MOTOR		
AREA (SMA) IN THE PREPARATION OF HAND MOVEMENT.		
133		
5.1	INTRODUCTION	133
5.2	METHOD	138
5.2.1	Participants.....	138
5.2.2	Stimuli, task and procedure	139
5.2.3	Apparatus	140
5.2.4	Localization of M1 for motor threshold.....	141
5.2.5	Localisation of TMS sites.....	141
5.2.6	TMS parameters.....	142
5.2.7	EMG recording and processing	143
5.2.8	Data and statistical analysis	144

5.3	RESULTS.....	148
5.3.1	Percentage of correct responses.....	148
5.3.2	RT.....	149
5.4	DISCUSSION.....	151
CHAPTER 6 - GENERAL DISCUSSION AND CONCLUSIONS.....		157
6.1	Summary of findings	157
6.2	How our findings meet previous literature	158
6.3.	Limitations.....	167
6.4	Future directions	168
REFERENCES.....		171
APPENDIX 2.....		193
APPENDIX 3.....		194
APPENDIX 4.....		197

List of abbreviations and acronyms

APB	Abductor pollicis brevis
C	Congruent
EDC	Extensor digitorum communis
EEG	Electroencephalography
EMG	Electromyography
ERP	Event-related potentials
FDI	First dorsal interosseus
FDS	Flexor digitorum superficialis
FPB	Flexor pollicis brevis
IES	Inverse efficiency score
LRP	Lateralized readiness potential
MEP	Motor evoked potentials
MoCA	Montreal Cognitive Assessment
M1	Primary motor cortex
MSO	Maximum stimulator output
OP	Opponens pollicis
RT	Reaction time
rMT	resting motor threshold
SD	Standard deviation

SE Standard error
SI Stimulus incongruent
SRI Stimulus response incongruent
SMA Supplementary motor area
TM Thenar muscles
TMS Transcranial magnetic stimulation

CHAPTER 1 - GENERAL INTRODUCTION

1.1 Interference control

Imagine you are at a grocery store trying to find a bottle of Coca-Cola on the shelves. There are many other drinks that are visually similar and could easily distract you. To find the item you're looking for, your brain needs to use interference control mechanisms to filter out the irrelevant items and focus the attention on the specific one you want. Here's another example: imagine you are driving your car while on holiday in the UK, where driving on the left side of the road is the norm. In this case, it's important to inhibit the automatic behaviour of driving on the right side of the road to avoid accidents and ensure safe driving.

Different cognitive processes are required in conflict situations. Activation of the appropriate response, resisting activation of inappropriate responses triggered by distracting information, and suppressing inappropriate responses which have been mistakenly activated. When the activation of appropriate responses competes with the activation of strong alternative responses, the function of interference control is crucial in resisting the interference from these alternatives.

Interference control is the ability to ignore irrelevant stimuli and inappropriate responses to maintain attention on a task. It is particularly important when competing information demands our attention. According to Diamond (Diamond, 2013), it plays a crucial role in everyday life for achieving effective behavior.

The study of the cognitive and neural processes involved in interference control has garnered a lot of attention and research in the field of cognitive neuroscience. Many researchers have been interested in understanding the mechanisms that the brain engages to mitigate and deal with interference of distracting information.

As interference can manifest at stimulus and/or response levels, Egner (Egner, 2008) reviewed the literature to determine whether interference control operates similarly across different interference tasks or if it shows task-specific characteristics. Although interference arises in all experimental paradigms (Eriksen & Eriksen, 1974; Simon & Small, 1969; Stroop, 1935), the involved cognitive mechanisms differ, and the paradigms are not interchangeable. Egner reported distinct interference mechanisms between relevant and irrelevant aspects associated with the stimulus, response, or a combination of both. Interference control functioning is specific to interference tasks and is likely facilitated by several distinct and parallel control mechanisms.

Different paradigms have been used to investigate interference control, particularly in assessing attentional control and manual response. In literature, the most used paradigms include the Stroop task (Stroop, 1935), the Simon task (Simon & Small, 1969), and the Flanker task (Eriksen & Eriksen, 1974). Researchers have to choose their task carefully based on the specific cognitive processes they aim to investigate.

The Stroop task (Stroop, 1935) has been used to measure the ability to suppress automatic processing of the stimulus meaning which is task-irrelevant. In the most common form, known as the word-colour version, participants are instructed to name the ink color of a color word, ignoring the meaning of the word. In *congruent* trials, the color word appears in the same color that it represents (i.e., the word "GREEN" shown in green ink). In *incongruent* trials, the color word appears in a different color than the one it represents, leading to a cognitive conflict (i.e., the word "RED" shown in green ink). The conflict which arises between the task-relevant (ink colour) and task-irrelevant information (word meaning) results in longer reaction times and decreased accuracy compared to congruent trials.

The Simon task (Simon & Small, 1969) has been classified as a spatial stimulus-response compatibility task because it is designed to assess the compatibility between the position of a stimulus and position of the response. Participants are

asked to respond to a specific feature of a stimulus while ignoring its spatial location. For example, a red or green circle may be shown to the left or right of a fixation cross. Participants are required to press the left button if the circle is red, while pressing the right button if the circle is green, regardless of the stimulus position. In *congruent* trials, the spatial position of the stimulus matches the response side (i.e., a red circle on the left), whereas in *incongruent* trials the spatial position of the stimulus and the response side do not match (i.e., a red circle on the right). In this paradigm, despite the irrelevance of the stimulus position to the task, participants tend to respond more quickly when the stimulus position and response position are congruent compared to when they are incongruent.

The Flanker task (Eriksen & Eriksen, 1974) is a well-known paradigm used to investigate the involuntary allocation of attention to distracting information which have to be ignored. In this task distracting information in the periphery ('flankers') interfere with the processing of relevant information in the centre (the target). The original version of the Flanker task consisted of a stimulus array made up of three letters from the set H, K, S, and G, and two response options (left and right buttons). Two letters were mapped to the same response option (i.e., H and K should be responded to by the left button while S and G should be responded to by a right button). In the *congruent condition (C)* the target (relevant information) and the flankers (irrelevant information) matched (i.e., HHH, SSS). In the *stimulus incongruent (SI)* condition, the target and flankers were different however they

were mapped to the same response hand (i.e., HKH, SGS). In the *stimulus response incongruent (SRI)* condition, the target and flankers were different and they were mapped to different response hands, evoking both stimulus and response conflict at the same time (i.e., HSH, SKS). In this paper, dating from 1974, the authors already established the basis for investigating both stimulus and response conflicts, using the distraction of the flankers to assess target processing.

Eriksen and Eriksen showed that resolution of interference between relevant and irrelevant information resulted in a cost. Slower and less accurate responses in the incongruent compared to congruent condition indicated that attention was allocated to the distracting information and the flankers have been processed. It was called the *Flanker effect*.

In the SI condition, the flankers interfered during visual processing of the target. In the SRI condition, the flankers automatically elicited a response that was different from the response required for the target. This automatic response must be suppressed before the correct response can be executed, leading to slower and less accurate responses.

Over the years, different versions of the original Flanker task have been developed. One of the most used in the literature is the arrow version of the Flanker

task. A series of stimuli consisting of arrows pointing either left or right are presented. The arrows can either be *congruent* (pointing in the same direction as the target) or *incongruent* (pointing in the opposite direction of the target). The task requires participants to indicate the direction of the central target arrow while ignoring the surrounding flanker arrows. Although a lot of studies used the arrow version to investigate interference control, one limitation is that it does not distinguish between interference at the stimulus and response level. Examining the mechanisms involved in stimulus and response interference control is crucial in clinical studies (Egner, 2008). Understanding which interference control domain is selectively impaired, for example in ADHD and Parkinson's disease, can help to design an effective rehabilitation program and help the quality of life of patients (Ridderinkhof et al., 2021).

Among different experimental paradigms, above presented, we focused on the Flanker task. We were interested in understanding the cognitive mechanisms involved in automatic processing of distracting information. We chose the Flanker task for different reasons. Firstly, it enabled us to explore interference control in presence of distracting stimuli and competing responses separately. Originally, we were interested to distinguish the timing between (early) stimulus processing and (later) response processing. Secondly, it was a simple task to be administered to children which normally show low levels of compliance. Thirdly, it was a

non-verbal test, therefore administrable to children who are not completely familiar with reading process. Lastly, the Flanker task allowed us to concentrate on manual responses - in which the Laboratory specialises - rather than verbal responses as requested in verbal naming version of the Stroop task.

1.2 Models of interference control

In literature, various theoretical frameworks have been proposed to elucidate the interference between target and distracting stimuli within the Flanker task. While these models have made valuable contributions to comprehending the underlying mechanisms of selective attention and motor control, they are not without limitations. Up to now, researchers persist in examining the Flanker effect from diverse perspectives because gaining insights into these limitations is crucial for refining and advancing our understanding.

In 2001, Botvinick, Braver, Barch, Carter, and Cohen (Botvinick et al., 2001) proposed the conflict monitoring model. It posited that the anterior cingulate cortex, in the frontal lobe of the brain, was involved in attention allocation and interference control. The model delineated a regulatory conflict-control loop, comprising a conflict-monitoring component, represented by the dorsal anterior cingulate cortex. This component was responsible for detecting various processing conflicts and subsequently transmitting a conflict signal to a strategic control component, embodied by the dorsolateral prefrontal cortex. The strategic control component,

in turn, endeavoured to resolve conflicts by strengthening top-down biasing processes linked to the task.

According to the conflict monitoring model, in the Flanker task, the anterior cingulate cortex plays a crucial role in detecting and resolving cognitive conflict between target and flankers. When flankers activate the incorrect response, the anterior cingulate cortex is believed to detect the interference and to conduct the conflict signal to the dorsolateral prefrontal cortex responsible for subsequent adaptations of cognitive control aimed to select the correct response.

While this model underlines the role of the anterior cingulate cortex in interference resolution, it has some important limitations. Firstly, the model oversimplifies the neural basis of conflict detection and resolution, as anterior cingulate cortex is part of a larger brain network involved in interference, as reported in a meta-analysis of neuroimaging tasks (Nee et al., 2007). Secondly, the model does not provide detailed explanations of how conflict, detected by the anterior cingulate cortex, is resolved or how adjustments in cognitive control occur. Specifically, the model does not consider the possible interaction between top-down and bottom up processes. Thirdly, the conflict monitoring model assumes the presence of one conflict-control loop responsible for conflict resolution in all interference tasks. As proposed by Egner (Egner, 2008), there is evidence to suggest the

presence of multiple independent conflict-control loops, each responsible for resolving different interference tasks.

In literature, different evidence underlined the role of bottom-up processes in conflict detection and resolution (Miller & Cohen, 2001; Miller, 1991). Kornblum and colleagues (Kornblum et al., 1990) suggested that the compatibility between the target and flankers on a relevant dimension, such as spatial location, resulted in decreased reaction times, while incongruence on this relevant dimension led to interference, consequently resulting in longer reaction times and reduced accuracy. In 2010, Egnér and colleagues (Egnér et al., 2010) proposed that attentional mechanisms operated at an early perceptual level to filter out irrelevant information. In the context of the Flanker task, attentional resources are deployed to attenuate the interference caused by distracting flankers, thus enhancing target processing.

Considering the role of both top-down and bottom-up processes in the Flanker effect, the dual process model has been largely used to understand cognitive processes involved in conflict situations (Frank et al., 2009; Ridderinkhof, 2014). In the model, there was a distinction between two different routes. The *indirect route* was intention-driven, top-down, controlled and voluntary, whereas the *direct route* was association-driven, bottom-up, automatic and involuntary. In conflict situations, the indirect route drove the activation of the correct response, whereas

the direct route activated the incorrect response triggered by distracting information. The studies were aimed to investigate how behaviour was influenced by integration of controlled and automatic processes when distracting information interfered with response.

In a recent and exhaustive review, Ridderinkhof and colleagues (Ridderinkhof et al., 2021) discussed the activation and integration of the indirect and direct routes during the Flanker task. For simplicity, we are going to refer to the indirect and direct routes as *controlled* and *automatic* routes, respectively.

In the Flanker task, the target activates the correct response in accord with instructions via the controlled route, while flankers prompt a response through the automatic route. The target response is voluntary and top-down activated, whereas the flanker response is involuntary and bottom-up triggered. There is also a difference related to the speed. The response to the flankers is normally faster than the response to the target. However, the speed of responses is not always an accurate indicator of good performance, as participants may respond quickly but still make errors when the flankers are shown. Ridderinkhof and colleagues (Ridderinkhof et al., 2021) presented two different situations that might arise before response execution. If the response associated with the target is the same as the response associated with the flanker, already activated by the auto-

matic route, the response can be executed quickly. If the flankers activate a different response from that associated with the target, both response options may be activated, creating a response competition. The brain needs to resolve the conflict between the two competing responses before choosing the appropriate one, resulting in slower and less accurate performance.

Stimulus response compatibility could influence the response selection process. When target and flankers are associated with the same response, as in the SI condition, flanker stimuli facilitate the activation of the correct response signalled by the target, leading to faster and more accurate responses. When target and flankers are associated with different responses, as in the SRI condition, flankers can prime the incorrect response, resulting in response competition that can slow down the selection of the correct response associated with the target.

Timing plays an important role during the response selection process. Ridderinkhof (Ridderinkhof, 2002) discussed the activation-suppression hypothesis as an explanation for integration of automatic and controlled processes in the Flanker task. During the initial stages, the selection of responses is primarily influenced by the response that is automatically associated with the flankers. This is followed by a gradual engagement of top-down controlled cognitive processes that selectively suppress the inappropriate responses conveyed by the flankers to select the target response in accord with task instructions. According to this hypothesis

faster responses are more vulnerable to high error rates in the incongruent condition compared to the congruent condition because more prone to the response capture, whereas slower responses are expected to have lower error rates because the passage of time allows for the suppression of flanker response.

The activation-suppression hypothesis provides a theoretical framework for understanding the cognitive mechanisms underlying the Flanker task. This is essential to explain how the appropriate response is selected successfully and in a timely manner (Miller & Cohen, 2001).

In this thesis, we chose the dual process model for two reasons. First, the model considered the crucial role of both top-down (controlled) vs bottom-up (automatic) processes, taking into account the evidence shown in literature. Second, the model proposed an interaction between (early) visual processing and (later) response processing which can explain complex mechanisms in conflict situations.

1.3 How does interference control develop across the lifespan?

At any age, a person's behaviour can be influenced by irrelevant information, however the ability to ignore distracting stimuli and competing responses may differ depending on a person's age.

In the available literature, children and older adults have showed more difficulties in suppressing irrelevant information than young adults (Li et al., 2009; Vu & Proctor, 2008). Although both children's and older adults' performances are significantly below those of young adults, the underlying neural mechanisms are different. The frontal brain network recruited for top-down control over behaviour in conflicting situations is not completely developed in children, whereas it declines in older people. It was assumed that interference control is not fully matured in children, whereas it starts to deteriorate in older adults (Menetre & Laganaro, 2023).

Different studies have focused on lifespan development of interference control (Cragg, 2016; Hirst et al., 2019; Jongen & Jonkman, 2008; Killikelly & Szucs, 2013). The authors investigated how people at different ages overcome interference of distracting stimuli and competing responses. Some of them investigated interference control comparing performance of children and young adults (Cragg, 2016; Jongen & Jonkman, 2008). Two studies focused on lifespan development of stimulus and response interference, directly comparing performances from childhood to late adulthood (Hirst et al., 2019; Killikelly & Szucs, 2013).

Jongen and colleagues (Jongen & Jonkman, 2008) investigated the stimulus and response interference in three groups of children aged 6-7, 8-9, and 10-12 years, and a group of young adults aged 18-28 years old. The authors reported that the

ability to ignore distracting stimuli was already developed at 6-7 years, because there were no differences in the size of the stimulus interference effect between groups. The response interference effect was equally strong in all age groups. Cragg (Cragg, 2016) recruited two groups of children aged 7 and 10 years old and a group of young adults of 21 years as mean age. The author showed that stimulus interference control was not fully developed at 7 years. Both groups of children experienced greater stimulus interference than the young adults, and 7 year old children showed more stimulus interference than the 10 year olds. There were no differences in the amount of response interference experienced across age groups. In a later study, Hirst and colleagues (Hirst et al., 2019) compared response time distributions of stimulus and response interference conditions. In children, between the age of 6 and 11 years, response interference occurred at the longest response times, while stimulus interference remained constant across different response times.

Few studies investigated the stimulus and response interference effects in older adulthood. In the same study, Hirst and colleagues reported that older adults aged 61-85 years showed slower responses and greater interference in the stimulus than the response interference condition. In a different study, Killikelly and colleagues (Killikelly & Szucs, 2013) recruited adolescents (16-17 years old), young adults (23-30 years old), and middle-age adults (45-62 years old). The

authors showed that stimulus and response interference were comparable among age groups.

In general, it is difficult to summarise the available findings to understand how stimulus and response interference control develops across the lifespan. Methodological differences complicate comparison between studies. Three studies did not consider the stimulus response compatibility when they measured the interference effects across different age groups (Cragg, 2016; Hirst et al., 2019; Jongen & Jonkman, 2008). Killikelly and colleagues (Killikelly & Szucs, 2013) did, therefore the authors balanced the proportion of congruent and incongruent trials at the response level, so flankers were associated with the same probability to a congruent or incongruent response.

As discussed above in the dual process model (Ridderinkhof et al., 2021), when target and flankers are associated with the same response, flankers facilitate the activation of the correct response, leading to faster and more accurate responses. The flankers of the SI trials – associated with the target response to the same response button – facilitate more accurate and faster responses, increasing the difference in performance between the SI and SRI trials. This mechanism alters the size of the response interference effect and the comparison between the interference effects. This methodological issue will be taken into account in our first study, presented in Chapter 2. Aimed to measure both the stimulus and response

interference effects across the lifespan, we first tested the hypothesis on whether the size of response interference effect is influenced by stimulus response compatibility.

1.4 How to measure the interference effect?

Interference of irrelevant information can arise at any or all phases of task processing. They can interfere during stimulus processing, response selection and/or response execution. In 2003, De Houwer (De Houwer, 2003) investigated the influence of irrelevant information during earlier and later stages of processing. The researcher highlighted the importance of separately examining the influence of distracting information that arises during the visual processing of stimuli, versus distracting information that arises during the selection and execution of a correct response among multiple distracting alternatives.

In the Flanker task, flankers influence the processing of the target at the visual level in the SI condition. It occurs because the flankers and the target are visually different but require the same response. In the SRI condition, the flankers not only interfere with the visual processing of the target, but also activate a response that competes with the activation of the target response at the response selection stage. In SRI condition, the flankers influence both the visual and response levels simultaneously. De Houwer (De Houwer, 2003) proposed to separate interference mechanisms at visual and response levels.

Performance can be measured using speed and accuracy, and those scores need to be converted into measures of interference. Ratio and absolute scores are two main ways used to isolate the interference effects (Cragg, 2016; Hirst et al., 2019; Jongen & Jonkman, 2008; Killikelly & Szucs, 2013). The ratio and absolute scores of the *perceptual (or stimulus) interference* effect were calculated by dividing or subtracting the SI and C conditions (SI/C or SI-C); the *response interference* effect was calculated by dividing or subtracting the SRI and SI conditions (SRI/SI or SRI-SI). *General interference* effect was calculated by dividing or subtracting the SRI and C condition (SRI/C or SRI-C).

There is a substantial heterogeneity between studies on how to measure interference effects. Some studies used the ratio scores (Cragg, 2016; Hirst et al., 2019), whereas other studies used absolute differences (Jongen & Jonkman, 2008; Killikelly & Szucs, 2013). Since measuring the interference effect is a crucial point, we aimed to determine whether absolute or ratio scores are better measures of interference effects. We measured the interference effect using both absolute and ratio score. However, we did not find evidence to suggest that measuring the interference effect using absolute scores is better than the ratio scores of the interference effect or vice versa. Without further exploration or modelling, both methods could reasonably be used to measure the interference effect. These findings will be discussed in detail in Chapter 2.

1.5 Continuous measure of interference control

In 1985, Coles and colleagues (Coles et al., 1985) discussed the importance of using neurophysiological methods to investigate covert processes which were not accessible to traditional behavioral measures. In their paper, the authors used neurophysiological techniques, such as the P300 latency of event-related potentials (ERP) and electromyography (EMG), to study the interference at central and peripheral level, respectively. In particular, they studied the interference of flankers during target processing, and the response competition between automatic response activated by flankers and top-down controlled response activated by target. In their study, the authors emphasised the use of neurophysiological methods to investigate, in both hands, interference across the time.

Despite this early approach, the vast majority of studies have investigated flanker interference by measuring reaction times using a keyboard (Bulger et al., 2021; Gutchess et al., 2021; Miller, 1991). Participants were instructed to press as quickly as possible two different key buttons using two hands, in accordance with the task instructions. Reaction time was measured as the time between stimulus presentation and key button pressing. It was a discrete measure of the time that the hand selected during the response competition took to press the response button. In these studies, in which a keyboard was used as a response device, it was difficult to investigate motor competition in any detail. To facilitate the reading

through the chapters, we will refer to the selected hand also as the *chosen or response hand*, and to the non-selected hand as the *unchosen or non-responding hand*.

There are two main problems. Firstly, motor processes in the unchosen hand were not recorded. In the dual process model, described above, the hand automatically associated with flankers is initially activated, and later suppressed at the response activation level to select the correct response associated with the target. This phenomenon is common in the SRI condition in which the flanker and target responses do not match. In accordance with the dual process model, it is possible to hypothesize an early neurophysiological activation of the hand associated with the flankers, which is later suppressed by activation of the hand associated with the target.

Secondly, the study of the temporal dynamics of response competition between the hands may be overlooked when relying solely on keyboard responses. In accordance with the dual process model, the activation of a response associated with the flankers is faster in comparison to activation of the target response. This is possible because the direct route is automatic and faster than the indirect route which is top-down controlled and slower. Based on this theoretical assumption, it is possible to investigate an early activation of the hand associated with flankers which is later substituted by the target response.

Filling these gaps, neurophysiological studies have aimed to investigate the response competition across time. In particular, understanding the ongoing motor processes in chosen and unchosen hands, from presentation of the stimulus until the response finished. In their neurophysiological study, Coles and colleagues (Coles et al., 1985) found a partial activation of the unchosen hand associated to the flanker in the incongruent condition compared to the congruent condition.

EMG is a fruitful method to investigate in a direct manner the motor processes in both hands simultaneously and continuously. Because it is applied to both hands, it allowed researchers to investigate simultaneously the motor activity directly in chosen and unchosen hands across time. A different method used to measure motor activity continuously in both hands is through dynamometer (or grip force) recording. It is a response device that can measure across time the amount of force applied by a person when responding. Participants usually hold two dynamometers, one in each hand. This is a powerful tool because the amount of force applied allows researchers to investigate the motor competition between the flanker and target responses continuously.

1.6 EMG studies

Although EMG shows powerful applications for continuous measures of interference effect, only a few studies have investigated the motor competition

between the flanker and target responses during the response stage at the peripheral level (Killikelly & Szucs, 2013; Szucs et al., 2009).

The studies reported activation of the hand associated with the target response in both congruent and incongruent conditions. The authors expected this result. In the congruent condition, there was no conflict, therefore the target response was easily selected and activated. In the incongruent condition, although motor competition between the target and flanker responses occurred, the response associated with the target was selected and activated according with task instructions.

New findings were reported in the hand which was unchosen during response competition. The researchers reported these results in the incongruent condition. Szucs and colleagues (Szucs et al., 2009) reported a significant EMG activation of the unchosen hand between 222-322 ms after the stimulus onset in incongruent trials. In a minority of trials, the EMG activity in the unchosen hand was detected prior to activity in the chosen hand, and in a majority of the trials, the chosen and unchosen response activations were simultaneous. In agreement with a previous study (Caldas et al., 2012), Caldas and colleagues found in 14% of correct trials, a detectable EMG activation in the unchosen hand which preceded the EMG activation in the chosen hand. In the reported studies, the activity of the

unchosen hand was investigated only in the incongruent condition, without any distinction between stimulus and response interference.

Later, Killikelly and Szucs (Killikelly & Szucs, 2013) investigated the activity of the non-selected hand during stimulus and response interference conditions. Theoretically, in the SI condition in which the flanker and target responses were associated to the same responses, it was unlikely to observe activation of the non-selected hand. Flankers and target activated the same hand response. In the SRI condition, in which the flankers and target were associated with different hand responses, it was likely to observe the activation of the non-selected hand. In their study, the authors reported significant EMG activation in the unchosen hand between 460-480 ms after stimulus onset, across all incongruent conditions. In this study, it seemed that the activation of the unchosen hand is a non-specific mechanism belonging to the incongruent conditions.

Taking these findings together, researchers have demonstrated that, in the condition where the stimuli presented were incongruent, there was the frequent activation of both chosen and unchosen hands. This interesting finding was possible to observe because the activity in both hands was measured simultaneously and continuously.

Looking at the timing of response activations, the results observed were mixed. One hypothesis suggests that both hands are activated simultaneously, leading to a competition between them, until one is selected for response. Alternatively, another hypothesis suggests that the unchosen hand is prematurely activated in response to the flanker stimuli, prior to the activation of the chosen hand, which is in response to the target. According to the dual process model, it is reasonable to hypothesise the initial activation of the hand associated with the flanker, which will be later suppressed, and the activation of the hand associated with the target.

These EMG studies contributed substantially to the study of motor competition at the response level between the chosen and unchosen hands, however the available findings are mixed. Up to now, it is still unclear how and when the motor competition between chosen and unchosen hands is solved. Moreover, it is still unclear the specific or non-specific nature of motor process in the unchosen hand in different interference conditions. On these open questions, we planned our study presented in detail in Chapter 3.

1.7 TMS studies

Different TMS studies have investigated functional and temporal aspects of interference control in the central nervous system (Bestmann & Duque, 2016; Guida et al., 2023; Klein-Flugge et al., 2013; Michelet et al., 2010; Parmigiani & Cattaneo, 2018; Pineda-Pardo et al., 2019; Verleger et al., 2009). Stimulating

brain areas which are supposed to be involved in specific stages of processing, researchers could reliably investigate the role and the timing of the brain areas from stimulus presentation up to motor response execution.

A complex architecture of neural systems supports different stages of interference processing. Cortical regions such as prefrontal and motor cortex, and sub-cortical areas such as substantia nigra and basal ganglia are involved in the activation of automatic and controlled motor responses (Isherwood et al., 2023).

Transcranial magnetic stimulation (TMS) is a helpful technique to study the brain mechanisms of motor responses in conflict situations. It is a non-invasive neurostimulation technique which interferes transiently with cortical activity under the scalp. Using an electromagnetic pulse, TMS creates a magnetic field which passes through the skull, and induces electrical current in neurons, which depolarises them, stimulating or interfering with activity (Rossini et al., 2015). TMS offers a reliable approach for examining the link between brain and behavior by enabling the establishment of causal connections between stimulated brain regions and subsequent behaviour. Establishing causal links is what makes TMS an important method in neuroscience.

1.7.1 TMS over primary motor cortex (M1)

In previous literature, TMS studies have stimulated over the M1 in order to investigate the temporal aspects of motor processes related to the chosen and unchosen responses during interference tasks. The M1 is an important brain area involved in execution of contralateral movements (Bhattacharjee et al., 2021).

Studying the interference effect at central nervous level allowed the researchers to investigate the stages that occur before the motor response onset. This aspect was interesting because it was not accessible at the behavioural level. Stimulating over M1, directly involved in movement execution, produces an involuntary and premature motor contraction which may alter subsequent reaction time measurements. To deal with this issue, researchers have used the motor evoked potential (MEP) as a reliable measure of corticospinal excitability recorded at a peripheral level in a time window between around 10-50 milliseconds after stimulation over M1 in the contralateral hemisphere. Changes in amplitude and latency of MEPs showed different functional and temporal aspects of motor process between chosen and unchosen hands (Klein-Flugge et al., 2013; Michelet et al., 2010; Verleger et al., 2009).

Klein-Flugge and colleagues (Klein-Flugge et al., 2013), using a decision-making task, showed a reduction of the MEP amplitude for the unchosen hand, and an increased amplitude for the chosen hand at 230 ms before the movement onset. In a different study, Verleger and colleagues (Verleger et al., 2009) investigated

MEP activity while participants performed the arrow version of the Flanker task. In the congruent condition, MEP amplitude increased when TMS was applied contralateral to the chosen hand. In the incongruent condition, MEP amplitudes in the unchosen hand gradually decreased in the first 90 ms after the lateralized readiness potential (LRP) peak, and mirror-symmetrically increased MEP amplitudes were seen in the chosen hand. These findings agreed with another study, in which Michelet and colleagues (Michelet et al., 2010) studied the motor competition in the first dorsal interosseus (FDI) and extensor indicis muscles in the same hand while participants performed the arrow version of the Flanker task. Their results showed that, in the congruent condition, MEP size increased when the muscle acted as agonist, and decreased when the same muscle acted as antagonist. The difference between MEPs became significant at 240 ms after the target. In the incongruent condition, MEP amplitude briefly increased in the (incorrect) muscle, which corresponded to an initial response to the flanker arrows. Later, at 240 ms after the stimulus, MEPs corresponding to the correct response were greater.

Taken together, TMS studies which stimulated over the M1 showed different motor processes, specifically an increased MEP amplitude related to the chosen response and decreased MEP amplitude related to the unchosen response. This difference of MEP amplitude was confirmed even when the same muscle in the hand acted as agonist or antagonist. Klein-Flugge and colleagues (Klein-Flugge

et al., 2013) supposed that motor processes in M1 related to the chosen and unchosen hand before the movement onset help preparation of movement. As a matter of fact, inappropriate responses in the incongruent condition are suppressed in one M1 hand area simultaneously with activation of the correct response in the other M1 (Verleger et al., 2009).

In the above TMS studies, it was not investigated whether different motor processes could arise when irrelevant stimuli and competing responses interfere with the motor response. According to the dual process model, it is reasonable to hypothesise in the SRI condition a premature increasing of MEP amplitude in the unchosen hand (because it relates to the flankers) and a later increasing of MEP amplitude in the chosen hand (because it relates to the target). Considering the lack of studies which investigate the motor processes in the SI condition, it is very difficult to predict the motor processes in the hand in which flanker and target responses correspond. Therefore, further studies are required in this direction.

These issues will be investigated in Chapter 4, in which we modelled how the MEP data changed over time in order to better understand the functional and temporal aspects of motor activity in chosen and unchosen hand in different interference conditions.

1.7.2 TMS over supplementary motor area (SMA)

Some studies were aimed to investigate selection and preparation of motor responses (Guida et al., 2023; Parmigiani & Cattaneo, 2018; Pineda-Pardo et al., 2019). Back to the dual process model, the researchers were interested in studying brain mechanisms involved in suppression of flanker response (via the automatic route) to select the correct target response (via the controlled route). In other words, the researchers investigated the stage preceding the execution of motor programs between hands in the M1.

Ridderinkhof and colleagues (Ridderinkhof et al., 2021) reported the role of pre-supplementary motor area (pre-SMA) and SMA in the dorsomedial frontal cortex. The pre-SMA, which is mostly interconnected with prefrontal areas, is involved in selection and preparation of motor responses. The SMA, densely connected with the M1 and spinal cord, is involved in the initiation of motor responses. In other words, the pre-SMA and SMA seem to be involved in earlier and later stages of motor processing, respectively. The distinction between pre-SMA and SMA has been reported also in a functional neuroimaging study (Lee et al., 1999).

