An investigation into the functional role of the dorsal premotor cortex in the control of rhythmic bimanual movements

by

Ronan Denyer

B. A., Trinity College Dublin, 2015

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Neuroscience)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

April 2024

© Ronan Denyer, 2024
The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the dissertation entitled:

An investigation into the functional role of the dorsal premotor cortex in the control of rhythmic bimanual movements.

submitted by Ronan Denyer in partial fulfilment of the requirements for
the degree of Doctor of Philosophy
in Neuroscience

Examining Committee:

Lara Boyd, Professor, Department of Physical Therapy, University of British Columbia
Supervisor

Todd Handy, Professor, Department of Psychology, University of British Columbia
Supervisory Committee Member

Nicola Hodges, Professor, School of Kinesiology, University of British Columbia
Supervisory Committee Member

Ian Greenhouse, Assistant Professor, Department of Human Physiology, University of Oregon
Supervisory Committee Member

Romeo Chua, Professor, School of Kinesiology, University of British Columbia
University Examiner

Hyosub Kim, Assistant Professor, School of Kinesiology, University of British Columbia
University Examiner
Abstract

The dorsal premotor cortex (PMd) and its connections with the primary motor cortex (M1) is thought to be important in the control of asymmetric rhythmic bimanual movements. However, it is uncertain if this brain-behaviour relationship arises because PMd is specifically tasked with programming of asymmetric bimanual movements, or more generally tasked with managing the increased cognitive load associated with asymmetric movements.

In the current dissertation, I conducted 4 experiments to probe these questions regarding the role of PMd in bimanual control. In experiment 1, I employed dual coil transcranial magnetic stimulation (TMS) to assess whether PMd-M1 interhemispheric communication differed during preparation of unimanual and bimanual movements. I discovered that PMd-M1 interhemispheric inhibition is released during unimanual but not bimanual movement preparation, suggesting PMd-M1 interhemispheric circuits may not be actively involved in controlling descending output during bimanual behaviours.

In experiment 2, I found that temporal coupling of rhythmic movements was enhanced when symmetric patterns were required compared to asymmetric patterns, and when spatially congruent timing cues were used instead of symbolic cues. These effects were restricted to high movement frequencies, and a follow up experiment 3 indicated this may have been driven by a change in movement strategy.

Results from experiments 1-3 provided a platform to interrogate the central question of the dissertation. If PMd is specifically responsible for programming asymmetric movement patterns, then (1) I would expect disruption of PMd by repetitive TMS (rTMS) to result in detriments to performance of asymmetric bimanual tapping patterns only, regardless of how movement
frequency is cued. If PMd is responsible for managing cognitive load, then (2) I would expect
detriments to performance to scale with the degree of cognitive load engendered by task
conditions. To test these competing predictions, in experiment 4 participants performed bimanual
rhythmic tapping tasks used in after receiving inhibitory rTMS over right PMd. Hypotheses (1)
and (2) were not supported. Instead, rTMS had no effect on behaviour. These results indicates that
asymmetric bimanual control is likely enacted by a distributed cortical network beyond PMd,
which is capable of compensating for neuronal challenge to right PMd to maintain behavioural
output.
Lay Summary

A part of the brain called the dorsal premotor cortex (PMd) is thought to be important for controlling difficult repetitive two-handed movements, such as rubbing your stomach while patting your head at the same time. However, it is not understood if PMd actively programs these kinds of movements, or if PMd manages the general increase in mental focus needed to accurately perform these movements. We addressed this question across 4 experiments using a variety of neuroscientific techniques. Surprisingly, PMd alone does not seem to exclusively actively program or manage mental focus during these tricky two-handed movements. Instead, PMd seems to one part of a broader network of regions that work together to control difficult two-handed movements. This broad network of regions may function as a backup system to help out when PMd isn't working properly, which has important implications for how we think about recovery from brain injuries.
Preface

This thesis was written and compiled by Ronan Denyer. Drs. Lara Boyd, Ian Greenhouse, Todd Handy, and Nicola Hodges, reviewed and provided feedback that was considered in the submitted version of this dissertation. All data collection and analysis were conducted in Dr. Lara Boyd’s Brain Behaviour Lab (BBL). All research described in this dissertation was approved by the University of British Columbia’s Clinical Research Ethics Board or the University of British Columbia’s Behavioural Research Ethics Board, certificate numbers: H19-02295, H20-02740 and H22-01613.