Although the researchers discussed functional dissociation between pre-SMA and SMA, a functional continuum rather than a discrete division between these areas (Ridderinkhof et al., 2021). Considering the functional continuity, we decided to focus on the SMA because it is anatomically and directly connected with

M1. We aimed to investigate the timing and role of SMA, using TMS, in the preparation of motor responses. If TMS disrupts activity in the SMA and leads to changes in flanker performance, this suggests that the targeted region is involved in the corresponding process.

In the literature, some neuromodulation studies have investigated the involvement of the SMA in preparation of motor programs (Guida et al., 2023; Parmigiani & Cattaneo, 2018; Pineda-Pardo et al., 2019). Guida and colleagues (Guida et al., 2023) explored the role of the SMA in automatic and voluntary response inhibition using theta-burst stimulation and transcranial static magnetic field stimulation over SMA, in two different experiments. The results showed no significant differences after applying either real or sham stimulation session, neither in automatic nor voluntary inhibition tasks. In the same laboratory, Pined-Pardo and colleagues (Pineda-Pardo et al., 2019) applied real or sham transcranial static magnetic field stimulation over the SMA in two double-blind subsamples. Results showed that the group undergoing real brain stimulation showed (within 30 minutes after stimulation) longer RTs in motor choice tasks, and increased accuracy in withholding predicted actions in comparison to the sham control group. Brain stimulation over SMA significantly affected the speed-accuracy trade off, particularly that initiation times increased in favour of increased accuracy. In these neuromodulation studies, the authors investigated the SMA functionality after the neurostimulation session using an 'offline' stimulation protocol.

Parmigiani and Cattaneo (Parmigiani & Cattaneo, 2018), using an 'online' stimulation protocol, applied single pulse or sham TMS over the SMA in a group of healthy volunteers. The authors showed no TMS effect, neither on the performance of STOP trials nor GO trials in the stop-signal task performed with the lips.

To the best of our knowledge, there are no studies that aimed to investigate the SMA functionality in the preparation of motor program to suppress irrelevant stimuli and inappropriate responses. In our experiment, presented in detail in Chapter 5, we used TMS to interfere with the activity of SMA, using an 'online' stimulation protocol. While participants performed the Flanker task, TMS was delivered over SMA in order to interfere with the preparation of motor responses.

1.8 Aims

We aimed to understand the behavioural and neural mechanisms of the flanker effect. We developed a coloured version of the Flanker task, to study the interference of distracting stimuli and competing responses. Using a coloured version of the task allowed us to investigate the interference mechanisms in younger children who are not fully familiar with reading alphabetic characters.

In sum, the following experiments will focus on four aims, all of which seek to fill in the gaps in the literature mentioned earlier. We aimed to:

1. Study the lifespan development of stimulus and response interference control at the behavioural level. We controlled for some methodological issues that might have confused previous findings.
2. Study motor processes in chosen and unchosen hands, investigating neuro-physiological aspects measured continuously at the peripheral level.
3. Use TMS over M1 to study at the central level the corticospinal excitability involved in motor processes of chosen and unchosen hands.
4. Use TMS over SMA to study the timing and the role of SMA in the preparation of motor responses.

CHAPTER 2 - FLANKER INTERFERENCE AT BOTH STIMULUS AND RESPONSE LEVELS DECREASES WITH AGE

2.1 INTRODUCTION

Irrelevant information can interfere at any or all phases of task processing, for example during stimulus processing, response selection, and/or response execution. Accordingly, interference control has been studied at the level of stimulus identification and target detection, as well as at the response level. Different and non-overlapping attentional mechanisms in the brain have been found for resolving these forms of cognitive interference (van Veen & Carter, 2005; van Veen et al., 2001).

Different researchers have discussed whether irrelevant information may interfere differently depending on a person's age. At any age, behaviour can be influenced by interference from irrelevant information, however the size of interference at both stimulus and response levels has been argued to differ with age. For example, children and older adults are reported to be less able to overcome distraction from irrelevant information than young adults (Li et al., 2009; Vu & Proctor, 2008). Several studies have aimed to understand how people at different ages resolve interference originating from competing stimuli and responses (Cragg, 2016; Hirst et al., 2019; Jongen & Jonkman, 2008; Killikelly & Szucs, 2013).

Jongen and colleagues (Jongen & Jonkman, 2008) recruited three groups of children aged 6-7, 8-9, and 10-12 years, and a group of young adults aged 18-28 years. They administered a colour object Stroop task in which participants had to identify, by pressing a button as quickly as possible, the printed colour of familiar objects presented in their usual or an unusual colour (e.g., a red strawberry vs. a blue strawberry). Stimulus and response interference were manipulated by the colour of the stimulus and the mapping of these colours to the response buttons. In the stimulus interference condition, the unusually-coloured stimuli were mapped onto the same response button as the object's usual colour. In the response incongruent condition, the unusual colour was mapped onto a different response button than the object's usual colour. The results suggested that interference at the stimulus level – the slowing of reaction times for stimulus incongruent compared to fully congruent stimuli – was already developed at 6-7 years old, because no differences in the size of the stimulus interference effect were found between the age groups. At the same time, the response interference effect was equally strong in all age groups, however, children were less accurate than adults.

According to more recent studies (Cragg, 2016; Hirst et al., 2019), control over competing stimuli has not fully developed by 7 years of age. Cragg (Cragg, 2016) tested two groups of children aged 7 and 10 years old, and a group of young adults of mean age 21 years, using a colour version of the Erikson Flanker task

(Eriksen & Eriksen, 1974). Participants were asked to indicate the colour of a central line while ignoring the colours of two flanking parallel lines. Both groups of children experienced greater stimulus interference than the young adults, and 7 year old children showed more stimulus interference (resulting in longer reaction times) than the 10 year olds. Regarding response interference, there were no differences in the amount of interference experienced across age groups. Interestingly, Hirst and colleagues (Hirst et al., 2019) observed in children between the ages of 6 and 11 years, that stimulus interference facilitated the correct response, while response interference facilitated the incorrect response. Comparing these two patterns across the response time distribution, the authors showed that response interference occurred at the longest response time latencies, while stimulus interference remained constant.

To our knowledge, few studies have investigated how people resolve the interference originating from competing stimuli and responses in later life. Hirst and colleagues (Hirst et al., 2019) suggested that stimulus interference control could be more susceptible to age-related changes than response interference, finding that adults aged 61-85 years showed slower responses and greater interference in the stimulus- than the response-interference condition. No specific age-related patterns in the response time distribution, as in childhood, were found. In another study, Killikelly and Szucs (Killikelly & Szucs, 2013) showed that both stimulus and response competition contributed to the overall interference effect, however

the interference was comparable among age groups. They tested adolescents (16-17 years old), young adults (23-30 years old), and middle-age adults (45-62 years old).

Looking across the reported studies, it is difficult to answer the question about how people at different ages resolve stimulus and response interference, because differences in experimental design and data analysis between studies complicate such comparisons.

In the reported studies, different measures have been used to calculate interference effects. Some studies used the ratios (relative scores) of incongruent:congruent responses as an interference measure (Cragg, 2016; Hirst et al., 2019), whereas other studies used differences (absolute scores) (Jongen & Jonkman, 2008; Killikelly & Szucs, 2013). In light of these considerations, it may be important to use the same measure of interference, making the comparison among studies easier.

The proportions of congruent and incongruent trials also influence the size of interference effects (Cragg, 2016). As reported by Hasshim and colleagues (Hasshim & Parris, 2014), the associations between stimuli and responses are learnt during an experiment, and participants may use them to predict or prepare

for upcoming stimuli, which may result in more accurate or faster responses, rather than interfering on the majority of trials. In the reported studies, the proportion of trials for each condition influenced the congruence in relation to stimuli and responses (**see Figure S1.1 in appendix 1**). In Cragg (Cragg, 2016), Hirst and colleagues (Hirst et al., 2019) and Jongen and colleagues (Jongen & Jonkman, 2008), the proportion of trials for each condition was equal. Looking at the stimulus level (the colour of target and distractors), 33.3% trials were congruent and 66.7% trials were incongruent, resulting in a stimulus imbalance. Looking at the response level (the response associated with the target and distractors), 66.7% trials were congruent and 33.3% were incongruent, resulting in a response imbalance at the same time. In Killikelly and Szucs (Killikelly & Szucs, 2013), the number of response incongruent trials was doubled in comparison to the other conditions, and this doubling gave an equal proportion of congruent and incongruent trials at the response level (50% and 50%), and an unequal proportion of congruent and incongruent trials at the stimulus level (25% and 75%, respectively). In these experiments, researchers must decide whether to balance the proportion of trials with respect to the stimuli or the responses.

To our knowledge, no previous study has taken into account these two aspects in the experimental design and data analysis, which may affect the interpretation of results, and complicate between-study comparisons. In the present study, the

first aim was to test whether the ratio or absolute scores are a better measure of flanker interference effects.

The second aim was to compare the interference effects in stimulus- and response-balanced conditions, in which there were different proportions of stimulus- and response-congruent trials. In the stimulus-balanced (response-imbalanced) condition, the number of congruent trials was doubled in order to balance at the stimulus level, so 75% of trials were response-compatible and 25% response-incompatible. In the response-balanced condition (stimulus-imbalanced), the number of SRI trials was double that of the other conditions in order to balance at response level, so 50% of trials were response-compatible and 50% response-incompatible. Increasing the proportion of SRI trials decreases the interference effect (Schmidt & Besner, 2008). We expected to find greater response interference effects in the stimulus-balanced condition because the distractors of the SI trials – associated with the congruent response – facilitate more accurate and faster responses, increasing the difference between the SI and SRI trials. In response-balanced conditions, there is the same probability that the distractors are associated with a congruent or incongruent response, which should prevent any facilitation of the response.

Taking into account these two methodological factors, the main aim was then to assess how stimulus, response and general interference effects differ across three age groups, from 6-14, 20-43, and 60-84 years.

2.2 METHOD

2.2.1 Participants

We recruited 150 participants in three age groups: 92 children (6-14 years old, mean \pm SD=8.8 \pm 2.1 years, 47 females, 83 right-handed by self-report), 25 young adults (20-43 years old, mean \pm SD=28.2 \pm 5.1 years, 16 females, 21 right-handed and 1 ambidextrous by self-report) and 33 older adults (60-84 years old, mean \pm SD=70.2 \pm 6.5 years, 19 females, 19 right-handed, 4 left-handed, 1 ambidextrous, 9 not reported). We recruited child and older-adult participants during two outreach events, the duration of which was set a priori, resulting mostly in convenience sampling; no power analysis was performed to justify the sizes of each group.

Young adults were students and staff at the University of Nottingham. They were recruited via posters and email advertisements to a mailing list of participants who had previously participated in other experiments. Children, and one third of the older adults, were recruited via public engagement events at the University:

children via 'Summer Scientist Week' (July 2019) and older adults via 'Silver Scientist Day' (June 2019) and a 'Wellbeing Age Conference' (October 2019). The remaining older adults were recruited via the University's volunteer database and word of mouth. Children received tokens to be spent on games at the event, and older adults who participated received a university gadget. Young adults and some older adults received an inconvenience allowance of £5.

All participants were fluent in English and reported no history of neurological or psychiatric disorders. All had normal or corrected to normal vision. Older adults tested in laboratory were examined with a 'Snellen chart' (Snellen, 1862) to test visual acuity (mean \pm SE=7.57 \pm 0.76). The Montreal Cognitive Assessment (MoCA) was used to test cognitive functioning (mean \pm SE=25.97 \pm 2.50). The correlations between the MOCA score and the perceptual, response and general interference effect are shown in **appendix 1**. The older adults understood the task instructions, and all performed the task better than chance (binomial test, $p \leq .05$). One participant did not perform the MoCA test due to a lack of time during the public event.

Written informed consent was obtained from each participant, and the children's carer before inclusion in the study. The study received ethical approval from the research ethics committee of the University of Nottingham (reference: SoPEC1196).

2.2.2 Stimuli and apparatus

Stimuli were coloured squares on a black background. The RGB codes of each colour were: red= [255 0 0], blue= [0 0 255], green= [0 255 0], yellow= [255 255 0]. The sides of each square were 2.5° from a viewing distance of 57cm, and there was 1.3° edge-to-edge separation between adjacent squares.

Stimuli were presented via a desktop Lenovo PC running Windows7 on a monitor 16" (resolution 1920x1080) for participants tested in the laboratory, and via a HP ProBook 430 G2 running Windows 7 on a monitor 15.4" (resolution 1366x768) for participants recruited via the public engagement events. Responses were collected with a standard high-speed USB2 computer keyboard. Stimulus presentation and response collection were controlled by custom scripts written using MATLAB and PsychToolBox 3 libraries (Brainard, 1997). IBM SPSS Statistics 21.0 was used for statistical analysis. All code and raw data are freely available at <https://osf.io/sx3je/>.

2.2.3 Task description

A coloured version of the Flanker task was administered. Two flanker squares of the same colour (red, blue, green or yellow) were presented to the left and right of, and 200ms earlier than, a middle square that was the target. In the *C condition*, the target and flankers were the same colour. In the *SI condition* the target and flankers differed in colour but were both mapped to the same response

hand and button. In the *SRI condition*, the target and flanker colours were different and were mapped to different response hands and buttons, evoking both stimulus and response interference at the same time. Participants were instructed to respond to the colour of the middle square (the target) using the left and right index fingers, and to ignore the surrounding squares (the flankers). They were encouraged to respond as quickly and as accurately as possible.

The colours were associated with two response buttons on a standard QWERTY keyboard; two colours were associated with a left key and left index finger response (button 'c') and two colours were associated with a right key and right index finger response (button 'm'). The four colours were combined in three response assignments (red/blue and green/yellow; red/green and blue/yellow; red/yellow and blue/green) that were counterbalanced across participants. Coloured square labels of the colours assigned to each key response were placed above the relevant key during the entire experiment to act as a reminder to participants.

2.2.4 Procedure

The participants were sitting comfortably in a quiet and dimly lit room, with their chin on a head-and-chin rest at a viewing distance of 57 cm from the monitor. Children did not use the head-and-chin rest because it was reported as uncomfortable by the first few children. Before starting the experiment, each participant

was asked to name the colour of four coloured squares (red, blue, green and yellow) shown on the screen, to check for gross colour-blindness.

As shown in **Figure 2.1a**, each trial started with the presentation of a fixation cross in the middle of the screen for a pseudorandom interval of uniform distribution 1000-3000 ms. Two flanker squares were presented, then 200 ms later, the target was presented between the two flankers until the participant's response or until a maximum of 2000 ms, before the stimuli were removed.

Children went through one practice block of 16 pseudorandomised trials. The practice blocks were counterbalanced between stimulus-balanced and response-balanced conditions between participants. Young and older adults went through two practice blocks of 32 pseudorandomised trials each. The practice blocks were counterbalanced between stimulus-balanced and response-balanced conditions. For the young adults, if accuracy in the practice blocks was lower than 75% and/or the mean reaction time (RT) was longer than 500 ms, feedback was shown to encourage them to improve accuracy and/or speed in the next block. In this case, each practice block was repeated up to three times, before the participant started the experiment. For children and older adults, feedback was shown when accuracy was lower than 75% and/or RT longer than 900 and 700 ms respectively. In this case, the blocks were not repeated, due to the lack of time during events, and the participants then went through the experiment.

During the experiment, stimulus-balanced and response-balanced conditions were blocked and presented in counterbalanced order across participants. In stimulus-balanced (response-imbalanced) blocks, the proportion of C trials was double that in the other conditions (50% C, 25% SI, 25% SRI) whereas in response-balanced (stimulus-imbalanced) blocks, the proportion of SRI trials was double that in the other conditions (25% C, 25% SI, 50% SRI) (**Figure 2.1b**). Children performed one stimulus-balanced block (16 C, 8 SI, 8 SRI trials) and one response-balanced block (8 C, 8 SI, 16 SRI trials), giving 64 trials in total. Young adults went through two stimulus-balanced blocks (48 C, 24 SI and 24 SRI trials) and two response-balanced blocks (24 C, 24 SI and 48 SRI trials), giving 384 trials in total. Older adults went through one stimulus-balanced block (32 C, 16 SI, 16 SRI trials) and one response-balanced block (16 C, 16 SI, 32 SRI trials), giving 128 trials in total.

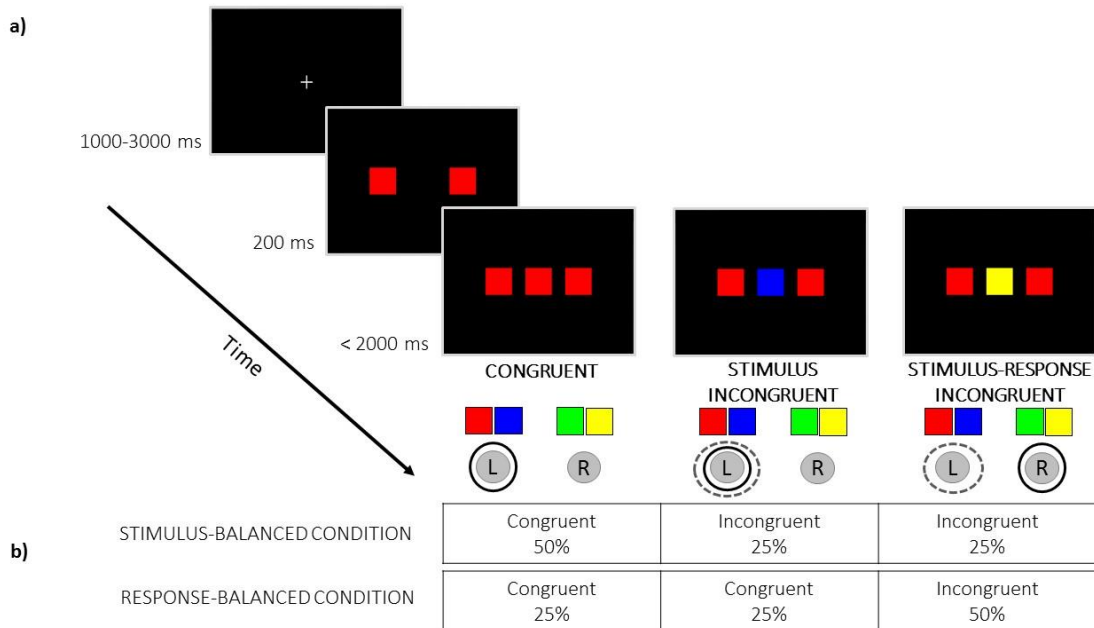


Figure 2.1 a) The illustration of trial sequence in the colour version of Flanker task. The stimuli are not to scale. L: left response button, R: right response button
b) The proportion of congruent trials in the stimulus-balanced and response-balanced conditions in our study.

2.2.5 Statistical analysis

Eleven children were excluded from the analysis. During the public engagement event, every child was allowed to take part in the study, but the data were not considered for analysis when the children were younger than six years old, as performance was very poor. Seven children were excluded for this reason. Two children were excluded because their careers reported diagnoses of developmental disorders, and two due to technical problems during the experiment. A

final sample of 81 children were considered for analysis. One young adult was excluded because they were unable to complete the study, therefore a sample of 24 young adults were considered for analysis. Two older adults were excluded: one reported a diagnosed colour blindness, and one was unable to complete the study. A sample of 31 older adults were included in the analysis. Using binomial tests, all participants performed better than chance at the $p \leq .05$ level: children were correct on at least 38 out of 64 trials, young adults on at least 208 out of 384 trials, and older adults on at least 73 out of 128 trials.

A log-10 transformation of the variable age was performed in order to address the skewed distribution when looking at age as a continuous predictor. Percentage of correct responses and mean RTs for correct responses were calculated. These measures were combined in inverse efficiency score (IES) (**see appendix 1**). IES consists of RTs divided by the proportion of correct responses (Bruyer, 2011; Townsend, 1978). Greater values of IES indicate worse performance. The mean of percentage of correct responses, RTs and IES were calculated for C, SI and SRI conditions for each participant within and between blocks. The descriptive statistics for the three groups are reported in **Table 2.1**.

Ratio and absolute scores between interference conditions were calculated to isolate the three interference effects using IES (De Houwer, 2003). We looked at all the interference measures, but only absolute scores were used to test the

main experimental hypotheses on the basis of the preliminary results (reported below). Respectively, the ratio and absolute scores of the stimulus or perceptual interference effect were calculated by dividing or subtracting the SI and C conditions (SI/C or SI-C); the response interference effect was calculated by dividing or subtracting the SRI and SI conditions (SRI/SI or SRI-SI). Lastly, a general interference effect was calculated by dividing or subtracting the SRI and C condition (SRI/C or SRI-C). Ratio scores greater than 1, and absolute scores greater than 0 indicate interference effects. These scores were calculated within and between blocks. Statistical significance was assessed with $\alpha = 0.05$. Degrees of freedom and p values were corrected using Greenhouse–Geisser estimates of sphericity where the assumption was violated. The partial eta-squared were calculated as an estimate of the effect size (Lakens, 2013). We performed a sensitivity analysis to calculate the smallest effect size of interest that could be detected with the sample size recruited ($\alpha=.05$, $\text{power}=.80$) (Lakens, 2014). In this Chapter, effect sizes larger than $d= 0.23$ will be statistically significant with 150 participants.

		Total		Congruent		Stimulus Incongruent		Stimulus Response Incongruent	
		M	(SD)	M	(SD)	M	(SD)	M	(SD)
Children (n=81)	Accuracy (%)	94.1	(6.14)	95.6	(5.84)	95.7	(7.03)	91.4	(9.40)
	RT (ms)	861	(181)	823	(172)	853	(197)	913	(193)
	IES (ms)	928	(260)	870	(217)	911	(287)	1028	(337)
Young adults (n=24)	Accuracy (%)	92.6	(4.92)	94.5	(4.39)	94.2	(4.52)	88.9	(7.63)
	RT (ms)	456	(66.2)	427	(69.6)	457	(69.0)	492	(66.4)
	IES (ms)	493	(72.5)	454	(76.8)	487	(74.5)	558	(84.2)
Older adults (n=31)	Accuracy (%)	97.2	(4.30)	97.1	(4.75)	98.1	(3.76)	96.8	(5.28)
	RT (ms)	630	(114)	616	(120)	631	(113)	649	(116)
	IES (ms)	654	(132)	640	(137)	647	(122)	677	(141)

Table 2.1 Mean (M) and standard deviation (SD) of proportion of correct responses (accuracy), reaction times (RT), and inverse efficiency scores (IES), for each age group, under total, congruent, stimulus incongruent, and stimulus response incongruent condition across blocks.

2.3 RESULTS

2.3.1 Measures of interference effect

The ratio scores and absolute scores of the general interference effect were significantly correlated ($r(133)=.865$, $p<.001$). Greater interference effect measured using ratio scores correlated with greater interference effect measured

using absolute scores. A bootstrap resampling technique with 10000 iterations was used to investigate whether ratio or absolute scores were better – in terms of their distributions – as a parametric measure of the interference effect. This analysis showed that resampled ratio and absolute scores were similarly normally distributed, although a Q-Q plot of the original raw data revealed a larger deviation from normality at the upper tail for general interference effects measured using ratio scores in comparison to absolute scores. Overall, there was no strong statistical reason to conclude that one measure was better than other, and both relative and absolute scores may be good measures of the interference effect. To simplify our analyses, and based on their marginally-better distributions, we used the absolute scores in the sequent sections.

2.3.2 Stimulus-balanced versus response-balanced

A repeated measures ANOVA with condition (*stimulus-balanced versus response-balanced*) as within-subject factor and $\log_{10}(\text{age})$ as a covariate was performed separately for perceptual, response and general interference effects. As shown in the following equation it was not possible to compare the perceptual (SI-C) and response interference (SRI-SI) effects in the two conditions using a multivariate ANOVA. Separate analyses are required because both perceptual and response interference effects share variance from the SI condition.

Percentual interference - Response interference

$$\begin{aligned}
&= (SI - C) - (SRI - SI) \\
&= SI - C - SRI + SI \\
&= 2(SI) - C - RI
\end{aligned}$$

For perceptual, response and general interference, there was no significant effect of condition ($F(1,134)=0.682$, $p=.410$, $\eta^2=.00506$, $MSE=15138$; $F(1,134)=0.087$, $p=.769$, $\eta^2=.000649$, $MSE=24646$; $F(1,134)=0.863$, $p=.355$, $\eta^2=.00640$, $MSE=25320$ respectively). Although the differences were in the predicted direction, interference effects were comparable in stimulus-balanced and response-balanced conditions (**Table 2.2**). There was no significant interaction between condition and age for perceptual, response and general interference effects ($F(1,134)=0.159$, $p=.691$, $\eta^2=.00119$, $MSE=15138$; $F(1,134)=0.002$, $p=.965$, $\eta^2=.0000149$, $MSE=24646$; $F(1,134)=0.070$, $p=.792$, $\eta^2=.000522$, $MSE=25320$, respectively) (**Figure 2.2**).

	Stimulus-balanced (75% response- congruency)			Response-balanced (50% response- congruency)		
	M	SD	<i>d</i>	M	SD	<i>d</i>
Perceptual interference	42.9	118	0.364	20.4	150	0.136
Response interference	100	174	0.575	78.6	139	0.565
General interference	143	179	0.799	98.9	215	0.460

Table 2.2 Mean (*M*), standard deviation (*SD*), effect size (Cohen's *d*) of interference effects in stimulus and response balanced conditions.

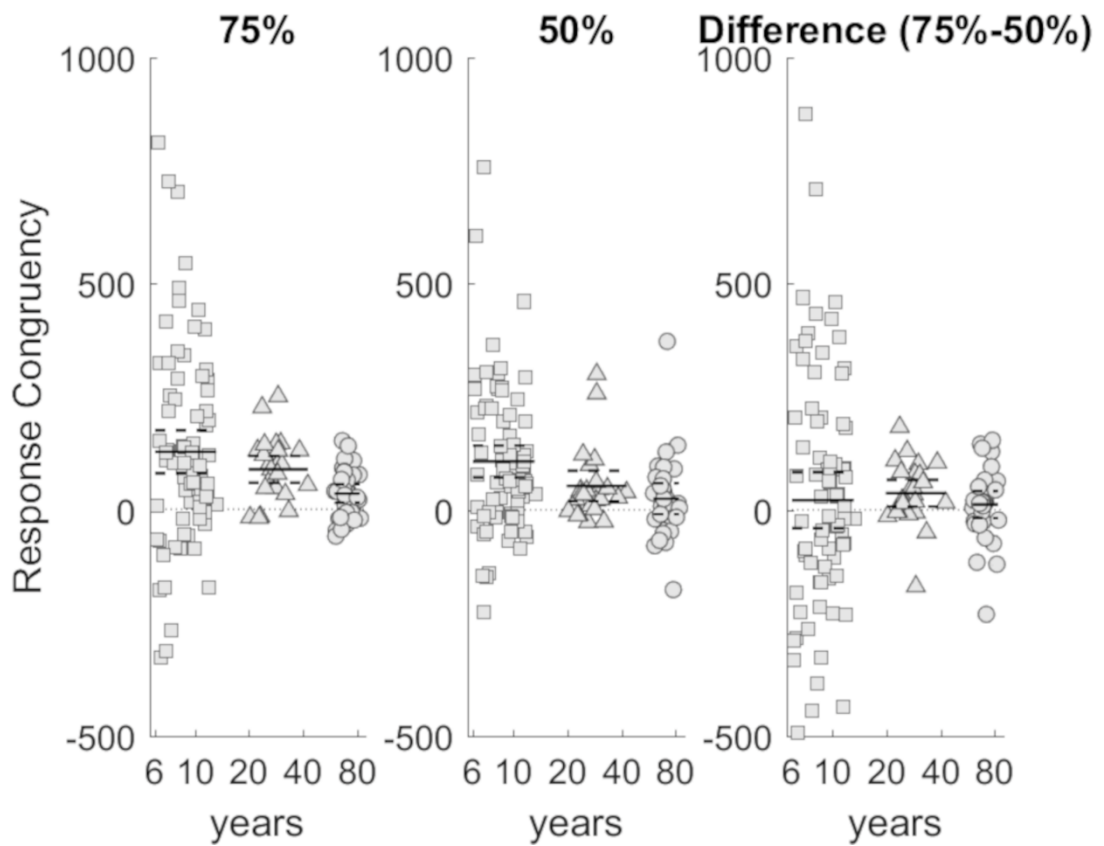


Figure 2.2 Response interference effect in stimulus-balanced (75% response-congruency) and response-balanced (50% response-congruency) condition. Children (squares), young (triangles) and older adults (circles). Mean and 95% confidence intervals reported for each age group.

2.3.3 Perceptual, response and general interference effects across age groups

A univariate ANOVA was performed, submitting the perceptual, response and general interference effects as dependent variables and $\log_{10}(\text{age})$ as a covariate. Significant effects of age were planned to be followed-up with independent sample t-tests to compare interference effects between the age groups.

For the perceptual interference effect, a significant effect of age was found, $F(1,134)=5.31$, $p=.023$, $\eta^2=.0381$, $MSE=10297$. A subsequent analysis between groups showed that children and young adults experienced more perceptual interference than older adults. Children and young adults showed comparable perceptual interference. For the response interference effect, a significant effect of age, $F(1,134)=16.9$, $p<.001$, $\eta^2=.112$, $MSE=11317$, found that children showed more response interference compared with both young adults and older adults, and young adults showed more response interference than older adults. For the general interference effect, a significant effect of age, $F(1,134)=19.2$, $p<.001$, $\eta^2=.125$, $MSE=23483$ showed that children had greater general interference compared to young adults and older adults. Young adults showed more interference than older adults (**Table 2.3**). As illustrated in **Figure 2.3**, younger people showed greater perceptual, response and general interference compared to older people.

	Children (N=81)			Young adults (N=24)			Older adults (N=31)		
	M	SD	<i>d</i>	M	SD	<i>d</i>	M	SD	<i>d</i>
Perceptual	40.8	129	0.316	32.7	19.9	1.64	6.84	47.9	0.143
Response	118	127	0.929	71.1	66.5	1.07	30.3	65.3	0.464
General	158	196	0.806	104	67.7	1.54	37.2	47.3	0.786

	Children vs Young adults				Children vs Older adults				Young adults vs Older adults			
	t	df	<i>d</i>	p	t	df	<i>d</i>	p	t	df	<i>d</i>	p
Perceptual	0.540	91.3	0.053	.591	2.03	110	0.192	.045	2.72	42.2	0.367	.009
Response	2.37	74.7	0.231	.020	4.75	101	0.449	<.001	2.28	53	0.307	.027
General	2.11	101	0.206	.037	5.18	99.9	0.489	<.001	4.30	53	0.580	<.001

Table 2.3 *Perceptual, response and general interference effects across age groups. Mean (M), standard deviation (SD), T-test (t), degree of freedom (df), effect size (Cohen's d).*

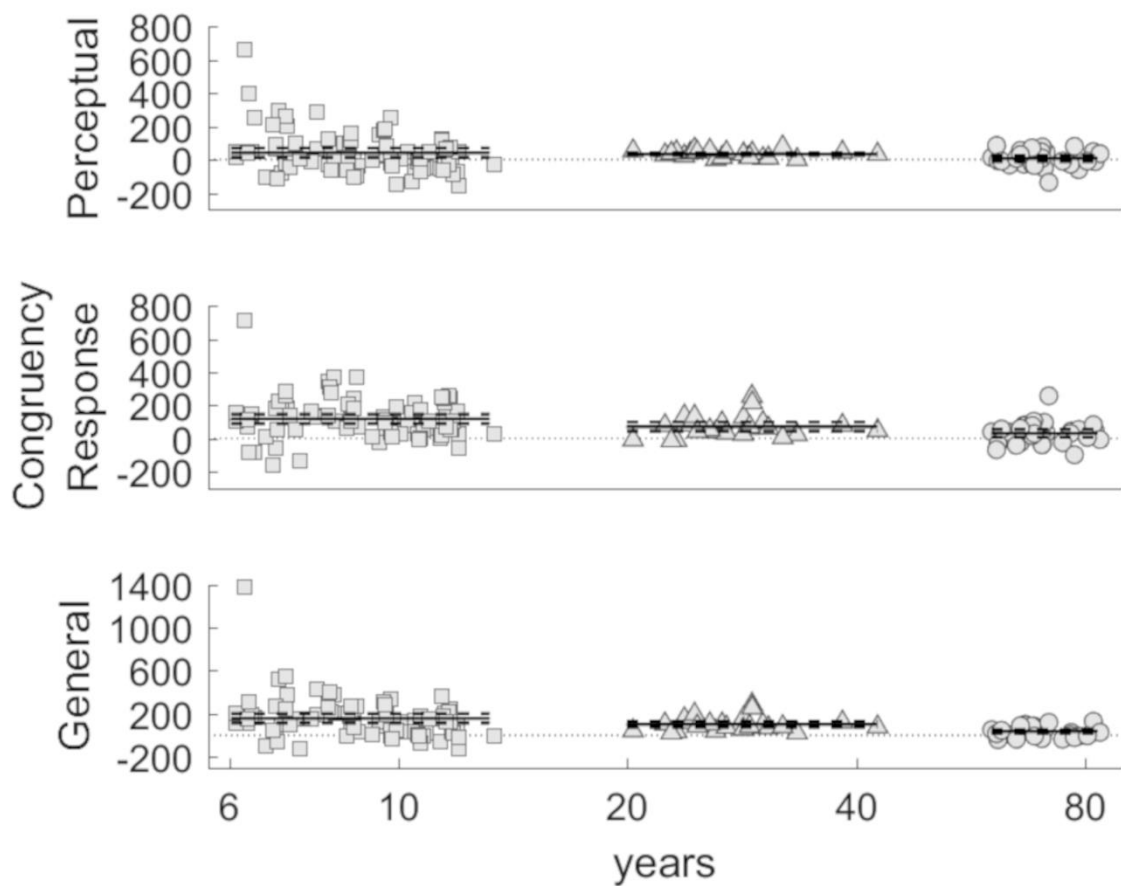


Figure 2.3 Perceptual, response and general interference effects are displayed across age groups: children (squares), young (triangles) and older adults (circles) Mean and 95% confidence interval are shown for each age group.

2.3.4 Comparing the current data with Cragg (2016) and Hirst et al. (2019)

We asked Cragg (Cragg, 2016) and Hirst and colleagues (Hirst et al., 2019) if we could reanalyse the raw data of their studies to clarify the differences between studies. We calculated IES and the absolute interference effects. We

calculated Cohen's d to allow comparison among studies. Summary data are available in **Table S1.1 in appendix 1**. Our findings were generally consistent with these previous studies and across age groups, however both the absolute (ms) and statistical (Cohen's d) effect sizes varied substantially, making definitive conclusions hard to draw.

2.3.5 Results using the same number of trials across groups

In response to a reviewer of this work, we analysed again the data using 32 trials of the stimulus-balanced block (the first 16 C, 8 SI and 8 SRI trials) and 32 trials of the response-balanced block (the first 8 C, 8 SI and 16 SRI trials) in young and older adults, so that each age group had the same number of trials. All trials of each condition were used for children. The ratio and absolute scores of the general interference effect were significantly correlated ($r(133)=0.863$, $p<.001$). Resampled ratio and absolute scores were similarly normally distributed, again the Q-Q plot revealed a larger deviation from normality at the upper tail for general interference effect measured using ratio scores in comparison to absolute scores. There was no significant effect of condition (*stimulus-balanced versus response-balanced*) for perceptual, response and general interference ($F(1,134)=0.693$, $p=.407$, $\eta^2=.00515$, $MSE=16817$; $F(1,134)=0.418$, $p=.519$, $\eta^2=.00311$, $MSE=27466$; $F(1,134)=1.66$ $p=.200$, $\eta^2=.0122$, $MSE=27863$ respectively) (**see Table S1.2 in appendix 1**). For perceptual, response and general interference effect, a significant effect of age was reported ($F(1,134)=7.52$,

$p=.007$, $\eta^2=.0531$, $MSE=10930$; $F(1,134)=12.7$, $p=.001$, $\eta^2=.0866$, $MSE=13024$; $F(1,134)=19.4$, $p<.001$, $\eta^2=.127$, $MSE=24743$ respectively) (**see Table S1.3 in appendix 1**). In summary, the interference effects decreased significantly with age when a reduced number of trials were considered (**Figure 2.4**).

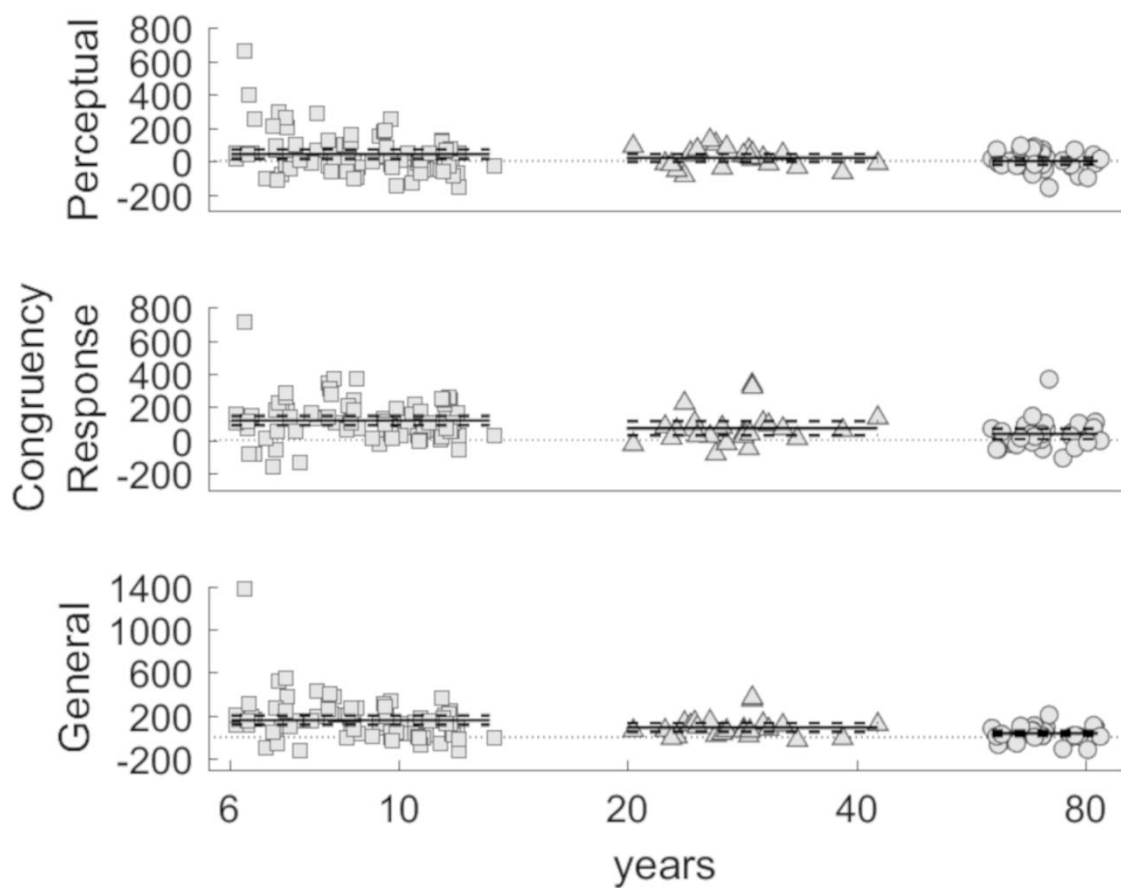


Figure 2.4 Using the same number of trials, perceptual, response and general interference effects are displayed across age groups: children (squares), young

(triangles) and older adults (circles). Mean and 95% confidence interval are shown for each age group.

2.4 DISCUSSION

In our study, we examined how children, younger and older adults resolve interference originating from irrelevant stimuli, competing responses, and from both competing stimuli and responses at the same time. Looking across the literature (Cragg, 2016; Hirst et al., 2019; Jongen & Jonkman, 2008; Killikelly & Szucs, 2013), it was difficult to answer the question because different measures of interference have been used. Furthermore, previous studies did not all take into account the observation that the proportions of stimulus-response congruence may influence the size of the response interference effect, and thus the comparison between this and others interference effects. In our study, we took into account each of these two methodological aspects, discussed in more detail below, in order to study the interference effect across age.

First, we focused on the specific measure of interference to disentangle whether the ratio of congruent and incongruent (ratio scores) or the difference between these conditions (absolute scores) was a more appropriate measure of the general interference effect. In our study, we chose the absolute scores to measure the interference effects because the data were marginally better distributed. However, we did not find evidence to suggest that measuring the interference effect

using absolute scores is better than the ratio scores of the interference effect or vice versa. Without further exploration or modelling both methods could reasonably be used to measure the interference effect.

Second, we examined whether response interference was greater in stimulus-compared to response-balanced conditions. Unexpectedly, we did not find a significant difference in interference effects between these conditions. Although the difference in response interference effects between stimulus- and response-balanced conditions was in the predicted direction, our manipulation of the proportion of response-congruent trials was not successful. Schmidt and Besner (Schmidt & Besner, 2008) reported a 27 ms and 5% advantage in their stimulus-balanced (609 ms and 32%) compared to the response-balanced condition (636 ms and 37%) when reaction times and percentage of errors were analysed separately. To facilitate the comparison between studies and interpret these results, we re-analysed the percentage of correct responses and reaction times separately. We found that interference effects were comparable between stimulus- and response-balanced conditions for percentage correct responses, but for reaction times the perceptual interference effect was 28 ms greater in stimulus- (41 ms) than response-balanced conditions (13 ms). Our results therefore match well the 27 ms differences in the previous study (Schmidt & Besner, 2008). We suppose that in our study facilitation in the stimulus-balanced condition may have been obscured in part by accuracy ceiling effects in each age group.

Taking into account the above methodological aspects, the main question was to understand how children, younger and older adults resolve perceptual, response and general interference. In our study, the interference effects significantly decreased with age: younger people showed more difficulties compared to older people when they had to ignore irrelevant information. Specifically, children experienced more perceptual, response and general interference compared to young adults, who showed more interference compared to older adults. Our findings are consistent with previous studies (Cragg, 2016; Jongen & Jonkman, 2008) in which a significant interaction between interference at the stimulus level and age was reported. The perceptual interference effect decreased with age; specifically, children showed longer reaction times (Cragg, 2016) in comparison to young adults. In the same direction for the response interference effect, Jongen and colleagues (Jongen & Jonkman, 2008) reported that children showed more errors in comparison to young adults when they had to solve the interference originating from conflicting responses. Our results, however, seem to be inconsistent with other findings (Cragg, 2016; Hirst et al., 2019; Killikelly & Szucs, 2013). Cragg (Cragg, 2016) reported that children and young adults showed comparable performance in the presence of interfering responses. Both Killikelly and colleagues (Killikelly & Szucs, 2013) and Hirst and colleagues (Hirst et al., 2019) did not find a significant effect of age with either perceptual or response interference effects in terms of both accuracy and reaction times. We could not compare the sample recruited in Killikelly and colleagues' study, because the authors

tested adolescents, younger and middle-age adults and our sample was not comparable in terms of age. In order to better compare the studies, we reanalysed the original data from two similar previous studies (Cragg, 2016; Hirst et al., 2019), adjusting for the measure of interference effect (absolute scores). In our study, children showed significant perceptual and response interference effects. These findings are in line with Cragg's results in which children of the same age showed significant perceptual and response interference. Regarding the middle group, we found that young adults showed interference at each level, also in this case our findings are similar to Cragg's findings which showed a significant response interference in the young adults group. Finally, our data showed that older adults are less vulnerable to the influence of interfering information. These data do not therefore agree with the finding of Hirst and colleagues in which older adults showed a significant perceptual interference effect.

Because in our study older adults did not show a significant perceptual interference it will be interesting to investigate in future studies the reasons behind this discrepancy with other studies. This result appears to contradict prevailing theories of cognitive decline in older adults (Li et al., 2009). We hypothesize that neural compensatory mechanisms may provide an explanation for this difference in behavioural performance.

In older adults, it is possible that increased neural activity, particularly in the dorsolateral prefrontal cortex and anterior cingulate cortex, may compensate for deficient mechanisms related to interference control (Salami et al., 2014).

In supplementary materials, we have re-analysed the data using ratio scores. We found that perceptual, response and general interference effects were comparable between the stimulus and response balanced conditions. In addition, we found an effect of age for the response and general interference, but we did not find the same influence for the perceptual interference effect. Looking at the data, young adults showed more interference compared to children who showed more interference compared to older adults. These findings are different from what we found when using absolute scores. At this stage, we find ourselves at a point where, without a well-developed theoretical model of the neurophysiological processes underlying these cognitive effects, it is difficult to know for sure how best to measure flanker interference across the lifespan.

In our study there are some limitations. First, there were differences in experimental setting between the different age groups, most importantly the number of practice blocks and the number of trials. In order to control for this aspect, we reanalysed the data using the same number of trials (64) for each age group, and we still found the same effect of age on the interference effects. Second, some participants were tested during public outreach events and others were tested

under stricter laboratory situations, using different screens and keyboards. While these differences should affect all three experimental conditions similarly (C, SI, SRI), it is possible that the within-participant effect-sizes depended partly on these experimental factors. Future studies should aim to minimise experimental differences between age groups wherever possible.

In sum, we found that people of all ages experienced significant interference when they had to ignore irrelevant information originating from interfering stimuli, responses and both at the same time. We also found that interference effects decrease significantly with age. However, comparing the data across three similar reports, the effect-sizes varied substantially both across studies and age groups. Perceptual interference effects were, overall, quite weak, while response and general interference effects were much stronger. Meta-analytic methods, modelling, or large-scale studies with identical methods across age groups may now be required to answer the question whether perceptual and response interference changes significantly across the lifespan.

CHAPTER 3 - TEMPORAL DYNAMICS OF THE MOTOR RESPONSE AT PERIPHERAL LEVEL IN THE FLANKER INTERFERENCE TASK.

3.1 INTRODUCTION

In the Flanker task, participants have to respond to the target (according to instructions) and ignore the distractors. Performance measured by discrete button presses is more accurate and faster in the C condition in comparison to the incongruent condition where target and distractors are associated with different responses resulting in a competition between hands which has to be solved before response execution.

Some studies have investigated the competition during execution of motor responses across time (Killikelly & Szucs, 2013; Szucs et al., 2009). EMG is a fruitful method to study, continuously, response competition at the motor level. Because it is applied to both hands, in these studies it allowed researchers to investigate the activity directly in chosen and unchosen hands simultaneously and continuously. Szucs and colleagues (Szucs et al., 2009) recorded EMG activity over the flexors of the thumb, specifically the flexor pollicis brevis of both hands in a group of adults. Participants had to press the left or right button on a video game controller using their thumbs, according to whether the physically larger number appeared on the left or right side of the screen. Their findings showed significant EMG activity in the chosen hand during the C trials. In incongruent trials, in which

participants pressed the correct response, the authors also reported a significant activation of the unchosen hand between 222-322 ms after the stimulus onset. Analysing the data of each participant, in minority of trials (30-50%), increased EMG activity in the unchosen hand was detected prior to a chosen hand response, and in majority of the trials (about 50-70%), the chosen and unchosen response activations were concurrent. The authors did not find any significant activity in the unchosen hand which was temporally posterior to the chosen hand activation. These findings agreed with a previous study (Caldas et al., 2012), in which the authors found, in 14% across correct trials, a detectable EMG activation in unchosen hand which preceded the EMG activation of chosen hand.

In the same laboratory, Killikelly and Szucs (Killikelly & Szucs, 2013) recorded EMG activity over the left and right flexors of the thumb in a group of young adults. The authors investigated how distracting stimuli and competing responses separately interfered with execution of the motor response. They used a modified version of the Stroop Task. Participants had to press, using their thumbs, the left button on a video game controller if the ink colour of a colour word was red or green and the right button if the ink colour was yellow or blue. Regarding EMG activity in the chosen hand, the results showed a significant activation across all conditions. In the unchosen hand, the authors reported activation between 460-

480 ms after stimulus onset across all conditions. The C condition had significantly lesser amplitude than the SRI condition, and the SI condition significantly lesser amplitude than the SRI condition.

Looking at the activation, the above authors showed in the incongruent condition a significant activation of the chosen hand. The authors expected this result because it was the hand which was supposed to respond. Unexpectedly, the authors also showed in the incongruent condition an activation of the unchosen hand, which was supposed not to respond. This surprising finding was possible due to continuous measurement of hand muscle activity. Looking at the timing, the above authors used different versions of the Flanker task, making difficult the comparison between findings. Szucs and colleagues (Szucs et al., 2009) used the number version of the Flanker task. They studied hand competition in the incongruent condition compared with congruent condition. Using a modified version of the Stroop task, Killikelly and colleagues (Killikelly & Szucs, 2013) investigated hand competition in SI, SRI and C conditions. The task allowed the researchers to investigate the motor mechanisms when irrelevant stimuli and competing responses interfered with response separately.

Bearing in mind that these findings are not fully comparable, different hypotheses arise: the first considers a simultaneous activation of both hands resulting in a

competition, until one hand is chosen for response. According to a different hypothesis, there is a premature activation of the incorrect hand, related to the flankers, which precedes the activation of chosen hand related to the target.

In this study, the aim was to investigate the time course of the activity in chosen and unchosen hands using the coloured version of the Flanker task. We hypothesised to find an activation of chosen hand across all conditions. Based on previous literature, the chosen hand will be prematurely activated in SI condition because flankers and target require the same response, whereas in SRI condition we predicted to find a premature activation of the unchosen hand, related to the flanker, and a later activation of the chosen hand, related to the target. We measured the hand activity with grip forces and the EMG activity over the flexor digitorum superficialis (FDS) and the extensor digitorum communis (EDC) muscles of both forearms. We chose these muscles because they were involved in flexing and extending, respectively, all the hand's fingers, and there were no signal artefacts because the grip force devices were not pressing against the electrodes.

3.2 METHOD

Methods were the same as described in the previous Chapter, except where indicated below.

3.2.1 Participants

We recruited 20 adults (19-44 years old, mean \pm SD=30.2 \pm 7.8 years, 13 females, 18 right-handed and 2 ambidextrous by self-report). They were students and staff at the University of Nottingham, recruited via posters and email advertisements to a mailing list of participants who had previously participated in other experiments. They received an inconvenience allowance of £10 for taking part. All participants were fluent in English and reported no history of neurological or psychiatric disorders. All had normal or corrected to normal vision. Written informed consent was obtained from each participant. The study received ethical approval from the research ethics committee of the University of Nottingham (reference: SoPEC1172).

3.2.2 Stimuli, task and apparatus

The coloured version of the Flanker task was administered (**Figure 2.1a**). A first computer was used for running the experiment under MATLAB and PsychToolBox 3 libraries (Brainard, 1997). Stimuli were presented via a desktop Lenovo PC running Windows 7 on a monitor 16" (resolution 1920x1080). Responses were collected with hand-held grip force sensors ('dynamometer', AD Instruments) and a standard high-speed USB2 computer keyboard (used for the first block of practice trials). Participants were instructed to maintain a steady grip of around 1.5% of maximum voluntary contraction during the task, and then to respond to the colour of the middle square (the target) by squeezing the response

dynamometer, and to ignore the surrounding squares (the flankers). All code for running the experiment are freely available at <https://osf.io/9tegc/>.

3.2.3 Procedure

Safety measures for COVID-19 were adopted according with the government guidelines.

Participants went through two practice blocks of 16 pseudorandomised trials each. In the first practice block, participants were instructed to press the left response key (button 'c') using the left index finger or a right response key (button 'm') using the right index finger on a standard QWERTY keyboard. If accuracy was lower than 87% and/or the mean RT was longer than 500 ms, feedback was shown to encourage them to improve accuracy and/or speed in the next practice block. In this case, the practice block was repeated up to three times. We increased the accuracy cut-off to reach the same probability of guessing. In the second practice block and during the experiment, participants were instructed to squeeze the dynamometers according to the instructions. During the experiment, participants went through six response-balanced blocks (16 C, 16 SI and 32 SRI trials), giving 384 trials in total.

3.2.4 Dynamometer and EMG apparatus and processing

Grip forces and muscle activity were recorded using dynamometers and EMG, respectively. The skin of participants' forearms was cleaned with alcohol wipes. Two electrodes were placed on the skin overlying the FDS muscle and two were placed along the EDC muscle of both forearms. The reference electrode straps were positioned around the bony region of both wrists.

A second computer was used to acquire EMG and grip force sensor data acquisition under LabChart 8 software (AD Instruments). Grip forces and EMG signals were continuously sampled at 2kHz. Grip force data were low-pass filtered at 5 Hz. The EMG data were band-pass filtered between 10 and 500 Hz. Offline, grip data were filtered with a second-order dual-pass Butterworth filter with a bandpass of 1–10 Hz and then adjusted for individual differences in force, by subtracting the minimum grip force and dividing by the maximum grip force within each dynamometer (to result in a relative force measurement from 0 to 1). EMG data were rectified and filtered with a second-order dual-pass Butterworth filter with a bandpass of 1–10 Hz and then adjusted for individual differences in EMG activity. We divided by the maximum EMG activity within each EMG channel (i.e., relative EMG activity). Dynamometer and EMG signals were corrected for baseline, which was calculated relative to the -250 to 0 ms interval preceding presentation of the flankers, and then subtracted from the data. Trigger signals were sent from the first computer at the onsets of the trial and flanker stimuli. Additional signals

before flanker onset encoded the correct response for the trial (one trigger=left response required, two triggers=right response required). The signals were digitized using LabChart 8 (AD Instruments) and data were analysed using custom scripts in MATLAB.

3.2.5 Data and statistical analysis

We analysed trials in which participants pressed the correct response. All descriptive results are reported in **Table 3.1 and Table 3.2**. Based on the sensitivity analysis ($\alpha=.05$, power=.80), effect sizes larger than $d= 0.63$ will be statistically significant with 20 participants (Lakens, 2014).

To calculate the RT from a continuously-varying signal (the mean and SD of the grip force), we had to set an (arbitrary) cut-off or threshold for what counts as a response (i.e., a change from the baseline signal). To do this, we decided to look for a level that led to approximately 90% 'hits' (correct responses to a target; e.g., right hand pressed when a right response was required) and 10% 'false alarms' (i.e., left hand pressed when a right response was required). This level of hits and false alarms corresponds to a d-prime score of 2.64. We then found the first time-point in the grip signal which led to a d-prime of at least 2.64. This d-prime level was first exceeded at the group level when with a mean+9 SD threshold to determine the RT (**Figure S2.1 in appendix 2**).

Analysis was carried out for two epochs of data. In the epoch relative to the stimulus (stimulus locked) we segmented a time window starting with presentation of the flankers and ending at 2000 ms after the target. In the epoch relative to the response (response locked) we segmented a time window which started 1250 ms before and after the response, so that the individual response was in the middle of the time window.

Firstly, we investigated the signal activity in chosen and unchosen hands in each dynamometer and EMG muscle. To test the deviation of data from zero, we performed independent t-tests on each sample, and then we calculated the lengths and onsets of segments made up of continuous sequences of significant samples, in two stages. In the first stage, statistical significance was assessed with $p < .005$ at each individual sample. Due to the large number of data points generated, to deal with false-positives, in the second stage, bootstrapping was carried out to provide 99% confidence intervals for any significant differences. In each bootstrap iteration, the participant's dataset (first condition) was randomly shuffled with datasets full of zeros (representing the second condition – i.e., after subtracting the baseline). In each iteration, the lengths and onsets of significant sequences was recorded. This process was repeated for 10,000 iterations, in order to find the null distributions of the lengths and onsets of significant segments. From the null distributions, p values were assigned to the sequences of significant

samples found in the real data. Segments of significant samples with statistical significance of $p < .01$ were considered.

Secondly, we replicated the interference effects in dynamometers and EMG muscles. We calculated the difference of activity between chosen and unchosen hands. In order to compare each sample with 0 difference, we performed independent t-tests on each sample and then we calculated the lengths and onsets of segments made up of continuous significant samples. Statistical significance at each sample was assessed with $p < .005$. Due to the large number of data points generated, to deal with false-positives, bootstrapping was carried out to provide 99% confidence intervals for any significant differences. In each bootstrap iteration, two experimental conditions were randomly shuffled within each participant, then significant differences between these random conditions were found at the group level. The null distribution for the perceptual interference effect was calculated by shuffling the SI and C conditions; for the response interference effect by shuffling the SRI and SI conditions; and for the general interference effect by shuffling the SRI and C condition. We calculated the lengths and onsets of segments made up of significant samples in each bootstrap iteration. This process was repeated for 10,000 iterations, in order to find the null distributions of the lengths and onsets of significant segments. From these null distributions, p values were assigned to the sequences of significant samples found in the real data.

Segments of significant samples with statistical significance of $p < .01$ were considered.

The described method is similar to the use of cluster-extent based thresholding in fMRI studies (Woo et al., 2014) and was already used in previous EMG studies from our laboratory (Naish et al., 2013; Reader et al., 2018).

		Total		Congruent		Stimulus Incongruent		Stimulus Response Incongruent	
		M	(SD)	M	(SD)	M	(SD)	M	(SD)
Participants	Accuracy (%)	90.0	(5.52)	91.7	(5.15)	91.5	(4.43)	88.4	(7.30)
(n=20)	RT (ms)	460	(71.9)	432	(71.6)	455	(75.1)	478	(71.7)

Table 3.1 Mean (M) and standard deviation (SD) of proportion of correct responses (accuracy) and reaction times (RT) under total, congruent, stimulus incongruent, and stimulus response incongruent condition.

		M	(SD)	t	df	p	d
Perceptual	Accuracy (%)	-0.215	(3.88)	-0.248	19	0.807	-0.055
	RT (ms)	23.2	(25.2)	4.13	19	<.001	0.923
Response	Accuracy (%)	-3.10	(4.66)	-2.98	19	0.008	-0.667
	RT (ms)	22.1	(23.0)	4.31	19	<.001	0.963
General	Accuracy (%)	-3.32	(5.66)	-2.62	19	0.017	-0.587
	RT (ms)	45.4	(18.4)	11.1	19	<.001	2.47

Table 3.2 *Perceptual, response and general interference effect at behavioural level calculated in dynamometers. Mean (M), standard deviation (SD), T-test (t), degree of freedom (df), effect size (Cohen's d).*

3.3 RESULTS

3.3.1 Stimulus locked analysis in chosen and unchosen hands (correct trials)

The dynamometer response in the chosen hand deviated significantly above baseline, as expected. After target presentation, a significant increase started at 344 ms (1526 samples long, bootstrap $p < .0001$) in C condition, 369 ms (1458 samples, $p = .0001$) in SI condition, and 401 ms (1512 samples, $p = .0001$) in SRI condition. In the unchosen hand, the dynamometer response deviated significantly below baseline. A significant decrease was observed starting at 960 ms (2180 samples, bootstrap $p = .0006$) in C condition, 53 ms (1172 samples, bootstrap $p = .007$) and 698 ms (2705 samples, bootstrap $p = .001$) in SI condition, 146

ms (738 samples, bootstrap $p=.009$) and 825 ms (2450 samples, bootstrap $p=.002$) in SRI condition (**Figure 3.1**).

In the FDS muscle, EMG activity was significantly different from baseline. In the chosen hand, EMG signal increased at 282 ms (1383 samples, bootstrap $p=.0006$) and 1028 ms (1997 samples, bootstrap $p<.0001$) in C condition, 280 ms (1329 samples, bootstrap $p=.0006$) and 1327 ms (533 samples, bootstrap $p=.009$) in SI condition, and 276 ms (3549 samples, bootstrap $p<.0001$) in SRI condition. In the unchosen hand, EMG signal deviated significantly below baseline only in the SRI condition, showing a significant decrease starting at 1698 ms (250 samples, bootstrap $p=.009$) and 1900 ms (247 samples, bootstrap $p=.009$). No significant EMG activity was found in C and SI conditions (**Figure 3.2**).

The EMG signal in the EDC muscle deviated robustly above baseline. In the chosen hand, EMG activity increased at 280 ms (3541 samples, bootstrap $p=.0001$) in C condition, 272 ms (3557 samples, bootstrap $p=.0001$) in SI and 266 ms (3569 samples, bootstrap $p<.0001$) in SRI condition. Activity in the unchosen hand started increase at 369 ms (280 samples, bootstrap $p=.010$) in the SRI condition. We did not find any significant EMG activity in the unchosen hand in C and SI conditions (**Figure 3.3**).

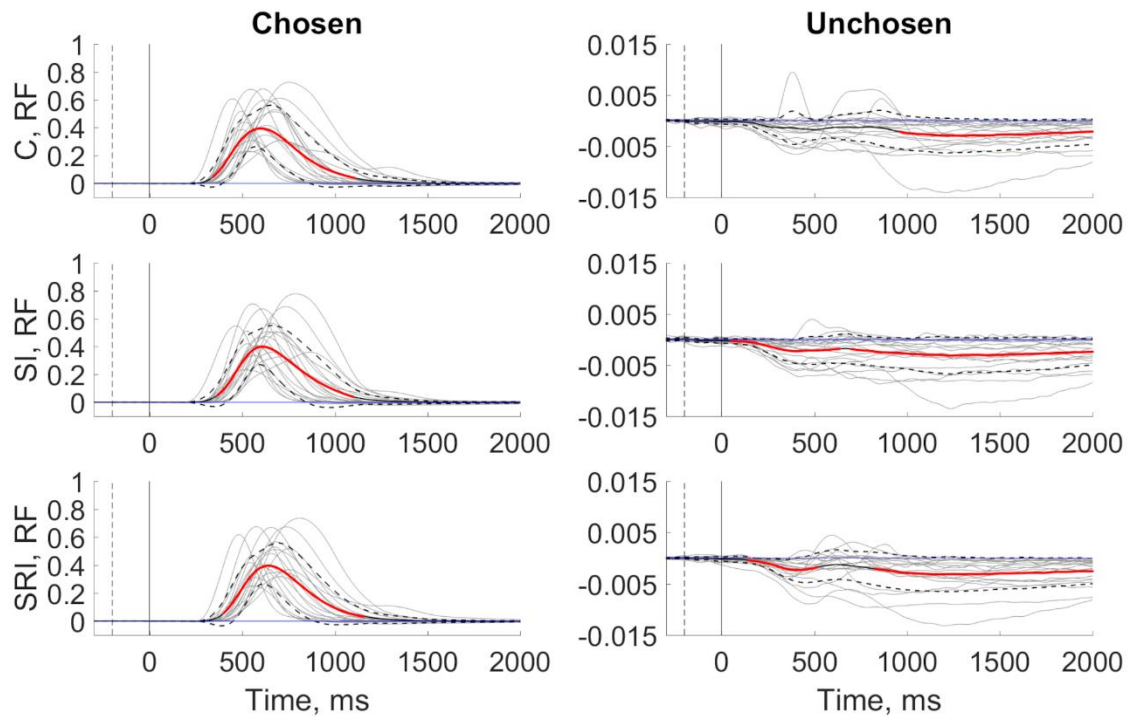


Figure 3.1 Relative force in dynamometers for chosen and unchosen hands in the congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) condition in correct trials. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline; vertical solid line at time = 0 ms shows onset of target; broken solid line at time = -200 ms shows onset of flankers.