Chapter 2 is based on work conducted by Ronan Denyer, Brian Greeley, Ian Greenhouse, and Lara Boyd. A version of this chapter is published [Denyer, R. et al. Interhemispheric inhibition between dorsal premotor and primary motor cortices is released during preparation of unimanual but not bimanual movements. Eur. J. Neurosci. 2023]. RD was responsible for conceiving the study, developing the analytic design, contributing to data collection, analyzing and interpreting the data, and writing and revising the manuscript. BG contributed to data collection, interpretation, and critical revisions of the manuscript. IG and LB contributed to interpretation of the data and critical revisions of the manuscript.

Chapter 3 is based on work conducted by Ronan Denyer and Lara Boyd. A version of this chapter is under preparation for publication. RD was responsible for conceiving the study, developing the analytic design, contributing to data collection, analyzing and interpreting the data, and writing and revising the manuscript. LB contributed to interpretation of the data and critical revisions of the manuscript.
Chapter 4 is based on work conducted by Ronan Denyer, Anjana Rajendran, Cristina Rubino, and Lara Boyd. A version of this chapter is under preparation for publication. RD was responsible for conceiving the study, developing the analytic design, contributing to data collection, analyzing, and interpreting the data, and writing and revising the manuscript. AR contributed to data collection and analysis, interpretation, and critical revisions of the manuscript. CR contributed to data collection, interpretation, and critical revisions of the manuscript. LB contributed to interpretation of the data and critical revisions of the manuscript.
Table of contents

Abstract ........................................................................................................................................ iii

Lay Summary .................................................................................................................................... v

Preface ................................................................................................................................................ vi

Table of contents ............................................................................................................................... viii

List of tables ......................................................................................................................................... xvi

List of figures ......................................................................................................................................... xvii

List of abbreviations .......................................................................................................................... xix

Acknowledgements ............................................................................................................................ xxii

1 General introduction and background .......................................................................................... 1

1.1 Preamble ......................................................................................................................................... 1

1.2 Bimanual coordination .................................................................................................................. 3

1.2.1 Experimental paradigms used to study bimanual coordination .................................................. 3

1.2.2 The egocentric principle and associated theoretical perspectives ........................................... 4

1.2.2.1 The dynamic pattern theory ................................................................................................. 4

1.2.2.2 Information processing perspectives ..................................................................................... 6

1.2.3 Cognitive-perceptual factors and the egocentric principle ......................................................... 6

1.2.4 Modifying the perceptual quality of the stimuli used to cue movement timing affects the stability of rhythmic bimanual movements .................................................................................. 7
1.2.5 Modifying the perceptual outcomes of rhythmic bimanual movements affects movement stability ................................................................. 8

1.2.6 Increasing cognitive load through additional task demands reduces rhythmic bimanual stability ........................................................................................................ 9

1.2.7 Event and emergent timing mechanisms in bimanual coordination ................. 10

1.2.8 Summary: a coalition of constraints ................................................................ 11

1.3 The dorsal premotor cortex: structural and functional connectivity with the primary motor cortex ........................................................................................................................................ 11

1.3.1 Structural connectivity between PMd and other motor regions ....................... 12

1.3.2 Functional connectivity between premotor and primary motor regions in non-human primates .................................................................................................................. 15

1.3.3 Functional connectivity between PMd and M1 in humans ................................ 16

1.3.3.1 Dual coil transcranial magnetic stimulation .................................................. 16

1.3.3.2 PMd-M1 intercortical circuits as assessed by dual coil TMS ....................... 19

1.3.4 Summary ......................................................................................................... 19

1.4 The functional role of the dorsal premotor cortex in behaviour ......................... 20

1.4.1 PMd activity during reach planning in non-human primates ............................ 20

1.4.2 The representational perspective on preparatory PMd activity ....................... 20

1.4.3 The dynamical systems perspective on preparatory PMd activity ................... 21

1.4.4 PMd: an integrator within a distributed motor system? ................................. 22

1.4.5 Summary ......................................................................................................... 23
1.5 Associations between dorsal premotor cortex activity and rhythmic bimanual coordination ................................................................. 23

1.5.1 Functional neuroimaging and rhythmic bimanual coordination .............. 24

1.5.2 TMS induced perturbations of PMd during rhythmic bimanual coordination ..... 25

1.5.3 Associations between PMd-M1 interhemispheric inhibition and rhythmic bimanual control ........................................................................ 26

1.5.4 Summary ......................................................................................... 28

1.6 Thesis overview ................................................................................... 28

1.6.1 Specific research aims and hypotheses .............................................. 31

2 Interhemispheric inhibition between dorsal premotor and primary motor cortices is released during preparation of unimanual but not bimanual movements. .................. 34

2.1 Introduction ....................................................................................... 34

2.2 Methods ............................................................................................ 37

2.2.1 Participants .................................................................................... 37

2.2.2 Experimental design ........................................................................ 37

2.2.3 Procedure ....................................................................................... 39

2.2.3.1 Experimental setup ..................................................................... 39

2.2.3.2 Magnetic resonance imaging ...................................................... 39

2.2.3.3 Electromyography ...................................................................... 41

2.2.3.4 Simple reaction time task .......................................................... 41
3 Movement quality moderates the effect of spatially congruent cues on the stability of symmetric and asymmetric rhythmic bimanual finger movements.

3.1 Introduction

3.2 Methods

3.2.1 Experiment 1

3.2.1.1 Participants

3.2.1.2 Experimental design

3.2.1.3 Procedure

3.2.1.4 Data processing

3.2.1.5 Dependent variables

3.2.1.6 Statistical analysis

3.2.2 Experiment 2

3.2.2.1 Participants

3.2.2.2 Experimental Design

3.2.2.3 Procedure

3.2.2.4 Data processing

3.2.2.5 Dependent variables

3.2.2.6 Statistical analysis

3.3 Results
3.3.1 Experiment 1 ......................................................................................................... 89
3.3.1.1 4-finger bimanual choice reaction time task ................................................. 89
3.3.1.2 4-finger bimanual rhythmic tapping task ..................................................... 91
3.3.2 Experiment 2 ........................................................................................................ 94
3.4 Discussion .................................................................................................................. 97
3.4.1 Spatially congruent cues reduce planning costs for asymmetric bimanual finger
movements ..................................................................................................................... 98
3.4.2 Increasing movement frequency drives a change in rhythmic movement quality
from a series of discrete movements to continuous movement ..................................... 99
3.4.3 Accuracy and stability of bimanual rhythmic finger tapping decreased
exponentially with increasing movement frequency ..................................................... 100
3.4.4 Effects of response symmetry and spatial congruence on rhythmic bimanual
tapping stability emerge at higher movement frequencies .......................................... 101
3.4.5 Limitations .......................................................................................................... 105
3.4.6 Conclusion .......................................................................................................... 106
3.5 Bridging statement ................................................................................................... 107