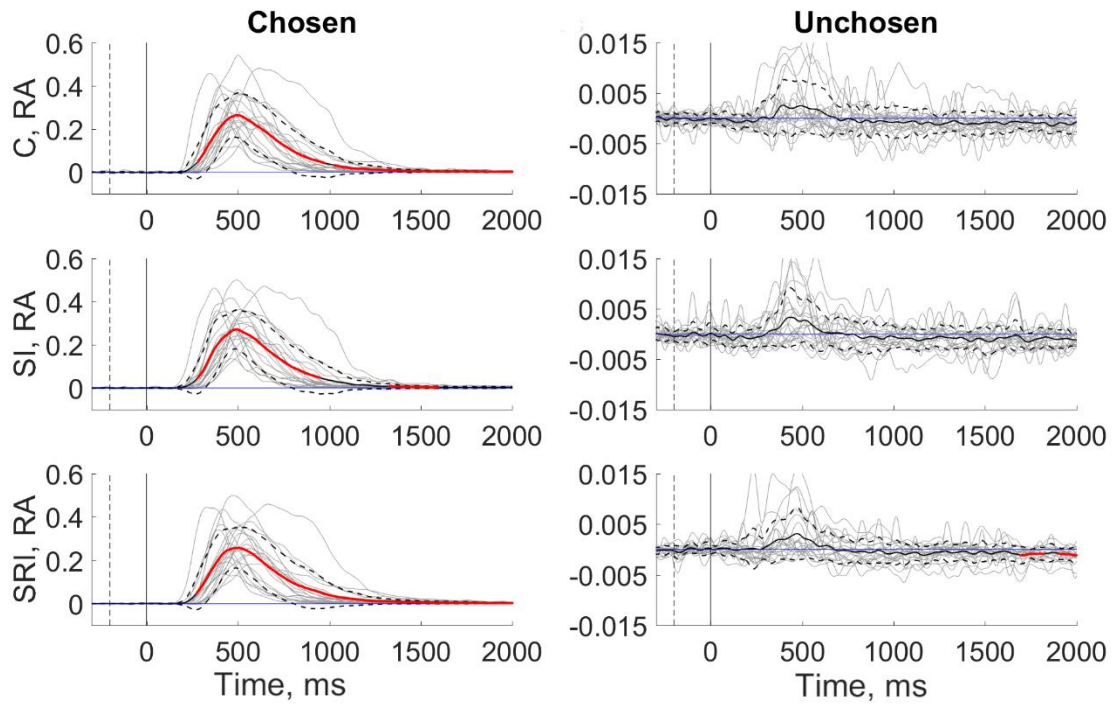


Figure 3.2 Relative activity in FDS muscle for chosen and unchosen hand in congruent (C), stimulus incongruent (SI) and stimulus response (SRI) condition in correct trials. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline; vertical solid line at time = 0 ms shows onset of the target; broken solid line at time = -200 ms shows onset of the flankers.

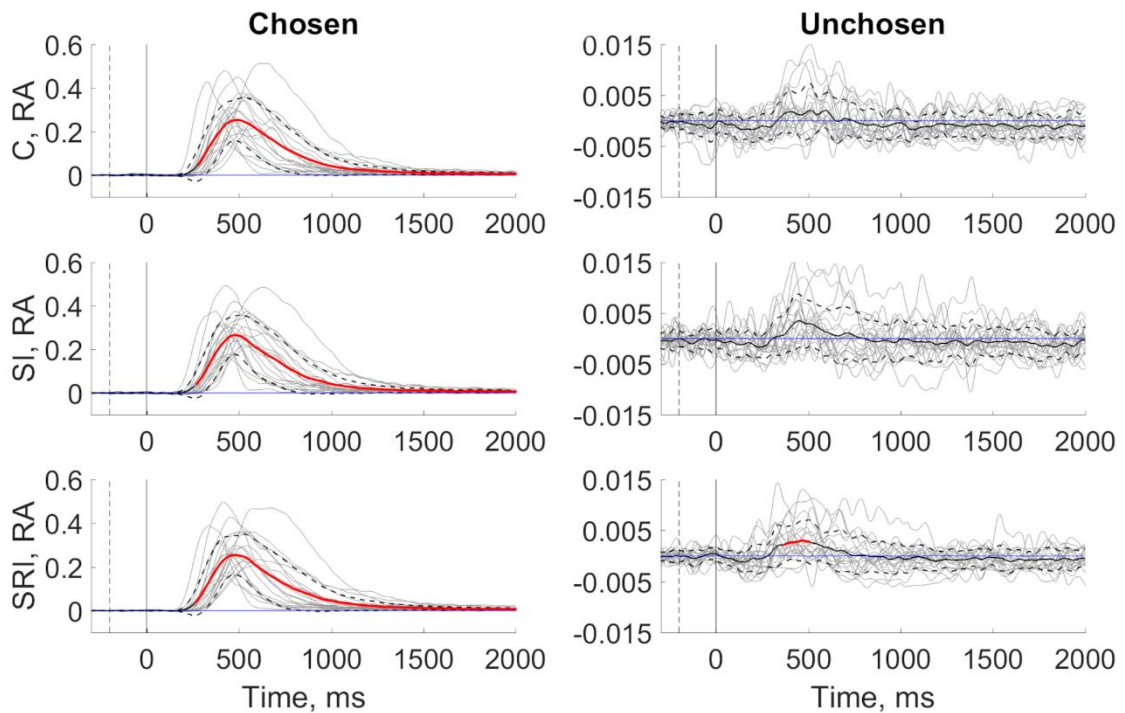


Figure 3.3 Relative activity in the EDC muscle for chosen and unchosen hand in congruent (C), stimulus incongruent (SI) and stimulus response (SRI) condition in correct trials. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline; vertical solid line at time = 0 ms shows onset of the target; broken solid line at time = -200 ms shows onset of the flankers.

3.3.2 Stimulus locked analysis in chosen and unchosen hands (incorrect trials)

This analysis was performed considering incorrect trials, in which participants pressed the incorrect response first. After target presentation, the dynamometer response was significantly above baseline in both chosen and unchosen response. In the chosen hand, we recorded a significant increase starting at 371 ms (1489 samples long, bootstrap $p < .0001$) in C condition, 431 ms (1035 samples long, bootstrap $p < .0001$) in SI condition and 288 ms (1315 samples long, bootstrap $p < .0001$) in SRI condition. In the unchosen hand, a significant increase started at 491 ms (1264 samples long, bootstrap $p < .0001$) in C condition, 524 ms (1274 samples long, bootstrap $p = .0001$) in SI condition and 586 ms (850 samples long, bootstrap $p < .0001$) in SRI condition (**Figure 3.4**).

In the FDS muscle, EMG activity recorded after target presentation in chosen and unchosen hand was significantly above baseline. In the chosen hand, significant increase was shown at 285 ms (1245 samples, bootstrap $p = .0003$) in C condition, 276 ms (1307 samples, bootstrap $p < .0001$) in SI condition and 253 ms (1405 samples, bootstrap $p < .0001$) in SRI condition. In the unchosen hand, a significant increase starting at 468 ms (1711 samples, bootstrap $p < .0001$) in C condition, 454 ms (1580 samples, bootstrap $p < .0001$) in SI condition and 451 ms (1286 samples, bootstrap $p < .0001$) in SRI condition were shown (**Figure 3.5**).

The EMG signal in the EDC muscle showed a significant increase from baseline. In the chosen hand, EMG activity increased at 246 ms (1805 samples, bootstrap $p < .0001$) in C condition, 258 ms (1151 samples, bootstrap $p < .0001$) and 879 ms (498 samples, bootstrap $p = .004$) in SI condition and 217 ms (1713 samples, bootstrap $p < .0001$) and 1193 ms (382 samples, bootstrap $p = .008$) in SRI condition. Activity in the unchosen hand started increase at 448 ms (1764 samples, bootstrap $p < .0001$) in C condition, 452 ms (905 samples, bootstrap $p = .0004$) and 931 ms (1122 samples, bootstrap $p < .0001$) in SI condition and 438 ms (1332 samples, bootstrap $p < .0001$) and 1375 ms (492 samples, bootstrap $p = .010$) in the SRI condition (**Figure 3.6**).

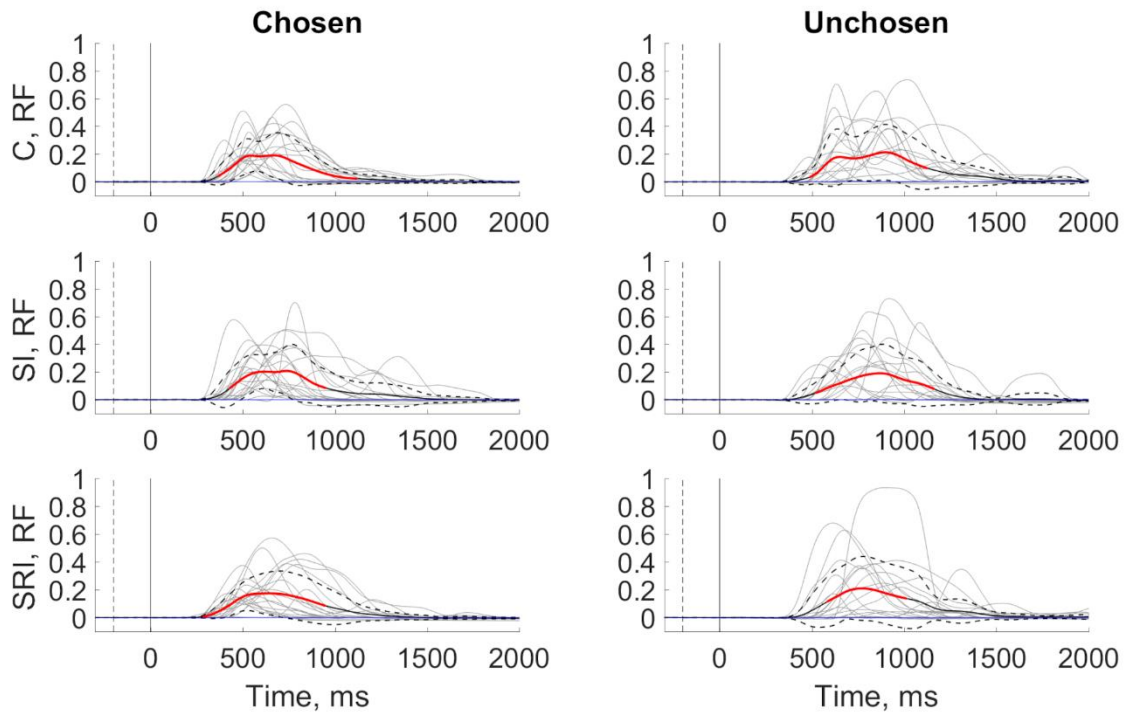


Figure 3.4 *Incorrect trials: relative force in dynamometers for chosen and unchosen hands in the congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) condition. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline; vertical solid line at time = 0 ms shows onset of target; broken solid line at time = -200 ms shows onset of flankers.*

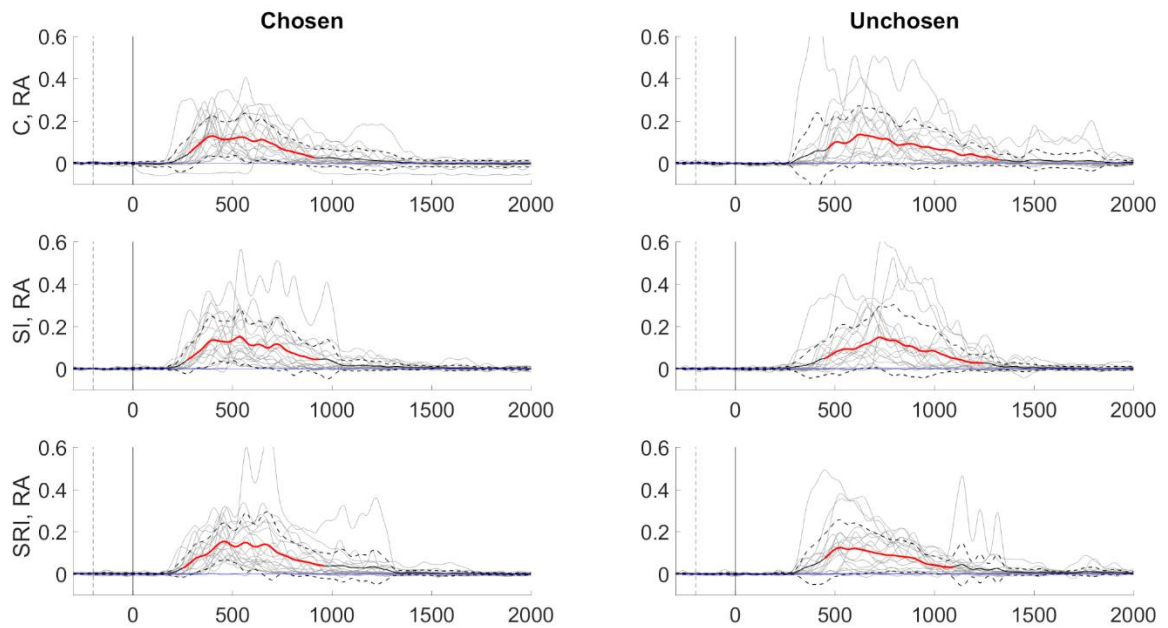


Figure 3.5 *Incorrect trials: relative activity in FDS muscle for chosen and unchosen hand in congruent (C), stimulus incongruent (SI) and stimulus response (SRI) condition. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline; vertical solid line at time = 0 ms shows onset of the target; broken solid line at time = -200 ms shows onset of the flankers.*

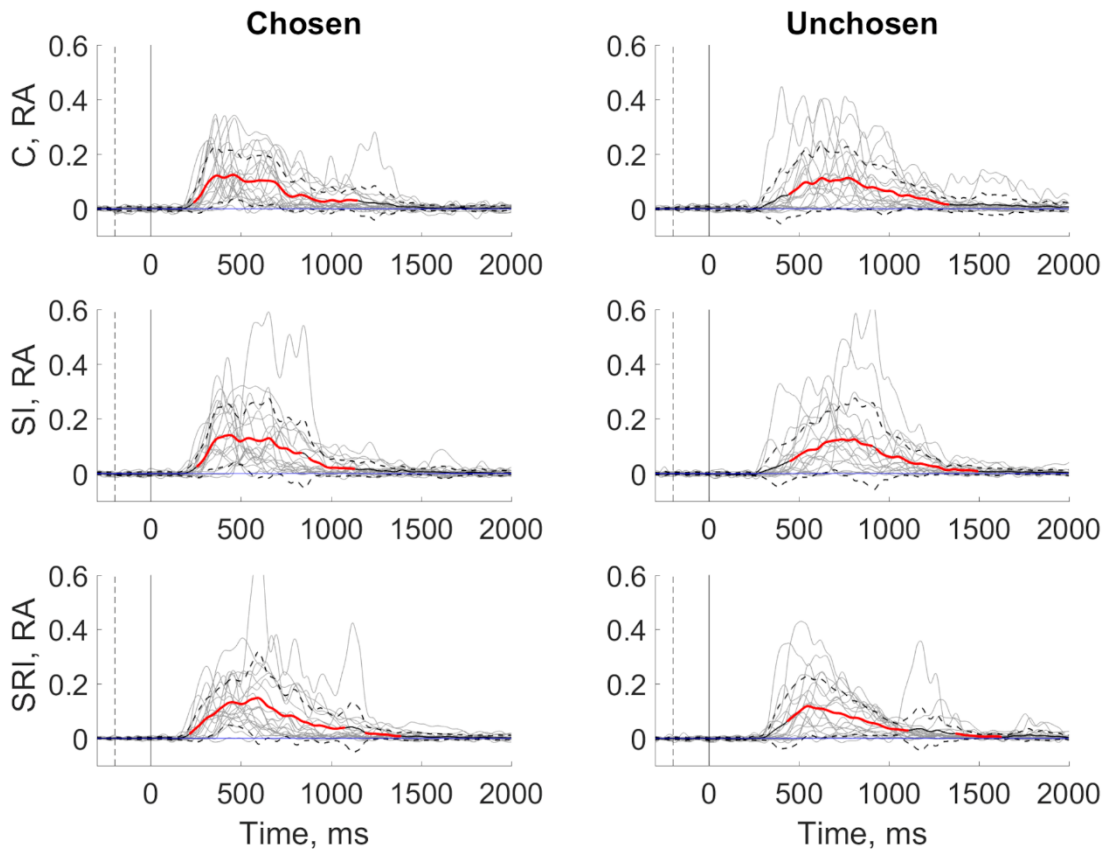


Figure 3.6 *Incorrect trials: relative activity in the EDC muscle for chosen and unchosen hand in congruent (C), stimulus incongruent (SI) and stimulus response (SRI) condition. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline; vertical solid line at time = 0 ms shows onset of the target; broken solid line at time = -200 ms shows onset of the flankers.*

3.3.3 Response locked analysis in chosen and unchosen hands

In response locked analysis zero corresponded to RT starting, therefore negative numbers corresponded to the time before the RT whereas positive numbers corresponded to the time after RT threshold. In the dynamometers, response in the chosen hand were significantly different from baseline. In C condition, a significant decrease appeared at -654 ms (1222 samples, bootstrap $p < .0001$) and a significant increase appeared at -36 ms (1049 samples, bootstrap $p = .0008$). We found a significant decrease at -358 ms (628 samples, $p = .008$) and a significant increase at -36 ms (989 samples, $p = .0007$) in SI condition. In SRI condition, a significant decrease starting at -492 ms (898 samples, $p = .004$) and a significant increase starting at -36 ms (1029 samples, $p = .001$) were shown. We found a significant response below baseline in the unchosen hand. A significant decrease started at -416 ms (923 samples, bootstrap $p = .004$) and 499 ms relative to the recorded RT (1503 samples, bootstrap $p < .0001$) in C condition. The unchosen hand force decreased at -459 ms (1045 samples, bootstrap $p = .007$) and 251 ms (1998 samples, bootstrap $p = .003$) in SI condition. We found a decrease of the unchosen hand at -535 ms (1176 samples, bootstrap $p = .005$) and 353 ms (1795 samples, bootstrap $p = .0007$) in the SRI condition (**Figure 3.7 and 3.8**).

For the FDS muscle, EMG activity of the chosen hand significantly deviated above baseline. In C condition, the activity in the chosen hand increased at -126 ms (1003 samples, bootstrap $p = .001$) and 538 ms relative to the response (1424

samples, bootstrap $p < .0001$). We found a significant increase of EMG activity at -124 ms (996 samples, bootstrap $p = .0001$) in SI condition. In SRI condition, EMG activity increased at -126 ms (1147 samples, bootstrap $p = .001$) and 471 ms relative to the response (1559 samples, bootstrap $p < .0001$). The unchosen hand decreased at -44 ms (239 samples, bootstrap $p = .005$) only in SI condition. We found no significant EMG activity in C and SRI conditions **(Figure 3.9)**.

Regarding the EDC muscle, EMG signal in the chosen hand deviated robustly above baseline. We found an increase at -136 ms (2771 samples, bootstrap $p = .0001$) in C condition, at -135 ms (2770 samples, bootstrap $p = .0001$) in SI condition and -135 ms (2769 samples, bootstrap $p = .0001$) in SRI condition. The unchosen hand increased the activity at -81 ms (265 samples, bootstrap $p = .005$) in the SI condition and -94 ms (381 samples, bootstrap $p = .002$) in the SRI condition. We found no significant EMG activity in the unchosen hand for the C condition **(Figure 3.10)**.

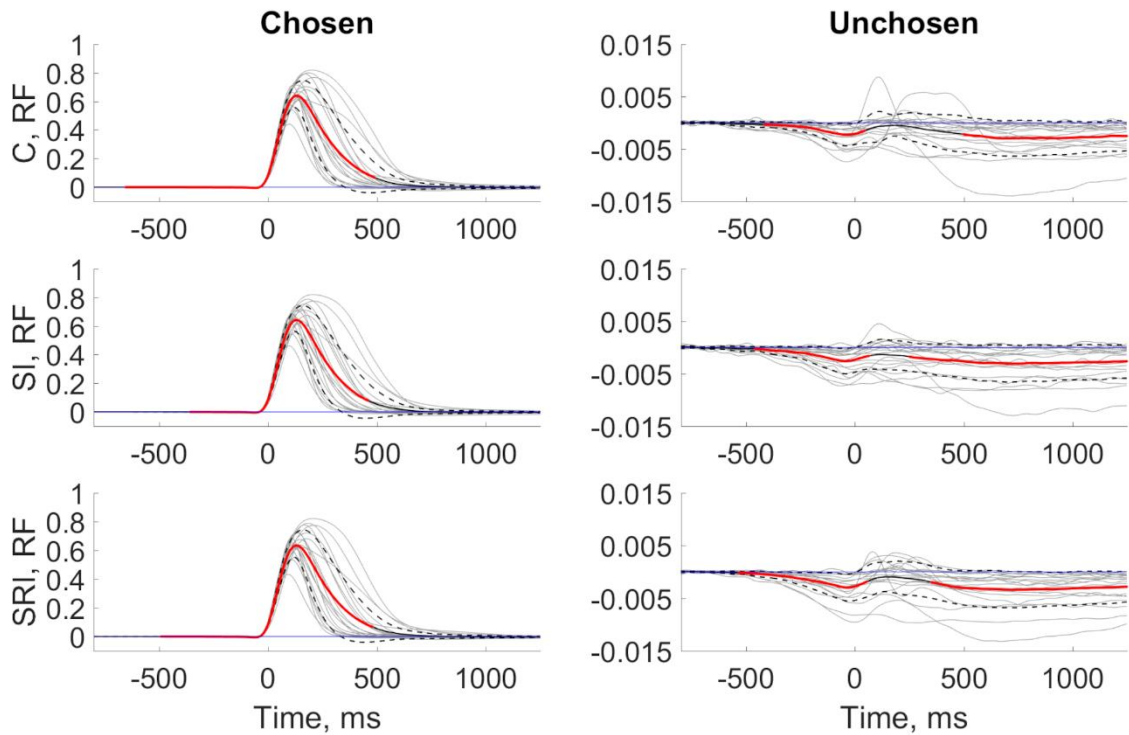


Figure 3.7 Relative force in dynamometer responses for chosen and unchosen hands in the congruent (C), stimulus incongruent (SI) and stimulus response (SRI) condition. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline.

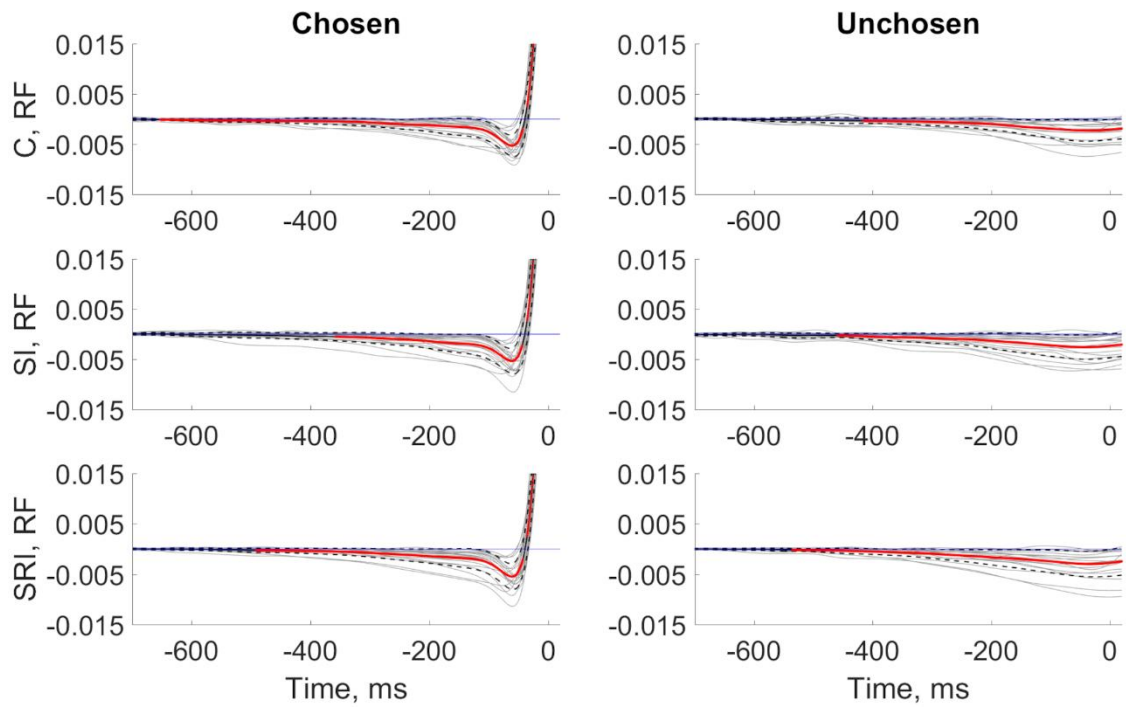


Figure 3.8 Zoomed-in image of the force in the dynamometers for chosen and unchosen hand in congruent (C), stimulus incongruent (SI) and stimulus response (SRI) condition. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline.

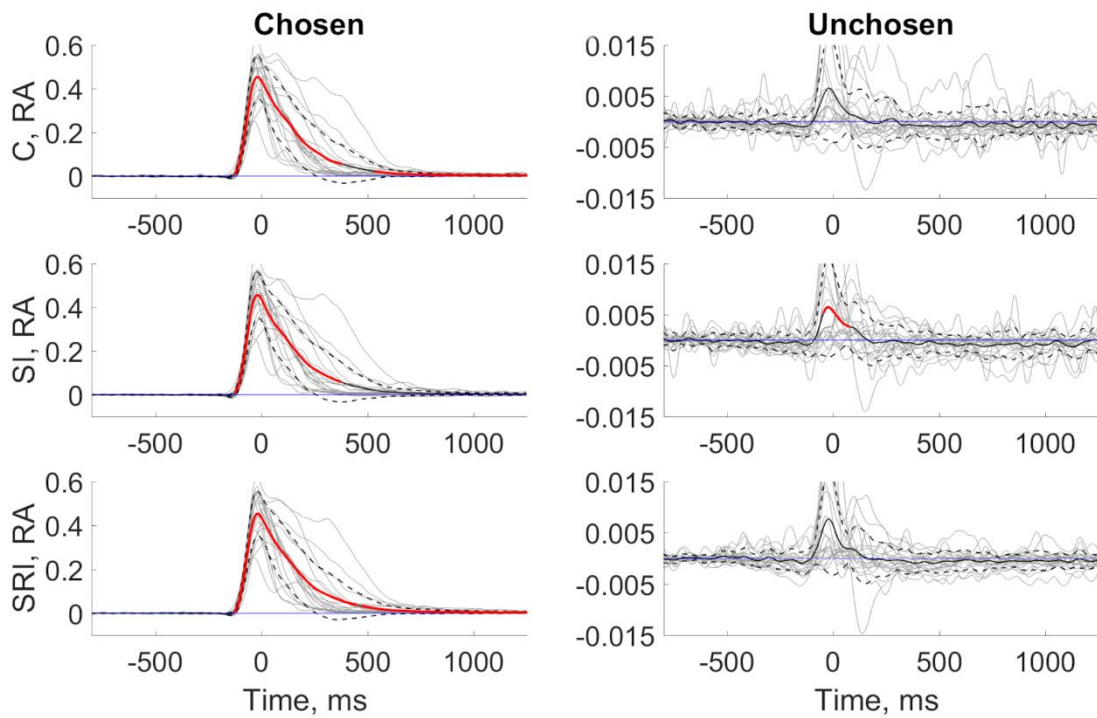


Figure 3.9 Relative activity in FDS muscle for chosen and unchosen hand in congruent (C), stimulus incongruent (SI) and stimulus response (SRI) condition. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline.

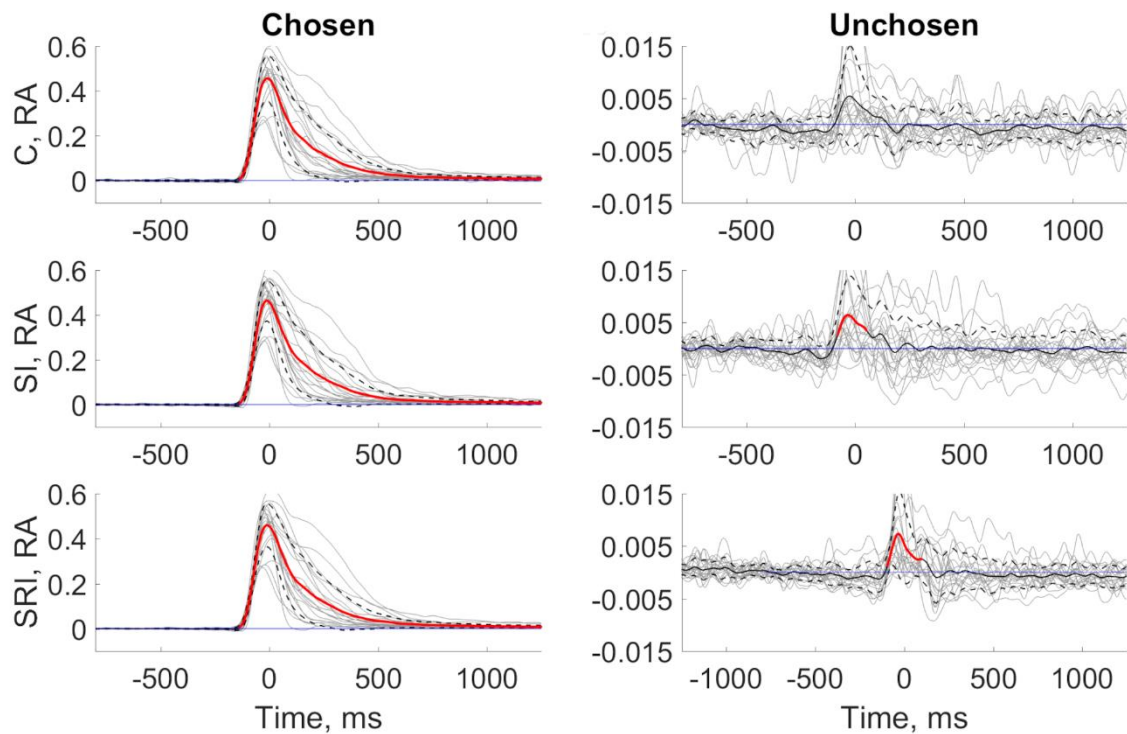


Figure 3.10 Relative activity in the EDC muscle for chosen and unchosen hand in congruent (C), stimulus incongruent (SI) and stimulus response (SRI) condition. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard error; red lines show significant samples; blue line corresponds to zero, the baseline.