4 Disruption of right PMd activity with repetitive transcranial magnetic stimulation has
no effect on the stability of rhythmic bimanual finger movements .............................. 108
4.1 Introduction ............................................................................................................. 108
4.2 Methods .................................................................................................................. 115
4.2.1 Participants ........................................................................................................ 115
4.2.2 Experimental design ........................................................................................................115
4.2.3 Procedure ........................................................................................................................116
4.2.4 Data processing ..................................................................................................................123
4.2.5 Dependent variables .........................................................................................................126
4.2.6 Statistical analysis ..........................................................................................................127
4.2.6.1 Primary analyses .........................................................................................................128
4.2.6.2 Supplementary analyses ...........................................................................................129
4.2.6.3 Exploratory analyses ..................................................................................................130
4.3 Results ...................................................................................................................................132
4.3.1 4-finger bimanual rhythmic tapping task .........................................................................132
4.3.2 4-finger bimanual choice reaction time task ....................................................................136
4.3.3 Simple reaction time task .................................................................................................138
4.3.4 Corticospinal excitability ................................................................................................138
4.3.5 Assessing the impact of movement frequency levels on stimulus type effects .. 139
4.4 Discussion .............................................................................................................................141
4.4.1 The brain can compensate for neuronal challenge to right PMd and maintain rhythmic bimanual behaviours ......................................................................................................141
4.4.2 The stabilizing effects of cueing movement frequency with spatially congruent stimuli are nosier than the stabilizing effect of movement symmetry .......................... 144
4.4.3 Limitations ......................................................................................................................145
5 Conclusion ................................................................................................................. 149

5.1 Summary of chapter aims and main findings ...................................................... 149

5.2 Synthesis of main findings .................................................................................. 152

5.3 Limitations ........................................................................................................... 154

5.4 Future directions .................................................................................................. 156

5.4.1 Indexing neural activity during rhythmic bimanual finger tapping after rTMS over PMd with fMRI ........................................................................................................... 156

5.4.2 Assessing the effect of rTMS induced inhibition of PMd on preparatory related activity in PMd-M1 interhemispheric circuits .............................................................. 157

5.4.3 Uncovering the limits of neural compensation ................................................. 158

5.4.4 Exploring the relationship between movement strategy and bimanual coupling accuracy using explicit instructions ................................................................. 159

5.4.5 Assessing how movement quality influences preparatory related changes in PMd-M1 interhemispheric circuits .............................................................. 160

5.5 Conclusion .......................................................................................................... 160

Bibliography .............................................................................................................. 162
List of tables

Table 2.1. Participant characteristics. ................................................................. 38

Table 4.1. Participant characteristics. ................................................................. 122
List of figures

Figure 1.1. Structural connectivity of the hand region of M1 and PMd. ............................................ 14

Figure 1.2. Dual coil transcranial magnetic stimulation during behaviour: from motor evoked potentials to inhibition ratios. ................................................................. 18

Figure 2.1. Experimental overview. .................................................................................................. 40

Figure 2.2. PMd-M1 interhemispheric inhibition across preparation ............................................. 51

Figure 2.3. Corticospinal excitability across preparation. .............................................................. 53

Figure 2.4. Reaction time. .............................................................................................................. 56

Figure 2.5. PMd-M1 interhemispheric and mean RT correlation .................................................... 58

Figure 3.1. Stimulus-response mappings and tasks outline. ............................................................ 77

Figure 3.2 Method to calculate relative phase of bimanual coupling from key press data ........... 81

Figure 3.3. Bimanual 4-choice reaction time task results. ............................................................. 90

Figure 3.4. Bimanual 4-finger rhythmic tapping task results. ......................................................... 93

Figure 3.5. Experiment 2: bimanual 4-finger rhythmic tapping task results. ............................... 95

Figure 3.6. Increasing movement frequency was associated with a shift in movement quality .. 96

Figure 4.1. Hypotheses representation. ......................................................................................... 111

Figure 4.2. Experimental overview. ............................................................................................... 114

Figure 4.3. Stimulus-response mappings ...................................................................................... 121

Figure 4.4. Method to calculate relative phase of bimanual coupling from key press data ...... 125

Figure 4.5. Bimanual rhythmic tapping task results. ................................................................. 135

Figure 4.6. Bimanual 4-choice reaction time task results. ......................................................... 137