3.3.4 Replication of interference effects

We found significant perceptual, response and general interference effects when we analysed stimulus locked epochs in the dynamometers. The perceptual interference started at 307 ms (388 samples, bootstrap $p=.004$) and the response interference started at 341 ms (468 samples, bootstrap $p=.003$) after target onset.

Regarding the general interference, we found two segments of significant consecutive samples, starting at 320 ms (465 samples, bootstrap $p=.002$) and 696 ms (506 samples, bootstrap $p=.001$), respectively (**Figure 3.11**). Unexpectedly, we found no significant interference effects in the FDS and EDC muscles (**Figure 3.12 and 3.13**).

When we analysed the response locked epochs, we did not find significant perceptual, response or general interference effects in either the dynamometers, FDS or EDC muscles.

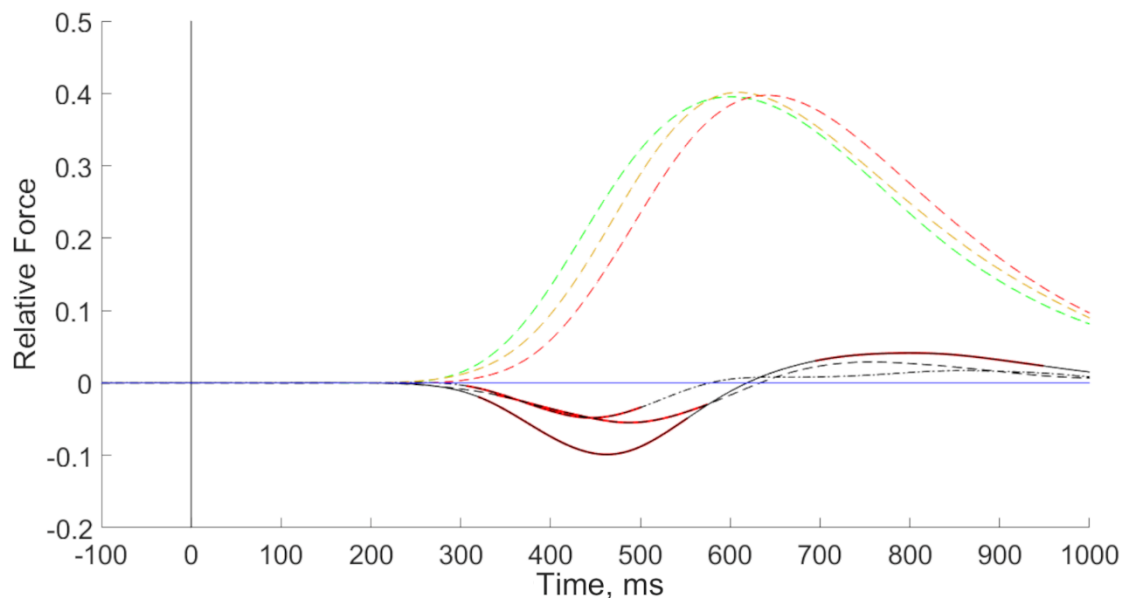


Figure 3.11 Dynamometer signals showing the perceptual interference (dash-dotted), response interference (dashed) and general interference (solid line) effects. Congruent (dashed-green), stimulus incongruent (dashed-gold) and stimulus response incongruent (dashed-red line). Thick red lines show significant samples; blue line corresponds to zero; vertical solid line at time = 0 ms shows the onset of target.

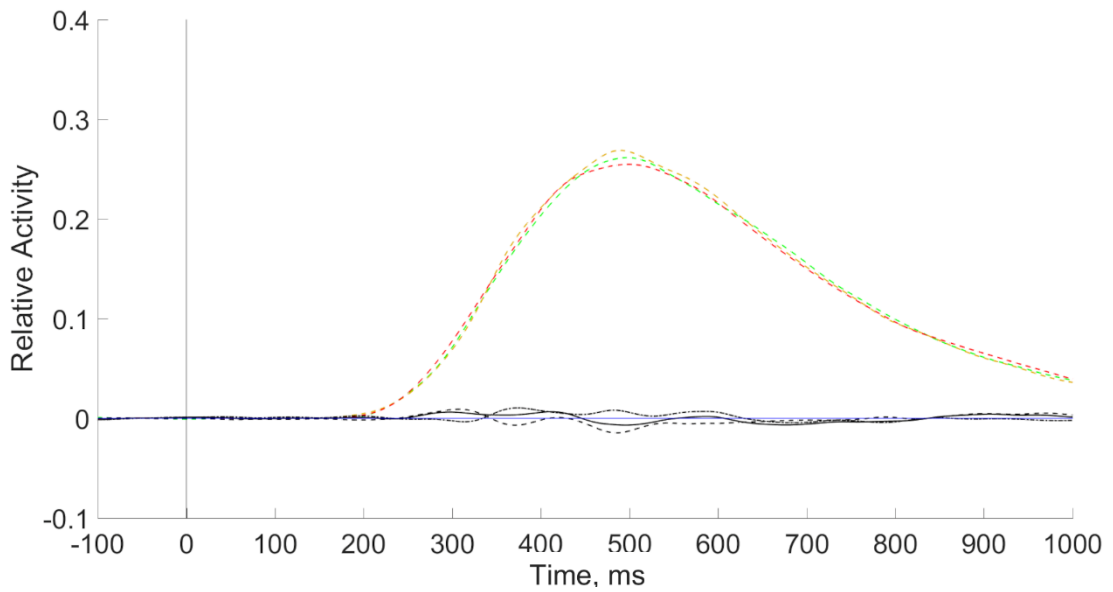


Figure 3.12 In the FDS muscles the perceptual interference (dash-dotted), response interference (dashed) and general interference (solid line) effects. Congruent (green), stimulus incongruent (gold) and stimulus response incongruent (red line). Blue line corresponds to zero; vertical solid line at time = 0 ms shows the onset of target.

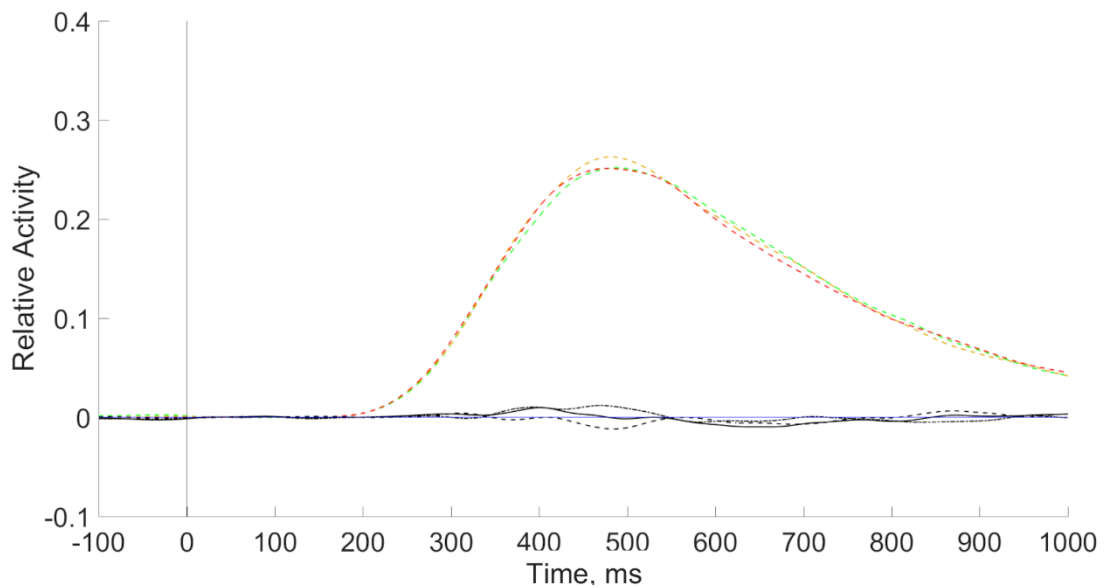


Figure 3.13 In the EDC muscles the perceptual interference (dash-dotted), response interference (dashed) and general interference (solid line) effects. Congruent (green), stimulus incongruent (gold) and stimulus response incongruent (red line). Blue line corresponds to zero; vertical solid line at time = 0 ms shows the onset of target.

3.4 DISCUSSION

In this study our aim was to investigate the activity of the chosen and unchosen hands when irrelevant information interfered with motor response execution at the peripheral level. We used the coloured version of the Flanker task because it allowed us to study the motor response activity when distracting stimuli

and competing responses interfered with the response. Using EMG, a few previous studies measured motor hand activity simultaneously and continuously, showing interesting results (Killikelly & Szucs, 2013; Szucs et al., 2009). In our study we used both dynamometers and EMG to measure the temporal and functional aspects of the motor responses. This was a novelty in the available literature, because it allowed us to investigate both force and EMG activity at the same time in chosen and unchosen hands.

3.4.1 Stimulus locked analysis in chosen and unchosen hands

We analysed the motor response in relation to stimulus processing. In the chosen hand, we found increased grip force and EMG activity across all conditions. In the C condition, the forces recorded in dynamometers started at 344 ms, whereas the activity recorded in arm muscles started around 281 ms after the target presentation. In the SI condition, we recorded significant forces starting at 369 ms in the dynamometers and significant activity starting around 276 ms in forearm muscles after the target presentation. In the SRI condition, the dynamometer response started at 401 ms, whereas muscle activities started around 271 ms after the target presentation. Taken together, we expected to find increased force and EMG activity in chosen hand, because it was supposed to respond in the task. Moreover, EMG activity in the FDS and EDC muscles was observed to initiate prior to the increase in grip force, indicating the temporal relationship between muscle activation and the subsequent hand force. We found

surprising results when we calculated the interference effects. In the dynamometer data, the perceptual and response interference effects were 25 ms and 32 ms respectively. These effects agree with our previous findings in young adults. In the arm muscles, however, both perceptual and response interference effects were equal (-5 ms) showing no interference effects.

We then focused on the unchosen hand. Looking at the dynamometers, force in the unchosen hand decreased across all conditions. The force decreased 960 ms in the C condition, 53 ms in the SI condition, and 146 ms after target onset in the SRI condition. We hypothesise that the force decrease, observed in the SI condition, was a response to the flanker rather than to the target, because it seems unlikely that people can respond so quickly to the target. The observed decrease in force in the unchosen hand is likely attributable to a non-specific motor process, as it was observed in both the congruent and incongruent conditions. Taking the results observed in both hands together, we showed that the unchosen hand started to decrease before chosen hand started to increase. These findings allow us to hypothesise a constructive process between hands which optimises the motor response execution.

Looking at the EDC and FDS muscles, we found only in the SRI condition that activity increased 369 ms and decreased 1698 ms after the target onset, respectively. Muscle activity (related to unchosen hand) was recorded later than muscle

activity (related to the chosen hand) started to increase. In general, these findings show that muscle activity is not significantly different in relation to interference conditions. It is likely the arm muscles are activated at all times and the activation is not different for different congruence conditions. Our findings reported in the unchosen hand were different from previous studies (Killikelly & Szucs, 2013; Szucs et al., 2009), in which the authors reported significant activation of the unchosen hand. At this point, we hypothesized that the difference was due to different instruments used to measure the participant's response. In previous studies, the authors used a single video game controller held by both hands, whereas we used two independent dynamometers, one per hand, to respond. It could be possible that, in previous experiments, both hands were involved in maintaining the posture and counter-acting forces produced by the other hand, whereas in our experiment, each hand and arm was involved in maintaining its posture independently.

After examiner's suggestion, we analysed the dynamometer and EMG activity in incorrect trials. It was interesting to investigate whether the chosen and unchosen hand followed the same response pattern in trials in which participants were sure to select the correct response. Because our sample achieved an accuracy higher than 90%, we analysed only few trials (less than 10 percent of the total). Looking at individual plots, some participants made only one or two incorrect responses

for each interference condition. Therefore, our discussion at this point has limitations. Looking at the dynamometers, the chosen (i.e., incorrect) hand was activated around 360 ms after target presentation, which happened before the recorded activation in the unchosen (i.e., correct) hand at 533 ms. The response pattern observed in dynamometers in incorrect trials showed discrepancies with the response pattern showed in correct trials. First, the chosen (incorrect) hand, was activated before the unchosen (correct) hand. Differently from correct trials, we reported that, the chosen hand (correct) hand was activated after the unchosen hand was deactivated. These data allowed us to assume presence of hand competition in incorrect trials instead a constructive process between hands. Looking at the EMG activity in FDS and EDC muscles, we found a similar response pattern, specifically activation of the chosen hand (incorrect) which started around 250 ms after target presentation, which was before activation of the unchosen hand (correct) hand which started at 450 ms. Although presence of important limitation, these data match well what we expected. We found activation of the chosen (incorrect) which was associated with the flanker before activation of the unchosen (correct) hand associated with the target like a hand competition process.

3.4.2 Response locked analysis in chosen and unchosen hands

We segmented the dynamometer and EMG signals in relation to the start of the RT, because we were interested in analysing the response related to motor

execution processes. In the chosen hand, grip force initially decreased and then it increased. The decrease started 654 ms, 358 ms and 492 ms before RT respectively in C, SI and SRI conditions. The increase started 36 ms before RT in all conditions. Zooming in on the graphs, we did not observe any variability in the signal shape of each participant. We hypothesise that there is a signal artifact related to the filtering process. Due to the presence of a strong response onset occurring 36 ms before the RT, there is a notable positive signal that is being filtered, leading to a 'ripple effect' prior to the strong onset signal. Because of this likely filter artifact, we are very cautious in any interpretation of any decreasing mechanism in the chosen hand. Focusing on the increased force, this result agrees with our hypothesis reported above in the stimulus locked analysis. We expected increased force in the chosen hand. When we analysed the EMG signal, it was observed that FDS and EDC muscles were both activated across all conditions. In particular, muscle activation started around 131 ms, 130 ms and 131 ms before RT in C, SI and SRI conditions. As expected, these findings showed the activation of arm muscle related to the chosen hand.

When we looked at the unchosen hand, we found a decrease of the dynamometer signal before the RT. The force decreased at -416 ms in C condition, -459 ms in SI condition and -535 ms in SRI condition. The decreased force produced by the unchosen hand was seen in all congruent and incongruent conditions. In this case, because there is not a strong onset response, there are no issues related

to the filter artifact. For this reason, it is reasonable to discuss a decreased force of unchosen hand. Because it was recorded before the target, but after the flanker, it is likely a relaxation before the motor response. When we analysed the activity in the arm muscles related to the unchosen hand, we found that the FDS was significantly activated at -44 ms in SI condition, and EDC muscle was significantly activated at -81 ms and -94 ms respectively in SI and SRI conditions.

Overall, we hypothesise the presence of a constructive process which optimizes the motor response execution, because when the force in the chosen hand increases, the force in the unchosen hand decreases. Our hypothesis is supported by the timing – the force in unchosen hand decreased before the chosen hand increased, likely to promote the chosen hand's activation. Activation of the arm muscles started a bit earlier than the RT onset, so it seems likely to be a useful process of hand stabilization. Taken together, these findings are in accord with results reported in the stimulus locked analysis. We could not compare our findings with previous EMG studies (Killikelly & Szucs, 2013; Szucs et al., 2009) for this part, because these authors did not perform a response locked analysis. Our findings are in accord with brain stimulation studies in the literature – presented in the next Chapter – in which they reported, at a central nervous level, an activation process related to the chosen hand movement and a decreasing process related to the unchosen hand movement (Klein-Flugge et al., 2013; Michelet et al., 2010).

3.4.3 Replication of interference effects

We aimed to replicate the interference effects using continuous measures of force and muscle activity. When we performed stimulus locked analysis in the dynamometer data, the perceptual interference started at 307 ms, the response interference began at 341 ms and the general interference effect started at 320 ms after target presentation. These results show the presence of clear congruency effects in the dynamometer signal. Increased effort is required when irrelevant stimuli and competing responses interfere with the response execution. Contrary to our expectations, we did not find any perceptual, response and general interference effects in either FDS or EDC muscles. The interpretation of this finding is that the FDS and EDC muscles are involved in maintaining the stability of the hand and are thus consistently active for both congruent and incongruent conditions. These muscles play a crucial role in providing support and control to the hand, ensuring its stability during the task. In other words, we hypothesise that FDS and EDC muscle are involved in a different motor function. One more hypothesis, which could explain why we did not find a significant interference effect in both FDS and EDC muscle, is related to the features of the EMG signal. It is smaller and it is noisier than the dynamometer signal. This could explain why we found interference effects in dynamometers but not in EMG activity.

When we investigated the congruency effects in a response locked analysis, we did not find any perceptual, response and general interference effects in either

the dynamometer or EMG activity. This was an interesting result, which allowed us to hypothesise that all interference effects are related to the stimulus processing. Once the response has begun, the influence of irrelevant information did not interfere with the motor response. As a matter of fact, we did not find any significant interference effect in the response locked analysis. This is a novel finding in literature, which was possible by use of the continuous measure of performance.

To summarise the above results, we found increased force in the chosen hand and a decreased force in the unchosen hand across all conditions. Looking at the timing, the unchosen hand started to decrease around 150 ms before the chosen hand increased. Moreover, we found interference effects in the dynamometer signals, but not in EMG activity. On this basis, we planned the experiment presented in the next Chapter.

CHAPTER 4 – STUDYING THE TIME COURSE OF MOTOR RESPONSES OVER THE PRIMARY MOTOR CORTEX (M1) USING TRANSCRANIAL MAGNETIC STIMULATION (TMS).

4.1 INTRODUCTION

Different studies have investigated the temporal dynamics of chosen and unchosen hands by stimulating over the M1 (Klein-Flugge et al., 2013; Michelet et al., 2010; Verleger et al., 2009). Using TMS, researchers have investigated the excitability of corticospinal pathway from motor cortex to chosen and unchosen hands. Measurements of temporal changes in MEPs can reliably reflect, at a central nervous system level, the time course of response processes between the hands.

Klein-Flugge and colleagues (Klein-Flugge et al., 2013) recorded EMG activity over the FDI of the right hand and applied single pulse TMS over M1 of the left hemisphere. A group of young adults were instructed to perform a decision-making task. At 230 ms before the movement onset, the authors observed a reduction of the MEP amplitude for the unchosen hand and an increased amplitude for the chosen hand. This difference in amplitude of MEP between chosen and unchosen hands increased progressively toward the overt response. The motor activation at 230 ms before the responses, evoked from TMS over left M1, increased

for right hand responses, which were contralateral to the stimulation, and decreased for ipsilateral left-hand responses. Klein-Flugge and colleagues (Klein-Flugge et al., 2013) supposed that increased activity in cortical representations of the chosen hand before the movement onset, and decreased activity in representations of the unchosen hand, may optimise preparation processes of movement.

In a different study, Verleger and colleagues (Verleger et al., 2009) recorded EMG activity over the FDI muscle in both hands and applied single pulse TMS over the left or right M1. During the experiment, participants performed the arrow version of the Flanker task. The stimulation was delivered at 0 ms and after 30, 60 and 90 ms relative to the LRP peak, measured in incongruent trials for each participant. The LRP is an electrophysiological indicator, measured using EEG, at a central level of motor response activation (Kornhuber & Deecke, 1965, 2016). In the congruent condition, the authors found that MEP amplitude increased when TMS was applied contralateral to the responding hand. In the incongruent condition, MEP amplitudes in the non-responding hand gradually decreased in the first 90 ms after stimulation, and mirror-symmetrically increased MEP amplitudes were seen in the responding hand. These results suggested that the inappropriate response, the flanker response, was suppressed in one M1 hand area simultaneously with activation of the appropriate response, the target response, in the

other M1. The authors recorded this dynamic in a time window of 174-264 ms relative to the target onset.

These findings agreed with a different study, in which Michelet and colleagues (Michelet et al., 2010) studied the motor dynamics between different muscles in the same hand. Participants performed the arrow version of the Flanker task. The researchers measured the EMG activity over FDI and the extensor indicis of the right hand to detect flexion (leftward) and extension (rightward) movements, respectively. In this experiment, participants had to respond by flexing the index finger if a central arrow pointed to the left or extending the index finger if the central arrow pointed to the right. Single pulse TMS was applied over the left M1 at one of five time points after the target stimulus onset, at 80, 160, 240, 320 and 400 ms, respectively. The results showed that, in the congruent condition, MEP size increased when the muscle acted as agonist and decreased when the same muscle acted as antagonist. The difference between MEPs became significant at 240 ms after the stimulus. In the incongruent condition, MEP amplitude briefly increased in the (incorrect) muscle, which corresponded to premature response to the flanker arrows. Later, at 240 ms after the stimulus, MEPs corresponding to the correct response were greater. The authors concluded that there was a selective increase of MEP amplitude in the agonist muscle, used for producing the movement, and a corresponding inhibition of the antagonist.

It is difficult to compare the above studies directly. The researchers investigated the corticospinal representation of the chosen and unchosen hands using different cognitive tasks. Klein-Flugge and colleagues (Klein-Flugge et al., 2013) used a decision-making task in which participants had to select a single option (forced choice condition) or choose between two options with different reward magnitude and probability (choice condition). Verleger and colleagues (Verleger et al., 2009) and Michelet and colleagues (Michelet et al., 2010) used the arrow version of the Flanker task in which MEPs of chosen and unchosen hands were studied in congruent and incongruent conditions. In these two studies, the authors did not investigate the temporal changes of MEP amplitude in stimulus and response interference conditions. To the best of our knowledge, no studies have investigated cortical representation of the chosen and unchosen hands using cognitive tasks in which interference originated from distracting stimuli and competing responses. Moreover, researchers processed the data using different strategies. Verleger and colleagues (Verleger et al., 2009), and Michelet and colleagues (Michelet et al., 2010) used a stimulus locked analysis in which motor processes were investigated according with stimulus onset. Klein-Flugge and colleagues (Klein-Flugge et al., 2013) used a response locked analysis in which corticospinal excitability was investigated in accord with response onset. Considering the different processing strategies, it is very difficult to compare the timing of excitability observed in these studies.

In this experiment, the aim was to study the time course of chosen and unchosen hand activation at the central nervous system level, using TMS over M1. Stimulating over M1, we investigated the total corticospinal excitability from which we could infer indirectly the M1 activity. We used the coloured version of the Flanker task, already used in our previous experiments, to study the corticospinal excitability in stimulus and response incongruent conditions separately. We delivered, simultaneously, two single-pulses of TMS, one over the left and one over the right M1, while the motor system was resolving response conflict at the stimulus and response levels. Using this double-coil TMS method over M1 to measure the corticomotor excitability in both hands simultaneously has been documented before (Grandjean et al., 2018). In our previous EMG experiment, reported in detail in Chapter 3, we found in the dynamometers an increased force in the chosen hand and a decrease in the unchosen hand, irrespective of condition. In the present experiment, we hypothesised to find a different representation of chosen and unchosen hands reflected by stimulation over M1. Specifically, we hypothesized an increased MEP amplitude for the chosen hand and a decreased MEP amplitude for the unchosen hand. We did not expect to find a different representation for different interference conditions. In terms of timing, because in the previous experiment we observed that decreased activity of the unchosen hand happened ~150 ms before activation of the chosen hand, in this experiment we hypothesized to find that decreased MEP amplitudes happen before the increasing of MEP amplitudes.

4.2 METHOD

The methods were the same as described in the previous Chapter, except where indicated.

4.2.1 Participants

We recruited 15 right-handed adults (20-45 years old, mean \pm SD=30.2 \pm 7.7 years, 9 females). They were students and staff at the University of Nottingham, recruited via email advertisements to a mailing list of participants who had previously participated in other experiments. They received an inconvenience allowance of £20 for taking part. All participants were fluent in English and reported no history of neurological or psychiatric disorders. All had normal or corrected to normal vision. Participant safety and suitability to undergo TMS was assessed using a self-administered questionnaire, checked by the experimenter. Side effects or discomfort were monitored using a follow up questionnaire over 24 hours following stimulation. No significant side effects or discomfort attributed to TMS were reported by participants. Written informed consent was obtained from each participant. The study received ethical approval from the research ethics committee of the University of Nottingham (reference: SoPEC1445).

4.2.2 Stimuli, task and procedure

A coloured version of the Flanker task, already presented in previous experiments, was administered (**Figure 2.1a**). Responses were collected with

hand-held grip force sensors ('dynamometer', AD Instruments) and a standard high-speed USB2 computer keyboard (used for the first block of practice only). Participants were instructed to maintain a steady grip of around 1.5% of maximum voluntary contraction during the task, and then to respond to the colour of the middle square (the target) by squeezing the response dynamometer, and to ignore the surrounding squares (the flankers). All code for running the experiment are freely available at <https://osf.io/9k4bj/>.

Participants went through two practice blocks of 16 pseudorandomised trials, the first using a standard QWERTY keyboard to respond. In the second block of the practice and experiment, participants used dynamometers to respond, EMG activity was recorded and TMS was applied as described below. They completed between four and eight response-balanced blocks (16 C, 16 SI and 32 SRI trials), giving in total between 256 to 512 trials per participant. Researchers gained more experience between participants in using the double-coil TMS method. This increased efficiency led to quicker data collection. In each case the experiment took a maximum of three hours.

4.2.3 TMS apparatus

TMS was carried out using a Magstim Bistim machine comprising of two Magstim 200² machines running independently (Magstim Company Ltd, Whitland, Carmarthen-shire, UK).

4.2.4 Localisation of M1

Participants chose a soft swimming cap which fitted the head well. Once the cap was on the head, measurements, in centimetres, were taken from nasion to inion and from left and right preauricular points to determine the correct location of vertex. Vertex is in the midpoint of the distances nasion – inion and left to right preauricular points.

We used two custom programs written in MATLAB to calibrate TMS intensity for each participant. During this phase, we used a figure-of-8 shaped TMS coil (7.5 cm outer diameter) held tangentially to the surface of the head with the handle pointing backwards at an angle of about 45° away from the sagittal plane on each side. Firstly, we used the 'TMS hotspot finder' program: we placed a 25-spot (5x5 cm) grid over both hemispheres, centred at 5 cm left/right and 1 cm forward relative to the vertex. For all participants, the starting TMS intensity was set at 75% of the maximum TMS stimulator output (MSO). We stimulated using two pulses over each of the 25 spots of the grid going from anterior to posterior and starting with the most medial row. We identified M1 of both hemispheres as the spot where we recorded the maximum mean MEP amplitude in the relaxed FDI muscle of hand. In our sample, mean±SD of M1 scalp coordinates were 5.20±0.86 cm lateral and 0.47±0.92 cm anterior of vertex in the left hemisphere, and 6.07±0.80 cm lateral and 0.67±0.98 cm anterior of vertex in the right hemisphere.

Next, the resting motor threshold (rMT) was determined using the QUEST algorithm implemented in our 'TMS threshold finder' program. The QUEST algorithm set the intensity for the next TMS pulse, in order to find the stimulator intensity that produced MEPs of around 0.15 mV peak-to-peak in the relaxed FDI muscle of the hand (measured between 10 and 40 ms after the TMS pulse). We used a higher threshold than is typically used (i.e., 0.05 mV) because of the background noise levels in our custom closed-loop TMS protocol hardware. The MATLAB code and hardware integration is under development by the HandLab and will be made available at our OSF project page.

4.2.5 TMS parameters

We used the two TMS machines independently. Each stimulator was connected to a figure-of-8 shaped TMS coil (7.5 cm) held tangentially to the surface of the head with the handle pointing backwards (flat coil) or upwards ('branding iron' coil), with the two loops of the coil held at an angle of about 45° away from the sagittal plane on each side. At this orientation, the monophasic TMS pulses induced a posterior-anterior current in the brain. Each stimulator delivered one single TMS pulse over the left or right hemisphere optimal location for the FDI muscle. TMS pulses were delivered at the same time over the two hemispheres at around 110% of participants' rMT. TMS intensity was occasionally adjusted during the experiment as required to maintain MEPs above the floor and below

the ceiling (i.e., at around 1 mV peak-to-peak). Based on the number of blocks completed, participants received between 512 and 1024 TMS pulses in total.

In the first TMS block, TMS was delivered randomly in a time window starting from the presentation of the target and ending 300 ms later. For subsequent blocks, TMS was delivered randomly in a time window starting from the presentation of the target and ending at a time point corresponding to the mean of the participant's RT in the preceding block. The mean RT was calculated at the end of each block, using a custom MATLAB script that found the first grip response that exceeded 9 SD from the pre-stimulus baseline.

The participants were provided with ear plugs to dampen the noise associated with the TMS pulse discharge. We used transparent, flexible, thin plastic TMS coil-shaped templates to keep the TMS coils in a constant position, and Manfrotto arms and clamps to hold the TMS coils. In order to prevent overheating of the coils, we placed on each coil two round ice packs. Two experimenters remained in the room throughout the study to locate and check the position of the coil, monitor participant safety, and ensure compliance with the instructions. All TMS parameters were annotated for each participant in the HandLab database.

4.2.6 MEP recording and processing

EMG activity was recorded from two muscles in both hands. Based on results obtained in the previous experiment, in which we did not find interference effects in the FDS and EDC muscles, in this experiment we recorded EMG activity in more distal muscles. Two electrodes were placed over the first dorsal interosseous (FDI) and two were placed over the opponens pollicis (OP), abductor pollicis brevis (ABP) and flexor pollicis brevis (FPB) which comprise the thenar muscles (TM). The skin of the hands was cleaned with alcohol wipes. The reference electrode straps were positioned around the bony portion of both wrists.

EMG signal was continuously sampled using LabChart 8 (AD Instruments) at an increased rate of 4kHz to allow for finer sampling of MEPs. In the first part of processing, we used EMG signal to extract peak-to-peak MEP amplitude by finding the minimum and maximum values within a search window, starting from 15 ms and ending 50 ms after TMS, while excluding TMS-related artefacts. As shown in **Figure 4.1**, we report the MEP extraction in one participant. MEP amplitude was adjusted for individual differences by subtracting from each MEP the minimum value and then dividing by the difference between the maximum and minimum MEP amplitude within each block of trials, giving a relative MEP amplitude from 0 to 1 per muscle and participant. In the second part of processing, EMG data were rectified and filtered with a second-order dual-pass Butterworth

filter with a bandpass of 1–10 Hz and then adjusted for individual differences. We divided by the maximum EMG activity within each EMG channel.

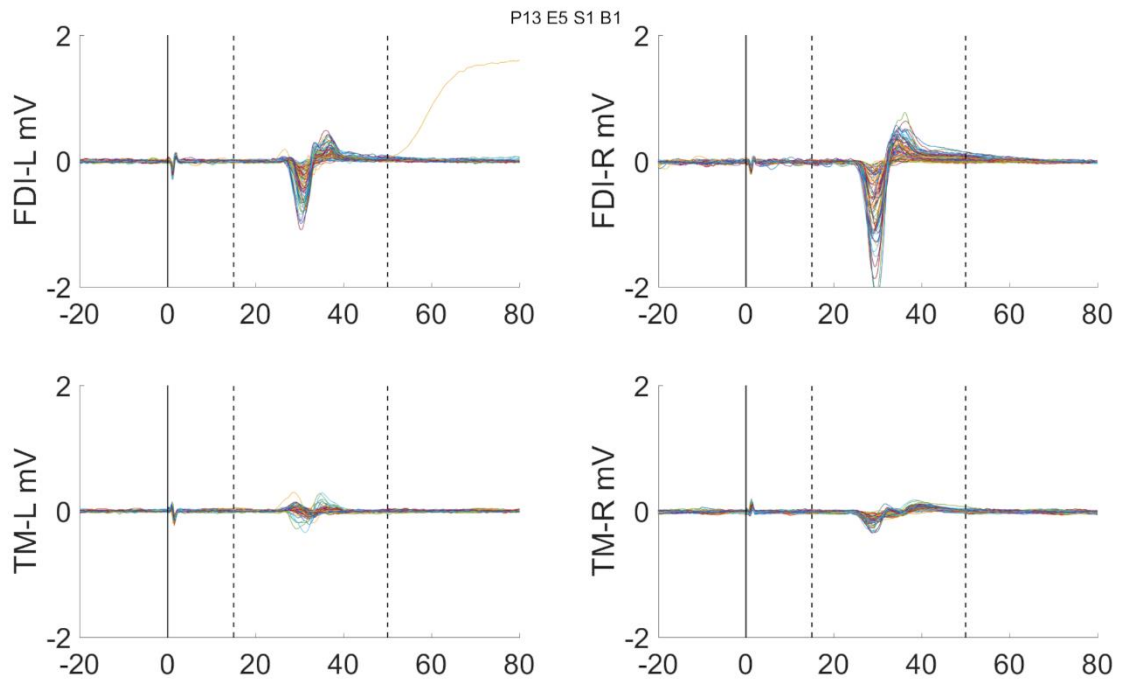


Figure 4.1 MEPs in left and right FDI and TM muscles in subject 1 in the first block. Vertical black line at time = 0 ms shows the onset of TMS. Vertical dashed black lines at time = 15 and 50 ms show time window for MEP extraction.

4.2.7 Data and statistical analysis

We investigate the presence of perceptual, response and general interference at behavioural level. All descriptive results are reported in **Table 4.1** and **Table 4.2**.

Based on the sensitivity analysis ($\alpha=.05$, $\text{power}=.80$), effect sizes larger than $d= 0.73$ will be statistically significant with 15 participants (Lakens, 2014).

Firstly, we segmented a time window starting with presentation of the flankers and ending 2000 ms after the target (stimulus-locked epoch), then we calculated RT for each trial. For subsequent analysis, we considered the trials in which participants responded correctly. Secondly, we looked at the MEP amplitude in a response-locked analysis, subtracting RT from stimulation time (when TMS pulse was delivered). In the plots, MEP amplitude was on y axis, and the RT was on x axis where zero corresponded to the starting of the RT.

We modelled the MEP data using a 'kneebend fit' model, from the HandLab ToolBox, in which data were fitting using two straight lines and three parameters. The model is graphically illustrated in **Figure 4.2**, in which we show the data for one participant. The horizontal line (parameter 'b') corresponded to the baseline level of relative MEP amplitude. The diagonal line (parameter 'c') was a slope, positive or negative, which modelled the changes in response after a particular time point. This parameter showed velocity of MEP changing, therefore how quickly participants made their decision. The vertical line (parameter 'a') corresponded to the time point which was the decision time. For parameter 'a', positive numbers corresponded to the time after the RT. Negative numbers corresponded

to the time before the RT, therefore more and less negative numbers are further and closer to RT respectively.

We investigated the rate of increase in MEP amplitude for chosen and unchosen hands in C, SI, and SRI conditions separately. We expected to find a positive slope for the chosen hand and a negative slope for the unchosen hand. It corresponded to increased MEP amplitude and decreased MEP amplitude after a decision time point for chosen and unchosen hand, respectively. We did not expect to find different representations for C, SI, SRI conditions. In terms of timing, we expected to find a start point in the unchosen hand which happened ~150 ms before the start point of the chosen hand.

For parameters 'a' and 'c' separately, we performed a repeated measures ANOVA with muscle (FDI and TM), hand (left and right) choice (chosen versus unchosen), and condition (C, SI, SRI) as within-subject factors. The baseline parameter 'b' was not analysed, because it had no theoretical significance in the model. It was not involved in modelling the changes in response after a particular time point. Considering the nature of the study, we were interested in main effects of choice, condition and interaction between these two factors (3 tests). For this reason, we decided for a hypothesis-driven approach and statistical significance was assessed with $p < .05$. Degrees of freedom and p values were corrected using Greenhouse–Geisser estimates of sphericity where the assumption was violated.

The partial eta-squared were calculated as an estimate of the effect size. We did not have a clear hypothesis about the remaining effects in the ANOVA. For this reason, we explored the data, then we corrected the significance for multiple comparisons (12 tests) using the Bonferroni's correction. Statistical significance was assessed for $p < 0.004$. Data were analysed using JASP (JASP Team, 2021).

		Total		Congruent		Stimulus Incon- gruent		Stimulus Re- sponse Incon- gruent	
		M	(SD)	M	(SD)	M	(SD)	M	(SD)
Participants (n=15)	Accuracy (%)	78.0	(13.0)	80.9	(14.2)	81.4	(12.7)	74.7	(13.0)
	RT (ms)	382	(54.5)	357	(56.4)	373	(48.8)	401	(59.1)

Table 4.1 Mean (M) and standard deviation (SD) of proportion of correct responses (accuracy) and reaction times (RT) under total, congruent, stimulus incongruent, and stimulus response incongruent condition.

		M	(SD)	t	df	p	d
Perceptual	Accuracy (%)	0.50	(4.83)	0.40	14	.696	0.103
	RT (ms)	15.5	(26.5)	2.26	14	.040	0.584
Response	Accuracy (%)	-6.63	(5.31)	-4.83	14	<.001	-1.25
	RT (ms)	27.6	(21.9)	4.88	14	<.001	1.26
General	Accuracy (%)	-6.13	(3.80)	-6.25	14	<.001	-1.62
	RT (ms)	43.1	(20.0)	8.34	14	<.001	2.15

Table 4.2 *Perceptual, response and general interference effect. Mean (M), standard deviation (SD), T-test (t), degree of freedom (df), effect size (Cohen's d).*

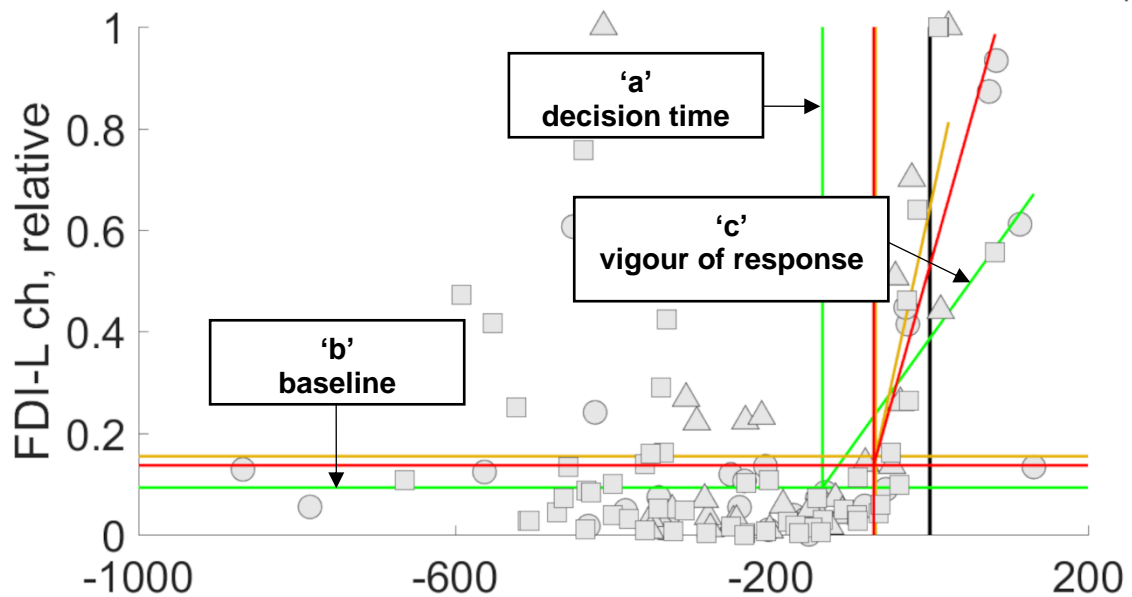


Figure 4.2 The ‘kneebend fit’ model in one subject in left FDI muscle, chosen hand. Congruent (circles, green line), stimulus incongruent (triangles, gold line) and stimulus response incongruent (squares, red line) conditions. Vertical solid black line at time = 0 ms shows the start of RT.

4.3 RESULTS

4.3.1 Slope parameter ‘c’

Means and standard errors (SE) are reported in **Table 4.3**. We found a significant effect of choice ($F(1,14)=42.3$, $p<.001$, $\eta p^2=.751$, $MSE=0.0000303$), suggesting that MEP amplitude in the chosen hand increased more than unchosen hand ($M=0.004$, $SE=0.000453$; $M=0.000371$, $SE=0.000453$ respectively) across time. One sample t tests were performed to compared the MEP amplitude

with zero for each muscle, hand and condition in chosen and unchosen hand separately. In the chosen hand, MEP amplitude increase were significantly different from zero, except for the right TM in the SRI condition ($F(1,14)=1.81$, $d=0.468$, $p=.091$) (**appendix 3, Table S3.1**). In the unchosen hand, the MEP amplitudes did not differ from zero (**appendix 3, Table S3.2**). Statistical analysis did not show other significant results for this parameter as reported below. We did not find significant effect of muscle ($F(1,14)=3.81$, $p=.071$, $\eta p^2=.214$, $MSE=0.00000805$), hand ($F(1,14)=0.278$, $p=.606$, $\eta p^2=.019$, $MSE=0.0000161$) and condition ($F(1,18)=0.136$, $p=.779$, $\eta p^2=.010$, $MSE=0.0000481$).

There were no significant effects for the interaction between muscle with hand ($F(1,14)=0.933$, $p=.351$, $\eta p^2=.062$, $MSE=0.0000105$), choice ($F(1,14)=0.589$, $p=.455$, $\eta p^2=.040$, $MSE=0.0000138$) and condition ($F(2,23)=0.765$, $p=.455$, $\eta p^2=.052$, $MSE=0.00000374$). We did not find significant interactions of the hand with choice ($F(1,14)=0.199$, $p=.662$, $\eta p^2=.014$, $MSE=0.0000187$) or condition ($F(2,23)=0.604$, $p=.522$, $\eta p^2=.041$, $MSE=0.0000117$). There was no significant interaction between choice and condition ($F(2,22)=1.71$, $p=.207$, $\eta p^2=.109$, $MSE=0.0000179$).

Analysis did not show any significant effects when interactions between three factors were performed. The interactions between muscle, hand and choice

($F(1,14)=0.804$, $p=.385$, $\eta p^2=.054$, $MSE=0.00000678$), muscle, hand and condition ($F(1,18)=0.305$, $p=.649$, $\eta p^2=.021$, $MSE=0.0000133$), muscle, choice and condition ($F(1,21)=1.47$, $p=.249$, $\eta p^2=.095$, $MSE=0.00000982$) were not significant. There was no significant interaction between hand, choice and condition ($F(1,17)=1.24$, $p=.293$, $\eta p^2=.081$, $MSE=0.0000312$).

The factors muscle, hand, choice and condition when combined showed no significant interaction ($F(2,23)=0.320$, $p=.688$, $\eta p^2=.022$, $MSE=0.00000605$). The results for slope parameter 'c' are shown in **Figure 4.3**.

Muscle	Hand	Choice	Condition	M	SE
FDI	LEFT	CH	C	0.006	0.002
			SI	0.005	0.000823
			SRI	0.004	0.000827
		UC	C	0.000192	0.000227
			SI	0.000446	0.000311
			SRI	0.001	0.000948
	RIGHT	CH	C	0.004	0.000701
			SI	0.004	0.000853
			SRI	0.005	0.001
		UC	C	0.000322	0.000329
			SI	0.000925	0.000717
			SRI	0.000135	0.000298
TM	LEFT	CH	C	0.004	0.001
			SI	0.003	0.000546
			SRI	0.004	0.000831
		UC	C	-0.0000230	0.000299
			SI	0.000563	0.000483
			SRI	0.0000507	0.0000833
	RIGHT	CH	C	0.004	0.0007183
			SI	0.002	0.0007198
			SRI	0.005	0.003
		UC	C	0.0000847	0.000220
			SI	0.000819	0.000410
			SRI	-0.000119	0.000179

Table 4.3 Mean (M) and standard errors (SE) for slope parameter 'c' in FDI and TM muscles. Chosen hand (CH); unchosen hand (UC), congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) conditions.

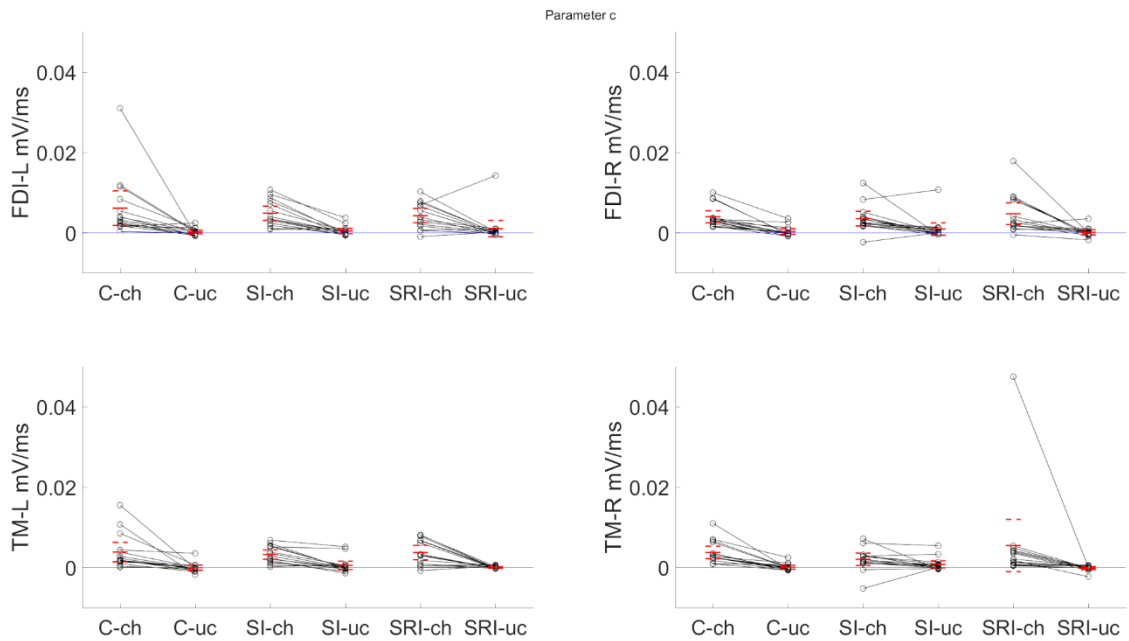


Figure 4.3 Mean and 95% confidence interval are shown for slope parameter 'c' in FDI and TM muscles. Chosen hand (ch); unchosen hand (uc), congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) conditions.

4.3.2 Kneepoint parameter 'a'

Means and SE are reported in **Table 4.4**. There was a significant effect of choice ($F(1,14)=12.3$, $p=.003$, $\eta^2=.468$, $MSE=97352$) showing that MEP amplitude in the chosen hand started increasing later (closer to the RT) in comparison to the unchosen hand ($M=-156$ ms, $SE=13.3$; $M=-271$ ms, $SE=29.9$ respectively). Muscle, hand and condition did not reach significant effects separately ($F(1,14)=0.006$, $p=.940$, $\eta^2=.0004$, $MSE=12986$; $F(1,14)=1.18$, $p=.295$,

$\eta^2=.078$, $MSE=39673$; $F(2,25)=3.15$, $p=.064$, $\eta^2=.184$, $MSE=28583$, respectively).

There was a significant interaction between choice and muscle ($F(1,14)=5.51$, $p=.034$, $\eta^2=.283$, $MSE=11793$). In order to explore the main effect of muscle, we performed in chosen and unchosen hands separately, a repeated measures ANOVA with muscle (FDI and TM), hand (left and right) and condition (C, SI, SRI) as within-subject factors. In the chosen hand, MEP amplitude of the FDI muscle increased later (closer to the RT) than the TM muscle ($M=-142$ ms, $SE=10.4$; $M=-170$ ms, $SE=17.0$ respectively; $F(1,14)=8.67$, $p=.011$, $\eta^2=.382$, $MSE=4009$), whereas we found no difference in starting time of the MEP amplitude changes between FDI and TM muscles in the unchosen hand ($M=-284$ ms, $SE=32.0$, $M=-258$ ms, $SE=31.5$ respectively; $F(1,14)=1.46$, $p=.247$, $\eta^2=.094$, $MSE=20770$). The interaction between choice and muscle was not significant when we applied the Bonferroni's correction (null hypothesis not rejected). The interaction between choice and condition reached significance $F(2,27)=4.54$, $p=.022$, $\eta^2=.245$, $MSE=20656$). In order to explore main effect of the condition, we performed in chosen and unchosen hand separately, a repeated measures ANOVA with muscle (FDI and TM), hand (left and right) and condition (C, SI, SRI) as within-subject factors. In unchosen hand, the kneepoints were significantly different between congruency conditions ($M=-319$ ms, $SE=33.0$; $M=-279$ ms, $SE=44.2$; $M=-216$ ms, $SE=26.1$, for SRI, SI and C respectively; $F(2,26)=5.09$,

$p=.015$, $\eta p^2=.267$, $MSE=33753$). There was no effect of condition in the chosen hand ($M=-165$ ms, $SE=18.0$; $M=-140$ ms, $SE=17.8$; $M=-163$ ms, $SE=19.1$; for SRI, SI and C respectively; $F(2,28)=0.807$, $p=.455$, $\eta p^2=.054$, $MSE=14443$).

We found a significant interaction between muscle and condition $F(2,22)= 4.13$, $p=.039$, $\eta p^2=.228$, $MSE=20291$). Post-hoc tests using Bonferroni correction were performed averaging over hand and choice factors. Results are summarized in **Table S3.3 in appendix 3**. For TM muscle the kneebend point in the SRI condition was significantly earlier compared to the C condition ($M=-268$ ms, $SE=23.2$; $M=-185$ ms, $SE=24.4$ respectively; $t(14)=3.14$, $d=0.503$, $p=.042$). No significant interactions emerged between muscle with hand ($F(1,14)=0.363$, $p=.557$, $\eta p^2=.025$, $MSE=17620$) as well as between hand with choice ($F(1,14)=0.033$, $p=.859$, $\eta p^2=.002$, $MSE=39782$) and condition separately ($F(2,27)=1.48$, $p=.246$, $\eta p^2=.095$, $MSE=19609$). The interaction between muscle and condition was not significant when we applied the Bonferroni correction (null hypothesis not rejected).

We did not find a significant interaction when we considered three factors. The interaction between muscle, hand and choice ($F(1,14)=1.88$, $p=.192$, $\eta p^2=.118$, $MSE=14209$), muscle, hand and condition $F(2,27)=1.63$, $p=.214$, $\eta p^2=.104$, $MSE=17656$) and muscle, choice and condition $F(2,21)=2.91$, $p=.088$, $\eta p^2=.172$,

MSE=17643). It was not significant the interaction between hand, choice and condition $F(2,27)=0.177$, $p=.830$, $\eta^2=.012$, MSE=34236).

Also the interaction with all four factors was not significant muscle, hand, choice and condition $F(2,27)=1.74$, $p=.196$, $\eta^2=.111$, MSE=17513). The results for kneepoint parameter 'a' in the whole group are summarized in **Figure 4.4**.

Muscle	Hand	Choice	Condition	M	SE
FDI	LEFT	CH	C	-140	26.1
			SI	-128	17.1
			SRI	-143	22.8
		UC	C	-268	45.5
			SI	-256	66.8
			SRI	-250	53.1
	RIGHT	CH	C	-150	13.3
			SI	-142	19.3
			SRI	-149	21.8
		UC	C	-221	46.4
			SI	-390	68.8
			SRI	-320	49.7
TM	LEFT	CH	C	-187	40.5
			SI	-139	21.3
			SRI	-142	20.4
		UC	C	-186	34.9
			SI	-254	65.0
			SRI	-334	58.2
	RIGHT	CH	C	-176	30.7
			SI	-151	25.1
			SRI	-224	37.1
		UC	C	-190	45.7
			SI	-216	48.0
			SRI	-370	57.9

Table 4.4 Mean (M) and standard errors (SE) for kneepoint parameter 'a' in FDI and TM muscles. Chosen hand (CH); unchosen hand (UC), congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) conditions.

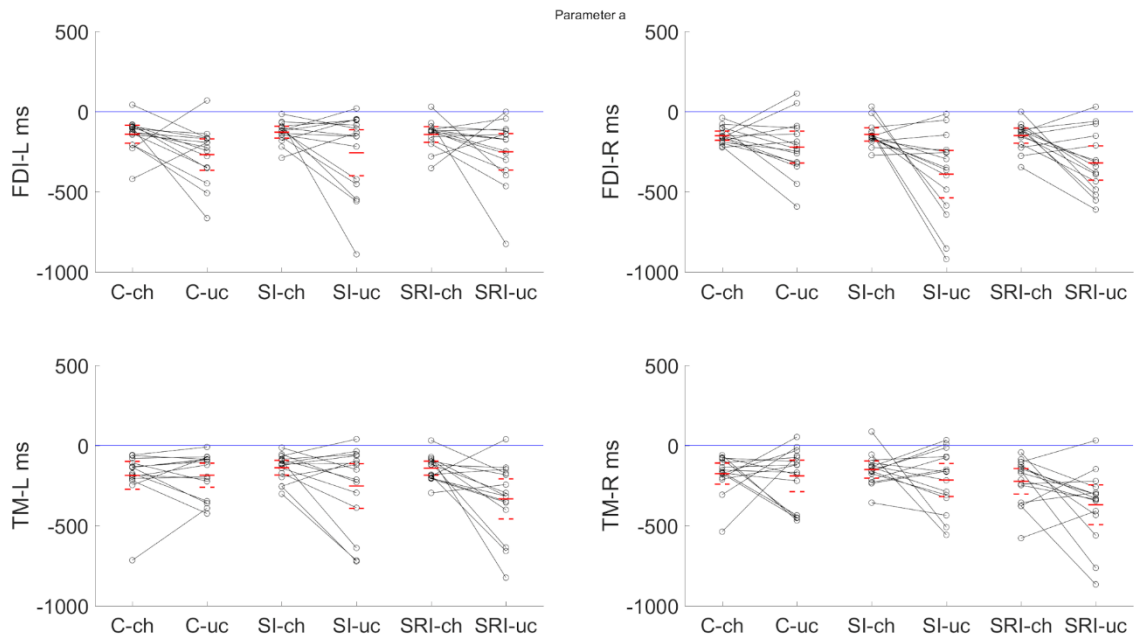


Figure 4.4 Mean and 95% confidence interval are shown for the kneepoint parameter 'a' in FDI and TM muscles. Chosen hand (ch); unchosen hand (uc), congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) conditions.

4.4 DISCUSSION

In our study we aimed to investigate, at the central nervous system level, the motor processes related to the chosen and unchosen hand. In particular, we investigated the neural mechanisms which preceded the onset of hand response. Stimulating over M1 we studied the temporal dynamics of MEP which is a reliable measure of corticospinal excitability. TMS pulse was delivered in a time window between the target onset and mean of RT for each participant. At behavioural

level, it is worth underlining that our sample did not show a significant perceptual interference effect. Previous studies found that MEP amplitude increased for chosen hand and decreased for unchosen response (Klein-Flugge et al., 2013; Michelet et al., 2010; Verleger et al., 2009). However, the researchers did not investigate the corticospinal excitability when irrelevant stimuli and responses interfere with movement. To fill this gap in literature, we used the colour version of the Flanker task. We modelled our data, using a 'kneebend fit' model, to study both functional and temporal aspects of the corticospinal excitability in chosen and unchosen hand under different congruency conditions. We conducted response-locked analysis, subtracting the individual RT from the stimulation time. This allowed us to investigate the motor representations before the RT started.

We found that MEP amplitude increased significantly more for the chosen than the unchosen hand. Moreover, increased amplitude in the chosen hand was significantly different from zero. In the unchosen hand, the MEP amplitude changing was not significantly different from zero. These results originated from the analysis of slope parameter 'c' through which we investigated the functional aspects of corticospinal excitability. Considering the results obtained in the chosen hand, our findings are in line with previous researches (Klein-Flugge et al., 2013; Michelet et al., 2010; Verleger et al., 2009) in which the authors reported an increased corticospinal excitability related to the hand chosen for the response.

These findings are in line with results of our EMG experiment, described in Chapter 3. The activation process of the chosen hand, observed at peripheral level, was in line from a functional point of view to the activation process observed at the central level. We did not expect the results observed in the unchosen hand. This finding was not in line with the previous studies (Klein-Flugge et al., 2013; Michelet et al., 2010; Verleger et al., 2009) in which the authors observed a decreased MEP amplitude related to the unchosen hand. It can be assumed that initially chosen and unchosen hands are close to the baseline, until one hand is ultimately chosen for the response. We did not find any significant interaction between choice and condition. The MEP amplitude in chosen and unchosen hands were not different in relation to C, SI and SRI conditions. Therefore, we can suppose that the time-course of motor excitability, once the response has begun, did not change for different congruency conditions.

Looking at the kneepoint parameter 'a', we analysed the temporal aspects of MEP activity in chosen and unchosen hands before hand gripping was made. Considering the response locked analysis, it is important to remember that zero corresponded to the RT. We found that MEP amplitude started increasing in chosen hand 156 ms before the RT. In the literature, Klein-Flugge and colleagues (Klein-Flugge et al., 2013) observed increased MEP amplitude in the chosen hand at 230 ms before the movement onset. To facilitate comparison between studies, we compared the two timing (156 ms and 230 ms) and then divided by the SE

(13.3 ms) in our sample. The obtained t-score (5.56) indicated a significant difference of timing between studies. We assume that time difference between studies is related to the different cognitive tasks used and cognitive processes involved. Participants in the previous study took ~70 ms more than our participants because they were involved in decision making processes. The model showed a significant result also for the unchosen hand, 271 ms before the RT. Because the MEP activity changes in the unchosen hand did not differ from zero as reported above, it is difficult to interpret the significant timing in the unchosen hand.

Initially, we found a significant interaction between choice and muscle. We considered the possible explanation. In the chosen hand, MEP amplitude increased 142 ms before the RT started in the FDI and 170 ms before the RT started in the TM muscle. In the unchosen hand, we did not find any significant difference of time activation between FDI and TM muscles. These results might show that muscles are involved at different times in hand responses. When we applied the Bonferroni's correction, the interaction between choice and muscle did not reach significance, therefore we are aware that our explanation could have limitations and further studies are required. We found a significant interaction between choice and condition. In unchosen hand, the model showed a significant time difference between congruency conditions. At this point, it is not possible to discuss about time difference between congruency conditions. When we analysed

the slope parameter 'c' in the unchosen hand, as reported below, the MEP amplitude did not significantly differ over time. Therefore, it is difficult to interpret this time difference between congruency conditions. It seems that cognitive load is higher for SRI, followed by SI and C condition: relative to RT, the motor process started earlier for SRI (319 ms) and progressively later for SI (279 ms) and C (216 ms) conditions. Finally, we found a significant interaction between muscle and condition. In TM muscle, the time point was significantly earlier for SRI (268 ms) compared to the C (185 ms) condition. Later, when we applied the Bonferroni's correction, the interaction between muscle and condition was not significant. At this point as well, other studies are required in this direction.

The results obtained from this experiment provide insights into the functional and temporal dynamics of the motor processes that occur prior to hand gripping. We hypothesise that both hands seem to undergo an inactivation process close to the baseline before one hand is selected for the response. In particular, the findings showed increased corticospinal excitability of the chosen hand 156 ms before the hand gripping passed the RT threshold. We hypothesise that this motor mechanism does not appear to be influenced by congruence conditions. It is an unspecific motor process, which does not change in relation to different interference conditions. It is difficult to link these findings with findings observed in our EMG experiment, in which we found at peripheral level the activation of the chosen hand and a decreased activation of the unchosen hand (~150 ms before). In

other words, the peripheral activation of the chosen hand is temporally anticipated by increased MEP amplitude. Unexpectedly, the deactivation of the unchosen hand (Chapter 3, EMG) was not replicated by a decreased MEP amplitude (this Chapter, MEP). The MEP amplitude was close to the baseline. At this point, it is very difficult to explain the motor mechanisms which happen along the spinal cord and that could explain this difference.

To conclude, we found at the central level an increased corticospinal excitability related to the chosen hand, starting at 157 ms before the hand grip RT. This was an unspecific process irrespective of interference conditions. On the basis, of this result, we planned the experiment presented in the next Chapter in which we tried to interfere with the preparation of motor processes to modulate the interference effect.

CHAPTER 5 - TIMING AND ROLE OF THE SUPPLEMENTARY MOTOR AREA (SMA) IN THE PREPARATION OF HAND MOVEMENT.

5.1 INTRODUCTION

Investigating the role and temporal dynamics of brain areas involved in preparation and execution of motor response has been a fundamental question in the literature.

The M1 is a crucial brain area involved in execution of movement (Bhattacharjee et al., 2021). Temporal dynamics of the MEPs elicited by TMS over contralateral M1 can reliably show different aspects of the hand and finger movement using different decision making and interference tasks (Klein-Flugge et al., 2013; Michelet et al., 2010; Verleger et al., 2009). As reported in our previous TMS experiment, we stimulated the M1 in order to study when movements related to chosen and unchosen hands originated in the corticospinal system. In the chosen hand, we found increasing MEP amplitude from 156 ms before the RTs, whereas MEP amplitude in the unchosen hand did not significantly increase from baseline. In this Chapter, we are interested in understanding brain mechanisms involved in suppression of flanker response to select the target response. In other words, the stage preceding the motor hand execution for which the M1 is responsible.

The SMA is an important area for contributing to preparation of motor response as well as to movement selection, initiation, coordination, execution and feedback monitoring of motor response (Makoshi et al., 2011; Oda et al., 2021; Passingham, 1996; Sadato et al., 1997). Motor preparation has been defined as processes that occur between a stimulus start cue and movement execution (Bestmann & Duque, 2016).

In a functional neuroimaging study, Lee and colleagues reported activation of specific SMA regions during a finger-thumb tapping movement. The anterior part of the SMA, with dense connections to the prefrontal cortex, was predominately involved in earlier stages of motor processing, such as selection and preparation of a motor response. The posterior part of SMA, which is directly connected to M1, was predominantly involved in later stages such as initiation and execution of the motor response (Lee et al., 1999).