Figure 4.7. Simple reaction time results. ....................................................................................... 138

Figure 4.8. Corticospinal excitability across sham and real rTMS. .......................................... 139
Figure 4.9. Re-analysis of mean absolute error values from Chapter 2 experiment 2................. 140
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIC</td>
<td>Akaike information criterion</td>
</tr>
<tr>
<td>BOLD</td>
<td>blood-oxygen-level-dependent</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CMA</td>
<td>cingulate motor area</td>
</tr>
<tr>
<td>CP</td>
<td>conditioning pulse</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyography</td>
</tr>
<tr>
<td>FDI</td>
<td>first dorsal interosseous</td>
</tr>
<tr>
<td>GLME</td>
<td>generalized linear mixed effects</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>IHF</td>
<td>interhemispheric facilitation</td>
</tr>
<tr>
<td>IHI</td>
<td>interhemispheric inhibition</td>
</tr>
<tr>
<td>LED</td>
<td>light emitting diode</td>
</tr>
<tr>
<td>LMER</td>
<td>linear mixed effect regression</td>
</tr>
<tr>
<td>M1</td>
<td>primary motor cortex</td>
</tr>
<tr>
<td>MEP</td>
<td>motor evoked potential</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurosciences Institute</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>PMd</td>
<td>dorsal premotor cortex</td>
</tr>
<tr>
<td>PMv</td>
<td>ventral premotor cortex</td>
</tr>
<tr>
<td>PPC</td>
<td>posterior parietal cortex</td>
</tr>
<tr>
<td>rmANOVA</td>
<td>repeated measures analysis of variance</td>
</tr>
<tr>
<td>RMT</td>
<td>resting motor threshold</td>
</tr>
<tr>
<td>RT</td>
<td>reaction time</td>
</tr>
<tr>
<td>rTMS</td>
<td>repetitive transcranial magnetic stimulation</td>
</tr>
<tr>
<td>S1</td>
<td>somatosensory cortex</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SMA</td>
<td>supplementary motor area</td>
</tr>
<tr>
<td>TMS</td>
<td>transcranial magnetic stimulation</td>
</tr>
<tr>
<td>TP</td>
<td>test pulse</td>
</tr>
</tbody>
</table>
Acknowledgements

First and foremost, to my supervisor Dr. Lara Boyd, thank you for taking a chance on a somewhat “green” prospect from across the ocean, and providing such excellent mentorship during my time at UBC. I really appreciate and admire the freedom, time, space, and encouragement you gave me to pursue my own ideas. I would also like to thank the members of my supervisory committee, Drs. Todd Handy, Nicola Hodges, and Ian Greenhouse. Todd, your big picture perspective was greatly appreciated, as was the UBC Psych participant pool connect, which saved me from recruitment woes and significantly elevated the quality of this work. Nikki, thank you for your terrific attention to detail, scientific rigour, and consistent reminders to remember what the actual question is. Ian, thank you for providing me with the tools to start this journey, and for entertaining so many muddled theory and data chats through the years. Those interactions really helped me to get my head around difficult concepts, and I look forward to the next edition.

Next, thanks to the wickedly talented cast of the Brain Behaviour Lab, past and present. I am regularly amazed at all the new ideas and achievements emerging from the team. Special thanks to Dr. Brian Greeley and Bev Larssen for their support and friendship throughout the years. Brian, your willingness to be a soundboard for ideas about data analysis and experimental design was invaluable to me throughout this process. And I can’t thank you enough for braving a (pre-vaccine) pandemic in full personal protective gear to help me get the data I needed for my first experiment. That will have its place in BBL lore. Bev, our regular “I have so much to do” and “what does this mean” check-ins by the printer were essential and will be remembered. Thank you also to Anjana Rajendran, Cristina Rubino, and Jordan Brocato for their help and support in conducting this thesis work. To the rest, thanks for the Tuesday drinks, the Friday drinks, the JJ Bean circuits, the Spanish Banks meetups, and the annual glass tray exchange at Secret Santa.
To my amazing family, thank you for all your love and support through the years! To Mum and Dad, thanks for encouraging me to forge my own path, for always accepting me for who I am, and for spoiling me (well, I am the youngest). To Niamh, thanks for your laughter and sense of perspective. To Myles, thanks for providing a space for my Arsenal anger (and joy). Most importantly, to my wonderful wife Dr. Iseult, thank you for being there with me every step of the way, from Madison to Vancouver, through the ins and outs, the ups the downs, and the highs and the lows. We made it!
1 General introduction and background