A few studies have investigated the involvement of the SMA in preparation of motor responses using TMS (Guida et al., 2023; Parmigiani & Cattaneo, 2018; Pineda-Pardo et al., 2019). In recent research, Guida and colleagues (Guida et al., 2023) explored the role of the SMA in automatic and voluntary response inhibition using two non-invasive brain stimulation techniques over the SMA. In the first experiment, a group of healthy volunteers, aged 22-36 years, underwent a

theta-burst stimulation protocol involving repetitive TMS. In the second experiment, a different group of healthy participants, aged 21-33 years, underwent a transcranial static magnetic field stimulation. In both experiments, the authors performed sham stimulation sessions as a control condition. In the training phase, participants performed a go/no go learning task in order to learn stimulus-response associations. After the brain stimulation sessions, both groups performed the go/no go learning task but with reversed stimulus-response associations, and the stop signal task in order to investigate the effect of brain stimulation on voluntary and automatic response inhibition, respectively. The authors expected to find deteriorated inhibition performance after brain stimulation sessions in comparison to sham stimulation session. The results of the experiment were unexpected - there were no significant differences after applying real or sham stimulation session, neither in automatic nor voluntary inhibition tasks. The authors supposed a hypothesis of indirect or reduced involvement of the SMA in the inhibitory control.

In the same laboratory, Pineda-Pardo and colleagues (Pineda-Pardo et al., 2019) previously conducted a similar experiment in healthy volunteers. The authors applied real or sham transcranial static magnetic field stimulation over the SMA in two double-blind subsamples. The study aimed to investigate role of the SMA in motor choice behaviour, specifically in withholding predicted actions, motor planning and stimulus-response compatibility using fully-cued, uncued-compatible

and uncued-incompatible tasks, respectively. The authors expected impaired motor performance after real brain stimulation in comparison to sham stimulation session. Results showed that the group undergoing real brain stimulation showed (within 30 minutes) longer RTs in all motor choice tasks, and increased accuracy in the fully-cued motor task in comparison to the sham control group. These findings suggested that brain stimulation over SMA significantly affected the speed-accuracy trade off, particularly that initiation times increased in favour of increased accuracy.

In the neuromodulation studies described above, the authors investigated SMA functionality after the neuromodulation session (i.e., 'offline' stimulation). They did not study when the SMA planned movement to resolve the motor reaction time and response inhibition tasks in an online setting. Therefore, it is fruitful to study the SMA functionality during the neuromodulation session (i.e., 'online' stimulation), because it allows to investigate the temporal aspects of SMA's functional role.

Parmigiani and Cattaneo (Parmigiani & Cattaneo, 2018) applied single pulse or sham TMS over the SMA in a group of healthy volunteers. The authors investigated the role of the SMA during responses in the stop-signal task performed with the lips. Participants were asked to respond as quickly as possible to the 'go' stimulus and inhibit responses to the 'stop' stimulus. The authors considered the

accuracy for analyses of performance. Results showed no TMS effect, neither on the performance of STOP trials nor on GO trials. The authors found these results as unexpected, therefore they proposed different hypothesis to explain the reasons of this negative result.

Looking at the literature, the results are mixed, therefore it is difficult to understand clearly in TMS studies the role of SMA in preparation of a motor program. To the best of our knowledge, there are no studies aimed to investigate the SMA functionality in the preparation of motor program to suppress irrelevant stimuli and inappropriate responses. In other words, when motor program related to the hand response is planned in the SMA.

In this Chapter, the aim was to study the timing and role of SMA in the preparation of motor responses related to the flanker and target stimuli. We delivered two (paired) pulses of TMS over the SMA in a time window from 60 to 300 ms after the target onset. We used a more frontal site to control for the TMS position, and a noTMS condition to control for the TMS sound. In the NoTMS condition, TMS was delivered away from the scalp. The sound pressure of TMS pulse was similar in the SMA and noTMS condition. Based on the findings observed in our previous TMS experiment (Chapter 4), we hypothesized that interfering with the preparation of the motor response associated with the chosen hand would result in changes in flanker performance. It will be difficult to hypothesise how TMS will

modulate the preparation of motor response. On this basis, we do not have a hypothesis whether TMS could facilitate or inhibit the preparation of motor response in chosen hand.

5.2 METHOD

The methods were the same as described in the previous Chapter, except where indicated.

5.2.1 Participants

We recruited 14 adults (20-46 years old, mean \pm SD=28.7 \pm 7.2 years, 7 females, 13 right-handed by self-report). They were students and staff at the University of Nottingham, recruited via email advertisements to a mailing list of participants who had previously participated in other experiments, and via word of mouth. They received an inconvenience allowance of £20 for taking part. All participants were fluent in English and reported no history of neurological or psychiatric disorders. All had normal or corrected to normal vision. Participant safety and suitability to undergo TMS was assessed using a self-administered questionnaire, checked by the experimenter. Side effects or discomfort were monitored using a follow up questionnaire over 24 hours following stimulation. No significant side effects or discomfort attributed to TMS were reported in the questionnaire by participants. During the experiment, one participant reported discomfort relating

to the TMS, and seemed to be developing a pre-syncope reaction of faintness and mild confusion. For this participant, we interrupted the experiment. Written informed consent was obtained from each participant. The study received ethical approval from the research ethics committee of the University of Nottingham (reference: SoPEC1445).

5.2.2 Stimuli, task and procedure

A coloured version of the Flanker task, already presented in previous experiments, was administered (**Figure 2.1a**). Responses were collected with hand-held grip force sensors ('dynamometer', AD Instruments) and a standard high-speed USB2 computer keyboard (used for practice only). If accuracy was lower than 87% and/or the mean RT was longer than 500 ms, feedback was shown to encourage them to improve accuracy and/or speed. To save time, the practice block was not repeated. Participants were instructed to maintain a steady grip of around 1.5% of maximum voluntary contraction during the task, and then to respond to the colour of the middle square (the target) by squeezing the response dynamometer, and to ignore the surrounding squares (the flankers). All code for running the experiment are freely available at <https://osf.io/7smux/>.

Participants went through two practice blocks of 16 pseudorandomised trials, the first using a standard QWERTY keyboard to respond. In second block of the practice and experiment, participants used dynamometers to respond, EMG activity

was recorded and TMS was applied as described below. All participants, except one, completed between nine to twelve response-balanced blocks (16 C, 16 SI and 32 SRI trials), giving in total between 576 to 768 trials per participant. In each case the experiment took a maximum of three hours. The participant, who reported discomfort relating to the TMS, completed only 4 blocks, giving in total 256 trials.

5.2.3 Apparatus

For eleven participants, we used a recent structural MRI scan to determine the position of the SMA and M1. The individual T1-weighted MRI scan was obtained using a 3 Tesla Philips Achieva MRI Scanner (MPRAGE, $1 \times 1 \times 1$ mm). We used the structural MRI image alongside theBrainsight stereotaxic neuronavigation (Rogue Research, Inc., Montreal, Canada) to obtain individual scalp measurements of brain targets. Three participants did not have a recent MRI scan; therefore, we used the mean scalp measurements of SMA and M1 of a previous unpublished study conducted in the HandLab. TMS was carried out using a Magstim Bistim machine comprising of two Magstim 200² machines yoked together (Magstim Company Ltd, Whitland, Carmarthen-shire, UK). EMG data were digitized using LabChart 8 (AD Instruments).

5.2.4 Localization of M1 for motor threshold

Participants wore a cap which fitted the head well. Then, we took measurements from nasion toinion and from left and right preauricular points to determine correct location of the vertex.

We localised the M1 position in left hemisphere. In the whole sample, mean \pm SD of M1 were 5.21 \pm 0.70 cm lateral and 0.36 \pm 0.57 cm anterior of vertex. In this experiment, we did not study MEPs, therefore we used the method reported by Rossini and colleagues (Rossini et al., 1994) to determine the rMT. It was a quicker method compared to the QUEST algorithm described in the previous experiment, and saved TMS pulses for each participant. With the coil placed over M1, we evaluated the rMT by gradually increasing and reducing single pulse TMS intensity. This threshold was defined as the minimum intensity needed to elicit MEPs in the relaxed right-hand FDI with a peak to peak of at least 0.05 mV in five of ten consecutive trials. We used a figure-of-8 shaped TMS coil (10 cm outer diameter) held tangentially to the surface of the head with the handle pointing backwards at an angle of about 45° away from the sagittal plane on each side.

5.2.5 Localisation of TMS sites

We selected two brain sites for TMS. The SMA position was localised using the individual structural MRI scan alongside stereotaxic neuronavigation. In the whole sample, mean \pm SD scalp measurements of SMA were -0.07 \pm 0.27 cm

lateral and 1.69 ± 1.02 cm anterior of vertex. Using the SMA position, we determined the TMS control site position, which approximately corresponded to the Fz site according to the 10-20 EEG system. We localised the TMS control site 0 cm lateral and 5 cm anterior of the SMA position, and identified the site halfway for the noTMS condition, specifically 0 cm lateral and 2.5 cm anterior of SMA position.

5.2.6 TMS parameters

The TMS stimulator was connected to a figure-of-8 shaped TMS coil (10 cm) held tangentially to the surface of the head with the handle pointing backwards (flat coil) at an angle of 0° from the sagittal plane. At this orientation, the monophasic TMS pulses induced a posterior-anterior current in the brain. The SMA was stimulated at around 120% of participants' rMT. Participants rated, using a 10 point rating scale, the subjective annoyance of receiving brain stimulation over SMA (Meteyard & Holmes, 2018). We reduced the MSO intensity used for SMA stimulation by 16%, then we stimulated the TMS control site. Participants rated, using the same 10 point rating scale, the annoyance of the TMS control site. In order to match annoyance between SMA and TMS control site, TMS intensity was adjusted in accordance with the participant's rating. The annoyance ratings were not systematically noted; therefore it was not possible to compare them statistically. In the noTMS condition, we used a foam TMS coil-shaped spacer of 5 cm thickness to keep the TMS coil away from the scalp. We measured

the sound pressure level (dB) of TMS, recorded at the participant's ear, so that it was approximately similar between the TMS and noTMS conditions.

Paired TMS pulses were delivered at 34 ms apart. In each trial, TMS pulses were randomly applied at one out of four possible time points (60 ms, 128 ms, 196 ms, 264 ms) covering a time window starting 60 ms and ending 300 ms after the presentation of the target. TMS was applied either over the SMA, on the control site or in the noTMS condition, in counterbalanced order across participants. Based on the number of blocks completed, participants received a total of 512 (N=1), 1152 (N=1), 1408 (N=1), 1536 (N=11) TMS pulses.

5.2.7 EMG recording and processing

As described in our previous TMS experiment, EMG activity was recorded from two electrodes placed over the FDI muscle and two placed over the TM muscles. The reference electrode straps were positioned around the bony portion of both wrists. EMG signal was continuously sampled at a rate of 4kHz. Offline, EMG data were rectified and filtered with a second-order dual-pass Butterworth filter with a bandpass of 1–10 Hz and then adjusted for individual differences. We divided the signals by the maximum EMG activity within each EMG channel.

5.2.8 Data and statistical analysis

The percentage of correct responses and mean RTs for correct responses were calculated. We considered RTs longer than 150 ms after presentation of the target, because it was unlikely possible to suppress interference information before that time. For each site and TMS time, the mean of percentage correct responses and RT under C, SI and SRI conditions were calculated. The descriptive results are reported in **Table 5.1 and 5.2**. Because the percentage of correct responses across the whole group was less than 90%, using IES was not recommended as a measure of performance. There was an outlier subject who we decided to consider in our analysis.

Three strategies were used to analyse data. Firstly, we used RTs and accuracy separately to perform a repeated measures ANOVA with site (SMA, control site, NoTMS), TMS time points (60 ms (t1), 128 ms (t2), 196 ms (t3), 264 ms (t4)), and condition (C, SI, SRI) as within-subject factors. Secondly, to simplify the analysis (from a 3x4x3 to a 2x4x3 design), we subtracted the NoTMS condition from SMA and control sites. Using RTs and accuracy separately, we perform a repeated measures ANOVA with site (SMA, control site), TMS time points (60 ms, 128 ms, 196 ms, 264 ms), and condition (C, SI, SRI) as within-subject factors. Thirdly, to match the analyses of previous Chapters, we subtracted the NoTMS condition from SMA and control sites and then we calculated the perceptual, response and

general interference effects. We used absolute scores as in Chapter 2. For perceptual, response and general interference effects separately, we perform a repeated measures ANOVA with site (SMA, control site) and TMS time points (60 ms, 128 ms, 196 ms, 264 ms) as within-subject factors.

The last two strategies showed results which were either non-significant or difficult to interpret, therefore in the following section, we will report the results obtained using the first strategy, which uses the raw data rather than differences. Statistical significance was assessed with $p < .05$. Degrees of freedom and p values were corrected using Greenhouse–Geisser estimates of sphericity where the assumption was violated. The partial eta-squared were calculated as an estimate of the effect size. Data were analysed using JASP (JASP Team, 2021).

Based on the sensitivity analysis ($\alpha = .05$, power = .80), effect sizes larger than $d = 0.75$ will be statistically significant with 14 participants (Lakens, 2014).

POSITION	TMS time	CONDITION	MEAN	SE
SMA	t1	C	85.9	3.78
		SI	87.5	2.70
		SRI	78.2	3.86
	t2	C	81.0	3.15
		SI	85.0	2.86
		SRI	81.1	2.59
	t3	C	87.3	1.94
		SI	84.8	2.24
		SRI	82.2	2.99
	t4	C	88.2	2.59
		SI	89.6	2.22
		SRI	87.9	2.51
NoTMS	t1	C	85.0	2.50
		SI	87.8	2.90
		SRI	80.4	3.02
	t2	C	83.1	3.52
		SI	89.6	2.40
		SRI	81.9	2.86
	t3	C	88.4	3.09
		SI	84.6	3.17
		SRI	84.5	3.72
	t4	C	90.4	3.20
		SI	87.2	5.05
		SRI	85.9	2.78
Frontal	t1	C	88.6	2.60
		SI	91.1	2.60
		SRI	80.9	2.49
	t2	C	90.1	2.50
		SI	82.9	4.74
		SRI	84.5	2.95
	t3	C	87.3	3.01
		SI	88.5	2.41
		SRI	83.6	3.05
	t4	C	86.3	4.55
		SI	90.7	2.33
		SRI	83.3	6.01

Table 5.1 Mean (M) and standard errors (SE) of percentage correct responses under congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) conditions for each TMS time and position.

POSITION	TMS time	CONDITION	MEAN	SE
SMA	t1	C	418	46.6
		SI	412	37.4
		SRI	427	27.2
	t2	C	401	31.2
		SI	412	28.2
		SRI	466	44.6
	t3	C	459	55.7
		SI	431	37.5
		SRI	445	33.1
	t4	C	428	35.4
		SI	448	31.6
		SRI	478	47.6
NoTMS	t1	C	393	38.1
		SI	409	26.5
		SRI	438	36.9
	t2	C	434	44.1
		SI	443	40.6
		SRI	477	42.9
	t3	C	384	30.1
		SI	459	39.4
		SRI	461	35.0
	t4	C	425	41.6
		SI	469	49.2
		SRI	464	43.4
Frontal	t1	C	375	31.5
		SI	418	42.4
		SRI	454	40.4
	t2	C	399	38.0
		SI	431	38.5
		SRI	450	34.5
	t3	C	441	44.1
		SI	431	32.5
		SRI	461	36.9
	t4	C	439	48.6
		SI	465	43.6
		SRI	461	35.0

Table 5.2 Mean (*M*) and standard errors (*SE*) of RT under congruent (*C*), stimulus incongruent (*SI*) and stimulus response incongruent (*SRI*) conditions for each TMS time and position.

5.3 RESULTS

In the whole sample, mean \pm SD of TMS intensity over SMA and the control site were 64.9 \pm 5.94% and 49.4 \pm 4.83% of the MSO, respectively. Mean \pm SD of rMT was 54.1 \pm 5.03 of the MSO.

5.3.1 Percentage of correct responses

We found a significant effect of condition (M=86.8, SE=2.10; M=87.4, SE=1.99; M=82.9 SE=2.74 for C, SI and SRI respectively; $F(2,26)=5.03$, $p=.014$, $\eta^2=.279$, MSE=205). We performed post-hoc tests using Bonferroni correction. Percentage of correct responses was significantly higher in SI compared to the SRI condition ($t(13)=2.93$, $d=0.783$, $p<.021$) showing a significant response interference effect. After comparing percentage of correct responses between C and SI condition ($t(13)=0.409$, $d=0.109$, $p=1$) and between C and SRI condition ($t(13)=2.52$, $d=0.673$, $p=.055$) we did not find significant perceptual and general interference effect. The factors site and TMS time did not reach significance separately ($F(2,26)=2.24$, $p=.127$, $\eta^2=.147$, MSE=47.8; $F(2,23)=2.49$, $p=.110$, $\eta^2=.161$, MSE=179 respectively).

No significant interactions emerged when site was combined with TMS time ($F(4,48)=0.944$, $p=.441$, $\eta^2=.068$, MSE=117) and condition separately ($F(2,30)=0.179$, $p=.866$, $\eta^2=.014$, MSE=135). There was no significant interaction between TMS time and condition ($F(4,49)=1.87$, $p=.134$, $\eta^2=.126$,

MSE=91.7). The interaction between position, TMS time and condition was not significant ($F(4,48)=1.21$, $p=.320$, $\eta^2=.085$, $MSE=264$). Results are summarised in **Figure 5.1**.

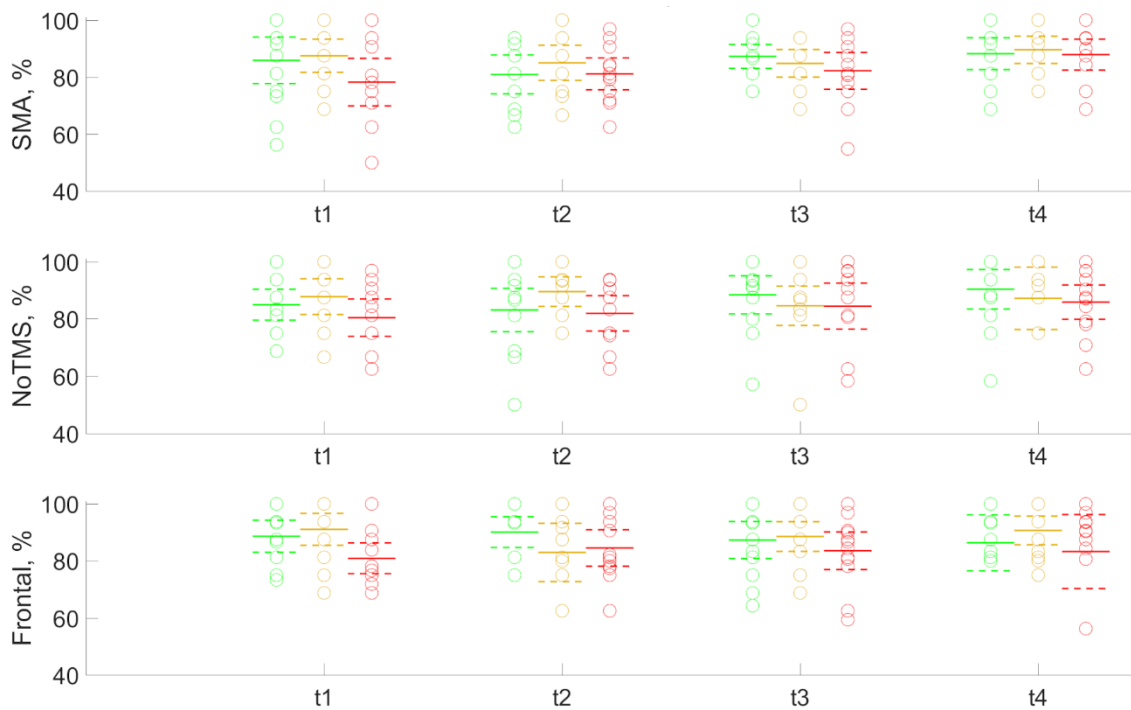


Figure 5.1 Percentage of correct responses under congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) condition for TMS times. Mean and 95% confidence interval are shown.

5.3.2 RT

We found a significant effect of TMS time ($M=416$ ms, $SE=34.5$; $M=435$, $SE=36.5$; $M=441$ ms, $SE=36.0$; $M=453$ ms, $SE=40.1$ for t1, t2, t3, t4 respectively;

$F(2,24)=10.3$, $p<.001$, $\eta^2=.441$, $MSE=4769$). Post-hoc tests using the Bonferroni correction showed that RTs at t1 were significantly faster compared with t3 ($t(13)=-3.70$, $d=-0.999$, $p=.004$) and t4 ($t(13)=-5.42$, $d=-1.45$, $p<.001$) separately. All comparisons are shown in **appendix 4 (Table S4.1)**. There was also a significant effect of condition, showing that RTs increased significantly across interference conditions ($M=416$, $SE=38.6$; $M=436$, $SE=34.9$; $M=457$, $SE=36.8$ for C, SI and SRI respectively; $F(2,23)=20.2$, $p<.001$, $\eta^2=.608$, $MSE=3902$). Post-hoc tests using the Bonferroni correction were performed. RTs reported in C condition were significantly faster compared to SI ($t(13)=-3.03$, $d=0.810$, $p=.016$) and SRI condition ($t(13)=-6.35$, $d=1.70$, $p<.001$). Moreover, RTs in SI were significantly faster in comparison with SRI condition ($t(13)=-3.32$, $d=0.887$, $p=.008$). These results showed in the sample significant perceptual, response and general interference effects. There was no significant effect of TMS site ($F(2,21)=0.112$, $p=.851$, $\eta^2=.009$, $MSE=4296$).

We did not find a significant interaction when we considered two factors. The interactions between site with TMS time ($F(3,39)=1.61$, $p=.202$, $\eta^2=.110$, $MSE=4183$) and condition did not reach significance ($F(2,24)=1.37$, $p=.273$, $\eta^2=.095$, $MSE=8029$). There was no significant interaction between TMS time and condition ($F(3,39)=0.856$, $p=.473$, $\eta^2=.062$, $MSE=5553$).

Also the interaction between all three factors, as site, TMS time and conditions did not reach significance ($F(3,41)=1.92$, $p=.138$, $\eta^2=.129$, $MSE=10063$). Results are summarised in **Figure 5.2**.

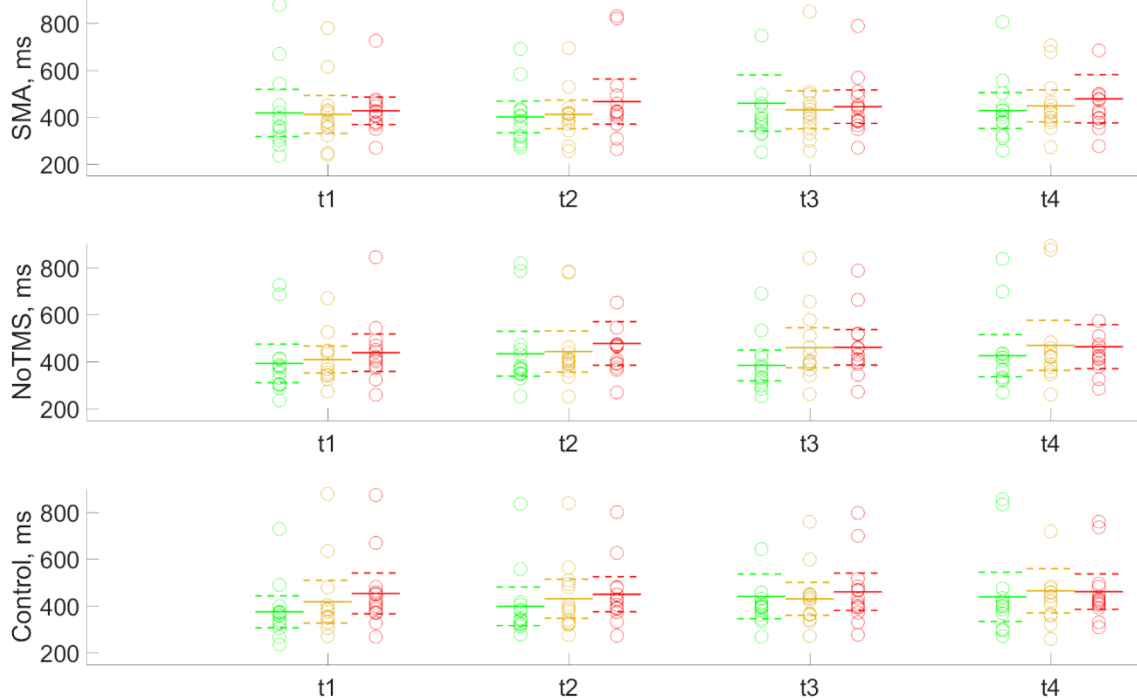


Figure 5.2 RT under congruent (C), stimulus incongruent (SI) and stimulus response incongruent (SRI) condition for TMS times. Mean and 95% confidence interval are shown.

5.4 DISCUSSION

In this study we aimed to investigate the involvement of SMA in the preparation of motor responses. We were interested in studying the role and temporal

aspects of SMA in suppressing irrelevant stimuli and inappropriate responses. Using paired pulses TMS, we stimulated the SMA in a time window between 60 ms and 300 ms after the onset of target. Previous TMS studies showed mixed results in either online or offline settings (Guida et al., 2023; Parmigiani & Cattaneo, 2018; Pineda-Pardo et al., 2019). Based on the findings observed in our previous TMS experiment in Chapter 4, we hypothesized that interfering with the preparation of the motor response associated with the chosen hand would result in changes in flanker performance. It was very difficult to hypothesise how TMS could modulate the preparation of motor response. We did not have a hypothesis whether TMS could facilitate or inhibit the preparation of motor response in chosen hand. This experiment was largely exploratory.

In this experiment, we analysed accuracy and RTs separately. Because we did not find high accuracy for all participants, using IES is not recommended to measure performance. Looking at the percentage of correct responses, we found a significant effect of interference condition. Participants showed more accurate performance in the SI compared with SRI condition. In SI condition, flanker stimuli facilitated the correct response because the flankers and target were associated to the same hand response. At this point, this was not a surprising result. It was already reported by Hirst and colleagues (Hirst et al., 2019) and discussed extensively in the Chapter 2. Looking at the RTs, there was a significant effect of TMS time. Further analysis showed that participants performed faster when TMS

was delivered at 60 ms in comparison to 196 ms and 264 ms after target onset. We interpreted this result as consequence of the clicking sound and other side-effects produced by the TMS machine and coil. It increased the participants' alertness, facilitating the speed of performance. TMS pulses delivered closer to the target onset speeded the RTs much more than TMS pulses delivered further away. This was already shown in a previous study (Duecker et al., 2013). The authors underlined the importance of differentiating between changes in behaviour that are linked to neural mechanisms and behavioural performance that resulted from the features of TMS itself. We found a significant effect of interference condition. The speed of performances increased in relation with the amount of irrelevant information. In other words, participants showed longer RTs in SRI condition than SI condition, which were longer compared to C condition. This result was in line with previous studies in which the authors showed that irrelevant information slowed the performance in comparison with congruent condition (Eriksen & Eriksen, 1974; Eriksen, 1995).

Contrarily to our expectations, we did not find a significant interaction between TMS site and condition when we analysed either the percentage of correct responses or the RTs. In other words, the effect of congruence condition was not different when TMS was applied on the SMA, control site or during NoTMS condition. TMS did not interfere with the motor process related to the chosen hand

response, therefore we did not modulate the interference effect, neither at stimulus nor response level. This result is line with previous studies in which delivering TMS over SMA did not interfere with the response preparation (Guida et al., 2023; Parmigiani & Cattaneo, 2018).

This result must be interpreted with caution. Although it was a negative result, we cannot exclude the possibility that the SMA plays a role in the motor response preparation. There are different possible explanations. Firstly, other brain areas are involved in the suppression of irrelevant information, therefore they could have compensated the SMA functionality. Considering anatomical and functional connectivity of SMA with cortical and subcortical brain structures (Isherwood et al., 2023), it is likely that interference control is the result of cooperation of different brain areas. This could explain why we did not observe an alteration of interference effect at the behavioural level. Secondly, the contribution of the SMA is likely to be more significant in other aspects of cognitive control. Because this is not a unitary construct, it is likely that SMA is involved in self-initiated inhibitory mechanisms (Ridderinkhof et al., 2014) or in inhibition of complex bimanual movements (Cincotta & Ziemann, 2008; Tanji, 1994; Toyokura et al., 2002). In our Flanker task, participants were required to respond to external stimuli pressing key buttons or squeezing dynamometers. Although the task required a bimanual movement, it was a simple movement and could explain why we did not observe the SMA involvement. This was also supposed by Parmigiani and Cattaneo

(Parmigiani & Cattaneo, 2018) as a possible explanation of negative results obtained in their research. Thirdly, TMS did not reach the deeper layers of SMA. Although, it is generally considered a superficial brain structure within the cortex rather than a deep subcortical structure, TMS was not able to stimulate appropriately the SMA interfering with its functionality. Spatial resolution makes TMS as not an ideal technique to investigate the role of SMA. Lastly, our study was sufficiently powerful to find the congruency effects, but we cannot exclude that TMS effect on the interference is much smaller. This could explain why we did find a modulation of the interference effect stimulating the SMA.

From a methodological point of view, there is a limitation in our study. Due to a coding error, we did not counterbalance the type of congruence trials across the four TMS time-points. As extensively reported in Chapter 1, number of SRI trials was doubled in comparison with C and SI trials. This prevented an automatic association of flanker stimuli with the correct response. Having a different number of trials was difficult counterbalance them across the TMS time (t1, t2, t3, t4). This likely influenced the variability of data observed in our study, as some participants had more trials per TMS time than others.

To sum up, in this study we did not show the involvement of SMA in the programming of motor responses during the Flanker task. We cannot exclude the role of SMA in the motor response preparation. Different lesion studies described the so

called SMA syndrome, supporting the SMA role in motor and speech response preparation (Della Sala et al., 2002; Palmisciano et al., 2022; Young et al., 2021). Further studies are required in this direction to assess the causal relationship between SMA and response motor preparation during interference control tasks.

CHAPTER 6 - GENERAL DISCUSSION AND CONCLUSIONS

6.1 Summary of findings

In this thesis we aimed to study the interference control. By using the coloured version of the Flanker task, which allowed us to separate the interference at stimulus and response level, we investigated the behavioural and neural aspects of interference control. The Flanker task is the 'train of thought' between Chapters. The main findings are summarised as follows:

A) All age groups showed significant interference effects. Younger people are more susceptible to both distracting stimuli and competing responses in comparison to older people.

B) Peripherally, we found decreased activation of the unchosen hand and increased activation of the chosen hand. The unchosen hand was deactivated around 150 ms before activation in the chosen hand. This was an unspecific motor process, which did not change across different interference conditions. We observed these findings in the dynamometers, but not in EMG signal recorded in FDS and EDC muscles.

C) Using single pulse TMS over M1, we found increased MEP amplitude, related to the chosen hand, starting 156 ms before RT. We did not detect specific motor processes related to the unchosen hand.

D) Paired pulsed TMS over SMA did not modulate the interference effect at both stimulus and response level. We reported different hypothesis, which likely might explain these findings.

6.2 How our findings meet previous literature

At this point, it is crucial to understand how our findings could be integrated in the dual process model and the wider literature. We will discuss them critically, so possible weakness can be used to improve knowledge of interference control in future studies.