1.1 Preamble

The ability to accurately coordinate two hands in time and space is critical for successful navigation of many of the problems that we face in daily living. Efforts to identify principles of bimanual coordination in the lab have revealed a seemingly innate preference for bimanual movements that are spatiotemporally symmetric as opposed to asymmetric, especially when repetitive movements are required\textsuperscript{1,2}. Subsequent attempts to understand the neural basis of this preference have suggested that the dorsal premotor cortex (PMd) is a particularly important brain region for the control of asymmetric bimanual movements. PMd is activated during performance of asymmetric bimanual movements\textsuperscript{3-5}, and perturbation of activity in PMd induces switches from asymmetric to symmetric movement patterns\textsuperscript{4,6}. Furthermore, changes in activity within interhemispheric circuits directly connecting PMd with the primary motor cortex (M1) during motor preparation have been shown to relate to our ability to maintain rhythmic asymmetric bimanual movements at high speeds\textsuperscript{7,8}.

But not all bimanual movements are created equal. Just as we find it difficult to simultaneously rub our stomachs and pat our heads, we find it effortless to repeatedly reach to two glasses at different distances to quickly clear the table before heading out the door after breakfast. These examples illustrate how differences in the way we perceive our environment can dramatically alter the manner in which we cognitively represent task goals, which in turn has powerful effects on our capacity to coordinate bimanual actions\textsuperscript{9,10}.

The role of such cognitive-perceptual factors in asymmetric bimanual control has not been fully elucidated. While PMd has been proposed to be specifically involved in the programming of
asymmetric bimanual movements, some models of PMd activity derived from single neuron recordings suggest a more general integrative function for PMd in motor control\textsuperscript{11--13}. Given that switching bimanual movement requirements from symmetric to asymmetric places a higher load on general cognitive resources\textsuperscript{14--17}, perhaps the apparent relationship between PMd and asymmetric bimanual control more generally reflects a critical role for PMd in the management of increased cognitive load.

PMd is capable of influencing activity in bilateral M1 via ipsilateral and interhemispheric projections. PMd-M1 interhemispheric circuits have been investigated much more frequently than ipsilateral circuits, since PMd-M1 interhemispheric circuits are more readily probed with commercially available TMS coils. The capacity to rapidly modulate activity within right PMd–left M1 interhemispheric circuits during motor preparation has been suggested as an important neurophysiological mechanism through which the brain coordinates descending motor output to control rhythmic asymmetric bimanual movements\textsuperscript{7,8}. However, there are still several unknowns regarding the precise functional role of this circuit in behaviour. Specifically, it is not fully established whether the observed patterns of change in activity during motor preparation is similar for both hemispheres, which limits any claim of hemispheric specialization. Furthermore, studies of changes in PMd-M1 interhemispheric circuits during movement preparation have largely focused on unimanual tasks; as such it is unknown if similar patterns of change are observed during preparation of bimanual movements. If PMd–M1 interhemispheric circuits do play a causal role in asymmetric bimanual movements then we would expect them to be active during bimanual movement preparation.

The overall objective of this thesis is to comprehensively examine the role of PMd in rhythmic bimanual control, with a specific focus on the moderating effects of cognitive-perceptual factors
in this relationship. A general introduction chapter presents key concepts before providing an overview of the 3 research chapters included in this thesis. Finally, a conclusion chapter synthesizes findings arising from the research chapters and suggests directions for future investigations.