In Chapter 1, we discussed the dual process model, to which different studies referred to understand cognitive processes involved in conflict situations (Frank et al., 2009; Ridderinkhof, 2014; Ridderinkhof et al., 2021). In conflict situations, such as in the Flanker task, the target activates the correct response in accord with instructions via the controlled route, while flanker stimuli prompt a response via the automatic route. At the response level, when the response associated with the target is the same as the response associated with the flanker, the response

is executed quickly. If the flankers activate a different response from that associated with the target, both response options may be activated, creating a response competition. In the literature, there is still a debate regarding the temporal course of response resolution. Specifically, it is debated whether the response activated from the flankers is faster than the response associated with the target. In other words, the dual process model hypothesised in incongruent trials the activation of the flanker (automated) response first, then activation of the target (controlled) response. Looking at findings reported in literature, it is possible to hypothesise a simultaneous activation of responses. As reported in Chapter 3, Szucs and colleagues (Szucs et al., 2009) detected, only in a minority of trials, increased EMG activity in the unchosen hand prior to a chosen hand response, whereas in majority of the trials, the chosen and unchosen response activations were concurrent. These data, which replicated a different previous study (Caldas et al., 2012), allowed to think about simultaneous activation of flanker and target responses rather than a sequential activation. The results showed in our EMG study (Chapter 3) do not support the hypothesis of simultaneous activation. In dynamometers, activation of chosen hand was preceded from deactivation of the unchosen hand as to promote a constructive motor preparation process in incongruent trials. Ours and findings showed in literature (Caldas et al., 2012; Szucs et al., 2009) highlight weakness of the dual process model in terms of timing and underlining mecha-

nisms. For this reason, we encourage researchers to investigate temporal aspects and dynamics underlining motor response in conflict situations so that other cognitive models of interference control might be more appropriate.

In Chapter 2, we found that all age groups experienced a significant interference effect. The response interference was significantly greater than the stimulus interference. In accord with the dual processes model, in the stimulus condition the flanker and target require the same response, whereas in the response condition the flanker and target responses do not match leading to a response competition and more interference. In this Chapter, we found that younger people were more susceptible to both stimulus and response interference in comparison to older people. This result seems in disagreement with the theories of cognitive decline in older people, however it is possible that they are two faces of the same coin. In accord with a previous study, we hypothesise neural compensatory mechanisms which could explain the behavioural performance. In older adults, greater neural activity, specifically in dorsolateral prefrontal cortex and anterior cingulate cortex, may compensate deficient mechanisms involved in the interference control (Salami et al., 2014). Moreover, Ménétré and Laganaro (Menetre & Laganaro, 2023) hypothesised that older people use crystallised intelligence to compensate a loss in the fluid intelligence abilities. While attentional and executive resources decline, the control of interference seems spare due to compensatory mechanisms. A matter to consider at this point is related to different interference tasks

used in literature. Hirst and colleagues (Hirst et al., 2019) used the Stroop test to investigate the stimulus and response interference in older people, whereas we used the coloured version of Flanker task. As remarked in Chapter 1, the interference paradigms are not exchangeable because different control processes are involved. For this reason, it is difficult to compare our findings with previous studies due to the interference tasks heterogeneity.

In Chapter 3 and 4, we moved on studying the motor processes at peripheral and central level respectively. In these studies, we used dynamometers to measure interference effects at the behavioural level. Dynamometers are helpful instruments to measure interference control because they allow to measure across the time motor processes in both hands simultaneously. In conflict situations, it is worth investigating the neurophysiological aspects of the unchosen hand. This aspect has not been detected in studies which used the keyboard to measure interference. In our EMG and TMS studies, participants showed a significant perceptual, response and general interference effect. These findings well replicated results observed in young adults' group in Chapter 2, in which we used keyboard, underlining worthiness of dynamometers to measure interference effect.

In Chapter 3, we focused on studying the motor processes at peripheral level. Using dynamometers, we found an increased activation of the chosen hand and a decreased activation of the unchosen hand. The deactivation of the unchosen

hand started around 150 ms before the chosen hand activation. This finding allows us to hypothesise a constructive process between hands rather than a response competition as hypothesised in the dual process model. The decreased signal of the unchosen hand optimises the motor activation of the chosen hand. Because we did not find differences related to the different interference conditions, we hypothesise this to be an unspecific motor process underlying decision making in general. When we analysed EMG signals in FDS and EDC muscles we found interesting results. As expected, we found significant activation of both muscles related to the chosen hand. Surprisingly, we did not find any significant EMG activity related to the unchosen hand. Furthermore, when we analysed the interference, we did not replicate the perceptual, response and general interference effects which we observed in dynamometers. These findings raise doubts regarding whether EMG measurement is a helpful method to measure neurophysiology of interference control. We hypothesise that any congruency signal in the EMG data is smaller than in the grip force data. For this reason, we encourage future studies aimed to investigate these EMG aspects.

In Chapter 4, we investigated the motor processes before the hand response execution. We focused on studying the motor processes at central level. Stimulating over M1 in left and right hemisphere, we found increased MEP amplitude related to the chosen hand which started 156 ms before the hand gripping. The literature reported a gradual increase in the amplitude of MEP, which started

around 100 ms prior to the initiation of volitional EMG (Bestmann & Duque, 2016; Chen & Hallett, 1999). Also at central level, the motor process did not change in relation to the interference conditions, suggesting it was an unspecific motor process of the decision-making process. Unexpectedly, we did not show a significant decreasing MEP amplitude related to the unchosen hand. In an exhaustive review, Bestmann and Duque (Bestmann & Duque, 2016) reported that specific postural requirements during the experiment could be responsible for suppression of MEPs in the unchosen hand. When the unchosen hand is not included in the initial response set, the suppression is frequently weaker. Up to now, it is still unresolved how MEP suppression in the unchosen hand is influenced by postural control, and further studies are required.

Findings showed in the TMS study partially replicated motor mechanisms reported in our EMG study. The peripheral activation of chosen hand (Chapter 3) was preceded by increased MEP amplitude at central level (Chapter 4). Furthermore, velocity of the MEP amplitude changing was not significantly different between stimulus and response interference. So far, TMS findings well replicated EMG results. On the other side, the peripheral deactivation of unchosen hand (Chapter 3) was not preceded by decreased MEP amplitude at the corticospinal level (Chapter 4). It is difficult to understand the reasons why we did not replicate our previous findings. Our data do not provide additional interpretations on the corticospinal pathway from M1 to motor hand response. In general, these findings

raise some doubts about whether TMS measure is an appropriate measure in studying the interference control. Specifically, TMS did not give important information about neurophysiological mechanisms, at the central level, which were specific of stimulus and response interference.

Finally, in Chapter 5, we tried to modulate the flanker interference effect. Using a paired pulse TMS protocol we stimulated over the SMA. In literature, different studies widely reported the involvement of SMA in the preparation of a motor program (Makoshi et al., 2011; Oda et al., 2021; Passingham, 1996; Sadato et al., 1997). The role of SMA has also been considered in the dual process model (Ridderinkhof et al., 2021). Unexpectedly, we did not find different performances, in terms of accuracy and RTs, among TMS over SMA and a more frontal control brain site, and the NoTMS condition. Looking at this negative finding, we cannot exclude that SMA is involved in the preparation of flanker and target responses. We interpreted our findings with caution. Firstly, when considering the anatomical and functional connections between the SMA and various cortical and subcortical brain regions (Isherwood et al., 2023), it becomes evident that interference control is likely an outcome of collaborative efforts among different brain areas. Secondly, the significance of the SMA's role is likely more pronounced in other aspects of cognitive control. Given that cognitive control is not a singular construct, it is plausible that the SMA plays a role in self-initiated inhibitory processes (Ridderinkhof et al., 2014) or in the inhibition of complex bimanual movements

(Cincotta & Ziemann, 2008; Tanji, 1994; Toyokura et al., 2002). Thirdly, looking at the anatomical features of the SMA, we assume that TMS is not a useful technique to reach deeper layer of this brain area, as already suggested (Parmigiani & Cattaneo, 2018).

Discussing with the examiners of this thesis, it is worth underlining that across all experiments, in young adults, accuracy data exclusively showed response interference, with Cohen's *d* effect-sizes of between -0.667 and -1.25, while reaction time showed perceptual (0.584-1.87), response (0.963-1.29), and general interference (1.48-2.47). (**Table 6.1**). At this point, we encourage meta-analytic methods to investigate this aspect of interference control.

Study		Perceptual interference			Response interference			General interference		
		M	(SD)	<i>d</i>	M	(SD)	<i>d</i>	M	(SD)	<i>d</i>
Exp 1	Accuracy (%)	-0.321	(3.30)	-0.097	-5.24	(6.43)	-0.815	-5.56	(6.33)	-0.878
	RT (ms)	29.6	(15.8)	1.87	35.0	(27.2)	1.29	64.6	(32.6)	1.98
Exp 2	Accuracy (%)	-0.215	(3.88)	-0.055	-3.10	(4.66)	-0.667	-3.32	(5.66)	-0.587
	RT (ms)	23.2	(25.2)	0.923	22.1	(23.0)	0.963	45.4	(18.4)	2.47
Exp 3	Accuracy (%)	0.50	(4.83)	0.103	-6.63	(5.31)	-1.25	-6.13	(3.80)	-1.62
	RT (ms)	15.5	(26.5)	0.584	27.6	(21.9)	1.26	43.1	(20.0)	2.15
Exp 4	Accuracy (%)	0.639	(5.84)	0.109	-4.57	(5.83)	-0.783	-3.93	(5.85)	-0.673
	RT (ms)	19.3	(24.2)	0.798	21.2	(19.3)	1.10	40.5	(27.3)	1.48

Table 6.1 Behavioural interference effects for accuracy and RTs across experiments. Mean (*M*), standard deviation (*SD*), effect size (Cohen's *d*).

6.3. Limitations

We are going to report some limitations that arose across the reported experiments. We hope that following issues will be the starting point of future research, so that knowledge on interference control can improve in the future.

Firstly, there are variations in the experimental settings across children, young and older adults. Some participants were assessed during public outreach events, while others underwent testing in a more controlled laboratory environment, which involved the use of different screens and keyboards. Furthermore, age groups were tested using different number of practice blocks and number of trials. In future investigations, efforts should be made to minimize such experimental differences between age groups wherever feasible.

Secondly, we would suggest to future studies to use dynamometers rather than EMG. When we used dynamometers, it was possible to investigate clearly the motor mechanisms of interference control. When we analysed the EMG activity, we did not find significant results in the unchosen hand, and we did not find significant interference effects. In the EMG study, we found dynamometers as a more useful technique than EMG to measure motor mechanisms at the peripheral level.

Thirdly, we would recommend a training of grip baseline contraction. As a potential improvement, we propose implementing a training for grip baseline contraction prior to start the experiment. At the beginning of all studies, we measured and instructed participants to maintain a grip at 3% of their maximum throughout the experiment. Subsequently, we lowered the threshold to 1.5% of their maximum grip due to participants' fatigue. It would be worthwhile for future research to investigate whether training in grip baseline contraction can lead to significant results. In other words, it will be interesting to investigate whether after training the grip baseline in each participant, it will be possible to observe stronger or clearer decreases of MEP amplitude changing related to the unchosen hand. If so, it will be possible to replicate the decrease of unchosen grip force reported in EMG study.

6.4 Future directions

In these experiments we helped to better understand the mechanisms underlying the interference effect. However, we believe that there is still much work to be done to improve the knowledge on behavioural and motor aspects of interference control when distracting stimuli and inappropriate responses interfere with the response.

It is worth investigating the stimulus and response interference control in children younger than 6 years old. Implementing a simplified form of the Flanker task it

will be important to study developmental trajectories of stimulus and response interference control. In the lifespan experiment presented in Chapter 2, we did not analyse the performance of seven children, because these were very poor. We supposed that the Flanker task was too difficult, because two colour labels were associated with each key press, and this required much cognitive load. Adapting and simplifying the flanker interference task, associating one colour label with each key press, it will be possible to investigate the interference control in younger children.

It will be interesting to investigate whether different mechanisms underline different cognitive tasks. In their review, Bestmann and Duque (Bestmann & Duque, 2016) described corticospinal excitability related to chosen and unchosen responses in different conflict situations. They investigated the motor mechanisms in situations like resolution of competition among different response alternatives, impulse control and conflict situations arising from distracting information. The authors showed that MEP amplitude related to the chosen and unchosen hand changed as function of cognitive interference. Looking at this interesting review, we encourage future research to investigate the motor mechanisms using different cognitive tasks.

Finally, future studies could focus on study of interference and motor control in neurodegenerative disorders as Parkinson's disease. Increased interference effects have been reported in patients with Parkinson's disease in association with motor symptoms as bradykinesia, rigidity and tremor (Guo et al., 2021; Praamstra et al., 1999; Praamstra et al., 1998). It will be interesting to investigate which aspects of cognitive control are impaired and whether they are correlated with the gravity of clinical symptoms, disease duration, symptoms onset, age and global cognitive functioning. Given that there is currently no cure for Parkinson's disease, existing treatments primarily aim to alleviate its symptoms. Advancing our understanding of motor and interference control may be critical in improving treatment approaches and mitigating the disease's impact on everyday life, ultimately enhancing the quality of life for patients.

In conclusion, this thesis explored how the brain manages interference arising from irrelevant information, making a modest contribution to the existing literature in this field. We hope that it can stimulate further research in the future.

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APPENDIX 1

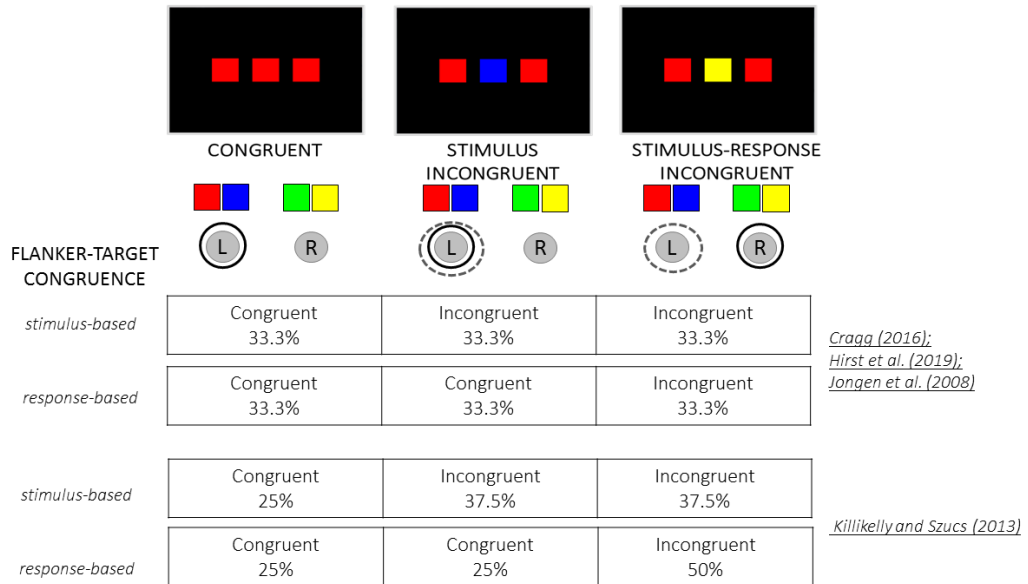


Figure S1.1 We used our experimental task as a schematic illustration to explain, in previous studies, how the proportion of trials for congruent, stimulus incongruent and stimulus response incongruent condition influences differently the flanker-target congruence when it was stimulus- and response-based. For each condition, correct response has been circled with a solid black line and the incorrect response in a broken grey line. L: left response button, R: right response button.

Correlation between MoCA score and perceptual, response and general interference effects

In the older adults, we did not find any significant association between the MoCA score (M=25.97, SD=2.50) and the perceptual (M=6.84, SD=47.9, $r(28)=.136$, $p=.472$), response (M=30.3, SD=65.3, $r(28)=.048$ $p=.799$) and general interference effect (M=37.2, SD=47.3, $r(28)=.214$, $p=.256$).

Correlation between accuracy and RTs

IES are recommended as measure of performance when the number of errors is low and when the accuracy and speed of performance are correlated. Furthermore, the importance of using combined scores of accuracy and speed to compare the performance among different ages has been supported before (Salthouse, 2010). In our study, a ceiling effect was shown for the accuracy, specifically all age groups reached at least 90% correct responses (older adults: M=97.2%, SD=4.30; children M=94.1%, SD=6.14 and young adults (M=92.6%, SD=4.92). A logistic transformation of the proportion of percentage of correct responses between blocks was performed to address the skewed distribution of the accuracy. We analysed in the total sample the association between the accuracy and the RTs using the Pearson's correlation. We found that better accuracy scores were associated with shorter RTs (M=3.36 ms, SD=1.15; M=737 ms, SD=221 respectively; $r(136)=-.211$ $p=.014$).

Study	Ages (years)	N	Perceptual interference				Response interference				General interference			
			IES	(SD)	SE	<i>d</i>	IES	(SD)	SE	<i>d</i>	IES	SD	SE	<i>d</i>
Our study	6-14	81	40.8	(129)	14.3	0.32	118	(127)	14.1	0.93	158	(196)	21.8	0.81
	20-43	24	32.7	(19.9)	4.1	1.64	71.1	(66.5)	13.6	1.07	104	(67.7)	13.8	1.54
	60-84	31	6.84	(47.9)	8.6	0.14	30.3	(65.3)	11.7	0.46	37.2	(47.3)	8.5	0.79
Cragg (2016)	7-10	76	176	(177)	20.3	0.99	259	(384)	44.1	0.67	435	(468)	53.7	0.93
	Adults	38	-2.76	(45.4)	7.4	-0.06	92.4	(62.8)	10.2	1.47	89.6	(58.5)	9.5	1.53
Hirst et al (2019)	6-11	49	41.1	(227)	32.4	0.18	80.1	(305)	43.6	0.26	121	(289)	41.3	0.42
	18-25	33	14.0	(50.9)	8.9	0.28	72.5	(118)	20.5	0.61	86.6	(107)	18.6	0.81
	61-85	38	69.4	(89.1)	14.5	0.78	194	(622)	101	0.31	263	(591)	95.9	0.45

Table S1.1 Inverse efficiency scores (IES), standard deviation (SD) and Cohen's *d*' calculated for each age group (Cragg, 2016 and Hirst et al. 2019). In Cragg, we calculated pooled IE and SD to combine 7 and 10 years olds in a single group. N: number of participants.

Results using the same number of trials across groups

We did not find any significant interaction between condition and age for perceptual, response and general interference effect ($F(1,134)=0.118$, $p=.732$, $\eta^2=.00088$, $MSE=16817$; $F(1,134)=0.284$, $p=.595$, $\eta^2=.00212$ $MSE=27466$; $F(1,134)=0.633$, $p=.428$, $\eta^2=.00470$, $MSE=27863$, respectively) (**Table S1.2**).

Children experienced more perceptual and response interference than older adults, whereas young adults showed comparable interference effects with children and older adults. Regarding the general interference, children and young adults showed comparable interference, and both groups experienced greater interference compared to older adults (**Table S1.3**).

	Stimulus-balanced (75% response-congruency)			Response-balanced (50% response-congruency)		
	M	SD	<i>d</i>	M	SD	<i>d</i>
Perceptual interference	41.0	125	0.328	14.0	155	0.090
Response interference	96.0	181	0.530	86.2	152	0.567
General interference	137	184	0.745	100	224	0.446

Table S1.2 Interference effects in stimulus and response balanced conditions, using the same number of trials across groups. Mean (*M*), standard deviation (*SD*), effect size (Cohen's *d*).

	Children (N=81)			Young adults (N=24)			Older adults (N=31)		
	M	SD	<i>d</i>	M	SD	<i>d</i>	M	SD	<i>d</i>
Perceptual	40.8	129	0.316	19.4	55.9	0.347	-0.919	58.6	-0.016
Response	117	127	0.921	71.0	101	0.703	36.4	85.5	0.426
General	158	196	0.806	90.4	96.3	0.939	35.5	67.0	0.530

	Children vs Young adults				Children vs Older adults				Young adults vs Older adults			
	t	df	<i>d</i>	p	t	df	<i>d</i>	p	t	df	<i>d</i>	p
Perceptual	1.17	89	0.114	.246	2.35	107	0.222	.021	1.30	53	0.175	.199
Response	1.65	103	0.161	.103	3.28	110	0.310	.001	1.38	53	0.186	.175
General	1.64	103	0.160	.105	4.93	109	0.466	<.001	2.49	53	0.336	.016

Table S1.3 Perceptual, response and general interference effects across age groups using the same number of trials across groups. Mean (M), standard deviation (SD), T-test (t), degree of freedom (df), effect size (Cohen's d).

Results using ratio scores

Stimulus-balanced versus response-balanced

We reanalysed the data using ratio scores as measure of interference. We found comparable perceptual, response and general interference effects between the

stimulus-balanced and response-balanced conditions ($F(1,134)=0.605$, $p=.438$, $\eta^2=.00450$, $MSE=0.021$; $F(1,134)=0.123$, $p=.726$, $\eta^2=.000917$, $MSE=0.029$; $F(1,134)=.406$ $p=.525$, $\eta^2=.003021$, $MSE=0.037$ respectively) (**Table S1.4**). We did not find a significant interaction between condition and age for any interference effect (perceptual: $F(1,134)=0.052$, $p=.819$, $\eta^2=.000388$, $MSE=0.021$; response: $F(1,134)=0.052$, $p=.820$, $\eta^2=.000388$ $MSE=0.029$; general: $F(1,134)=0.084$, $p=.772$, $\eta^2=.000626$, $MSE=0.037$).

	Stimulus-balanced (75% response-congruency)			Response-balanced (50% response-congruency)		
	M	SD	d	M	SD	d
Perceptual interference	1.06	0.142	0.423	1.03	0.160	0.188
Response interference	1.15	0.200	0.750	1.11	0.162	0.679
General interference	1.21	0.222	0.946	1.14	0.239	0.586

Table S1.4 Interference effects in stimulus and response balanced conditions using ratio scores. Mean (M), standard deviation (SD), effect size (Cohen's d)

Perceptual, response and general interference effects across age groups

No significant effect of age was found for the perceptual interference effect $F(1,134)=2.10$, $p=.150$, $\eta^2=.0154$, $MSE=0.013$. For response and general interference, a significant effect of age was shown ($F(1,134)=7.15$, $p=.008$, $\eta^2=.0507$,

MSE=0.018; $F(1,134)=8.29$, $p=.005$, $\eta^2=.0583$, MSE=0.033, respectively). Children and young adults showed comparable response and general interference, and both groups showed more response and general interference compared to older adults (**Table S1.5; Figure S1.2**). In summary, using the ratio score we found an effect of age only for the response and general interference. We did not find the effect of age for the perceptual interference effect.

	Children (N=81)			Young adults (N=24)			Older adults (N=31)		
	M	SD	<i>d</i>	M	SD	<i>d</i>	M	SD	<i>d</i>
Perceptual	1.05	0.135	0.370	1.08	0.046	1.74	1.02	0.071	0.282
Response	1.15	0.132	1.14	1.16	0.168	0.952	1.05	0.090	0.556
General	1.19	0.196	0.969	1.25	0.197	1.27	1.07	0.080	0.875

	Children vs Young adults				Children vs Older adults				Young adults vs Older adults			
	t	df	<i>d</i>	p	t	df	<i>d</i>	p	t	df	<i>d</i>	p
Perceptual	1.86	101	0.182	.065	1.40	99.0	0.132	.166	3.80	51.7	0.512	<.001
Response	0.559	103	0.055	.577	4.34	79.1	0.410	<.001	3.20	53	0.431	.002
General	1.41	103	0.138	.162	4.79	110	0.453	<.001	4.43	28.9	0.597	<.001

Table S1.5 Perceptual, response and general interference effects across age groups using ratio scores. Mean (M), standard deviation (SD), T-test (t), degree of freedom (df).

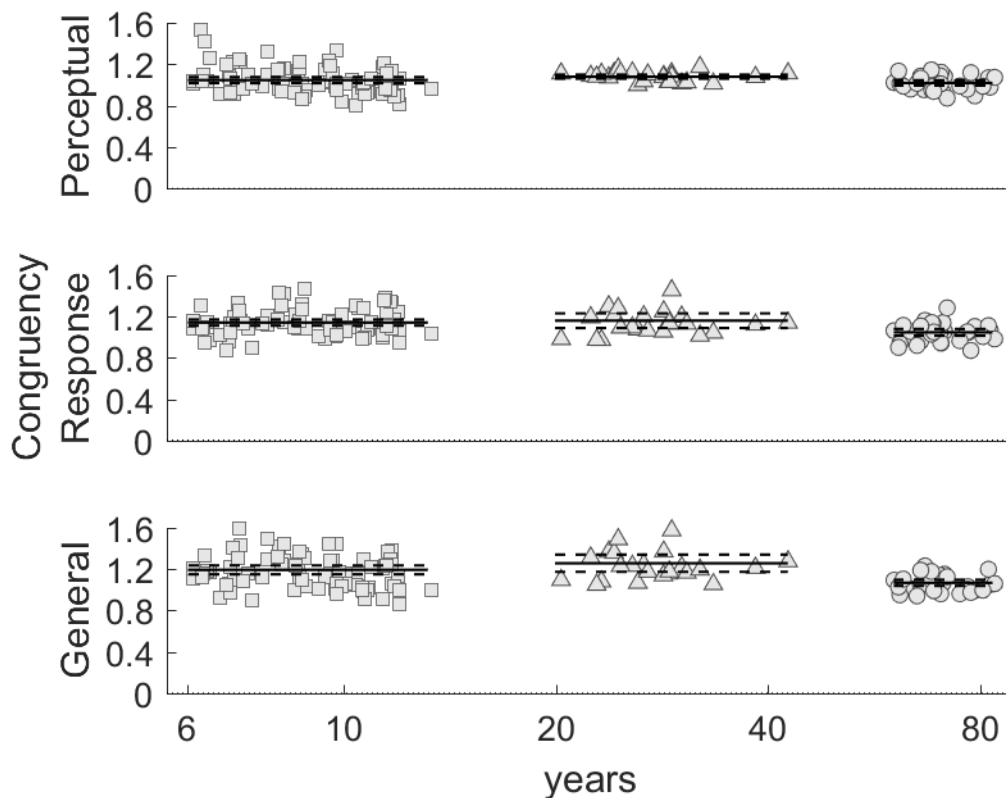


Figure S1.2 Perceptual, response and general interference effects are displayed across age groups using ratio scores: children (squares), young (triangles) and older adults (circles). Mean and 95% confidence interval are shown for each age group.

APPENDIX 2

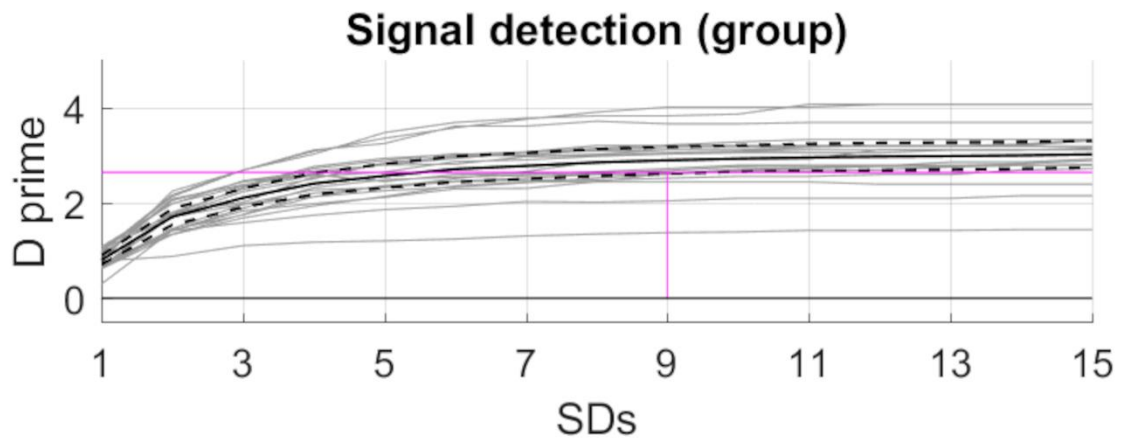


Figure S2.1 *D prime threshold in the whole group. Thin grey lines show individual data; solid black line shows the group mean data; broken black lines show the group standard deviations; purple line corresponds to chosen $D prime (= 2.64)$ and chosen $SD (=9)$.*

APPENDIX 3

	t	df	d	p
FDI-L C	3.06	14	0.790	.008
FDI-R C	5.67	14	1.46	<.001
TM-L C	3.44	14	0.888	.004
TM-R C	5.25	14	1.36	<.001
FDI-L SI	5.91	14	1.53	<.001
FDI-R SI	4.12	14	1.06	.001
TM-L SI	5.94	14	1.53	<.001
TM-R SI	2.86	14	0.738	.013
FDI-L SRI	5.17	14	1.34	<.001
FDI-R SRI	3.79	14	0.979	.002
TM-L SRI	4.52	14	1.17	<.001
TM-R SRI	1.81	14	0.467	.091

Table S3.1 One sample *t*-test in chosen hand. *T*-test (*t*), degree of freedom (*df*), effect size (Cohen's *d*)

	t	df	d	p
FDI-L C	0.846	14	0.218	.412
FDI-R C	0.981	14	0.253	.343
TM-L C	-0.077	14	-0.020	.940
TM-R C	0.385	14	0.099	.706
FDI-L SI	1.432	14	0.370	.174
FDI-R SI	1.290	14	0.333	.218
TM-L SI	1.166	14	0.301	.263
TM-R SI	1.998	14	0.516	.066
FDI-L SRI	1.113	14	0.287	.284
FDI-R SRI	0.454	14	0.117	.657
TM-L SRI	0.609	14	0.157	.553
TM-R SRI	-0.664	14	-0.171	.517

Table S3.2 One sample *t*-test in unchosen hand. *T*-test (*t*), degree of freedom (*df*), effect size (Cohen's *d*).

		t	d	p Bonferroni
	TM, C	-0.45	-0.116	1.000
	FDI, SI	1.30	0.336	1.000
FDI, C	TM, SI	-0.19	-0.049	1.000
	FDI, SRI	0.79	0.204	1.000
	TM, SRI	2.82	0.728	.102
	FDI, SI	1.71	0.442	1.000
TM, C	TM, SI	0.19	0.049	1.000
	FDI, SRI	1.19	0.307	1.000
	TM, SRI	3.14	0.811	.042
	TM, SI	-1.76	-0.454	1.000
FDI, SI	FDI, SRI	-0.51	-0.132	1.000
	TM, SRI	1.50	0.387	1.000
TM, SI	FDI, SRI	1.00	-0.116	1.000
	TM, SRI	2.95	0.336	.072
FDI, SRI	TM, SRI	2.34	-0.049	.365

Table S3.3 Post-hoc tests to explain the interaction muscle*condition for knee-point parameter 'a'. *T-test (t), effect size (Cohen's d).*

APPENDIX 4

		t	d	p Bonferroni
t1	t2	-2.742	-0.733	.055
	t3	-3.700	-0.989	.004
	t4	-5.420	-1.45	<.001
t2	t3	-0.959	-0.256	1.000
	t4	-2.678	-0.716	.065
t3	t4	-1.719	-0.459	.561

Table S4.1 Post-hoc tests related to the effect of TMS time. T-test (*t*), effect size (Cohen's *d*).