1.2 Bimanual coordination

The advent of bipedalism in the evolutionary history of humans liberated the upper extremities for complex interactions with the surrounding world. This is reflected in the seemingly limitless capacity we have for using our upper limbs in the production of voluntary actions. Simple skills can be learned solely through observation or a few trials of practice, while complex skills are learned after extensive practice. While it is common to think in terms of hand dominance conferring greater capacity for skill and motor learning, in reality many of the tasks we encounter in daily living require use of two hands in coordination. For example, steering, cutting a loaf of bread, tying shoelaces, and buttoning a shirt all require the integrated action of two hands. Given the prevalence of bimanual action in human life, investigating the behavioural principles underlining the coordination of the two hands and testing how the nervous system implements these principles has become an active area of interdisciplinary research.

1.2.1 Experimental paradigms used to study bimanual coordination

Attempts to study bimanual coordination in the lab has involved deployment of a diverse range of behavioural tasks, reflecting the many types of bimanual actions humans produce in daily life. These range from bimanual reaching paradigms\textsuperscript{18–21}, to bimanual finger sequence learning paradigms\textsuperscript{22–24}, to “real-world” paradigms where task requirements require mimicking skills used in daily living, such as cutting a loaf of bread\textsuperscript{25}. However, attempts to uncover principles of bimanual coordination have been dominated by behavioural paradigms in which repetitive
rhythmic bimanual movements are required, such as rhythmic wrist movements, rhythmic finger tapping, and rhythmic circle drawing. Within these rhythmic movement paradigms, the focus has been centered on identifying constraints on bimanual coordination, with the reasoning that by determining these constraints, fundamental principles governing the coordination of bimanual actions can be identified.

1.2.2 The egocentric principle and associated theoretical perspectives

A cardinal finding from studies of rhythmic bimanual movement is that accuracy improves when both hands move symmetrically in time (“in phase”) with one another compared to when both hands move in an asymmetric manner (“anti phase”). Furthermore, as movement frequency is increased, asymmetric patterns inevitably transition to symmetric patterns. In contrast, transition from symmetric to asymmetric rarely occurs. This effect has been named the “egocentric principle” and been shown across a wide array of tasks, including bimanual circle tracing\textsuperscript{26}, bimanual wrist supination and pronation\textsuperscript{27–29}, and bimanual finger tapping\textsuperscript{4,6} (for reviews, see\textsuperscript{1,2,30,31}). Two major theoretical perspectives have been developed which attempt to explain why the egocentric principle arises, namely the dynamic pattern perspective and the information processing perspective\textsuperscript{1,2}.

1.2.2.1 The dynamic pattern theory

The dynamic pattern theory formally describes biological systems in terms of their time-dependent changes\textsuperscript{1}. Such systems are conceptualized as being composed of many sub-components that self-organize into coherent global patterns. In the context of rhythmic bimanual movements, the marked preference for certain phase relationships, the dependency of pattern stability on movement frequency, and the asymmetry in the transitions between different stable states on the “order parameter” (e.g., the relative phase between the position of bimanual effectors) are well
captured by a dynamical systems equation in which each effector is modelled as non-linear oscillators, with its stability described in terms of limit cycle dynamics\textsuperscript{27,31}. Interactions between the limbs arise due to non-linear coupling terms that connect the dynamics of each oscillator. These equations deliver a mathematical description of an attractor landscape as a function of cycling frequency, which is regarded as the critical “control parameter”\textsuperscript{2}. According to the dynamical pattern perspective, individual differences in the ability to maintain bimanual rhythmic movements outside of the attractive states of in phase (relative phase = 0 degrees) and anti phase (relative phase = 180 degrees), and to maintain anti phase patterns at high movement frequencies is due to differences in the shapes of these attractor landscapes which emerge within the central nervous system. Learning is similarly regarded as a dynamic process whereby the attractor landscape is reformed through novel behavioural experience.

While such a component model of rhythmic bimanual coordination provides an elegant descriptive account for the spatiotemporal properties of rhythmic bimanual movement, critics have argued it is limited in its capacity to generate predictions regarding the neural basis of bimanual coordination, since its formulation is abstract\textsuperscript{9}. For example, on a representational level it remains unclear whether the component oscillators refer to the position and velocity of a limb, the time-varying contraction of agonist and antagonist muscles, or the generation of more abstract spatial codes, or indeed across all these levels. Similarly, the theory is neutral in terms of the neural implementation of the required sub-components\textsuperscript{9}, which has made it difficult to generate specific predictions on the neural control of rhythmic bimanual movements and integrate the theory with others concerned with explaining the neural control of movement.
1.2.2 Information processing perspectives

Perceived issues with the dynamical systems perspective as well as several findings demonstrating a role for cognitive-perceptual factors in influencing coordination dynamics of rhythmic bimanual movements motivated the formulation of accounts which conceptualized coordination constraints observed in rhythmic bimanual coordination as an information-processing problem. Within the information-processing perspective, rhythmic bimanual movement is considered a class of dual-task performance, with the egocentric principle considered to emerge due to structural interference caused by limited neural resources\(^{32-35}\). Failure to spatiotemporally couple asymmetric movements as accurately as symmetric movements is said to be caused by “crosstalk” within neural processes. Crosstalk can emerge within different levels of neural processing, for example within processes responsible for perceiving task-relevant stimuli, within processes responsible for planning movement, and within processes responsible for executing movement\(^2\). Since asymmetric movements necessitate the simultaneous specification of distinct spatial codes to effectively plan movements and the activation of potentially distinct motor programs to execute the intended behaviour, the risk for crosstalk is increased. Motor learning is said to be achieved through successful suppression of neural crosstalk across multiple levels of processing, through practice and/or by integration of task demands into a meaningful global control structure\(^{1,2,9}\).

1.2.3 Cognitive-perceptual factors and the egocentric principle

If the instability of asymmetric versus symmetric rhythmic bimanual coordination is downstream of fundamental limitations in our capacity to process multiple sources of information in parallel, then manipulating task demands to promote more efficient information processing should reduce the instability of asymmetric control. This is indeed the case, with several lines of evidence
demonstrating that manipulating how task goals are represented in cognitive -perceptual terms can profoundly affect rhythmic bimanual accuracy.

1.2.4 Modifying the perceptual quality of the stimuli used to cue movement timing affects the stability of rhythmic bimanual movements

Manipulating the perceptual quality of stimuli used to cue movement timing/frequency can affect the stability and accuracy of rhythmic bimanual movements. Simply requiring participants to perform rhythmic wrist flexion and extension movements in time with an auditory metronome significantly reduces relative phase variability compared to conditions in which participants are required to generate movements based on a memorized frequency. Furthermore, using a double metronome structure that communicates flexion and extension timing with distinct tones significantly reduces transitions to symmetric patterns. When the movement frequency of rhythmic bimanual movements is communicated using flickering visual stimuli, spatially congruent stimuli increased rhythmic stability. For example, in rhythmic bimanual finger circling, asymmetric circling is more stable, and transitions from asymmetric to symmetric patterns are delayed, when the desired circling pattern is cued with flashing light emitting diodes (LEDs) that are spatially congruent with the direction of motion. Likewise, deficits normally observed when switching from symmetric to asymmetric rhythmic circling patterns based on abstract symbolic stimulus-response mappings (letters or shapes) are abolished when spatially congruent stimuli (directional arrows) are used to cue switches.

The benefits of spatial congruence on task performance are also evident in the domain of discrete bimanual reaching. Reaches are planned faster when location is communicated directly with LEDs, compared to when location is communicated with an abstract symbol. Furthermore, asymmetric reaches are planned significantly slower than symmetric reaches when movement is cued with...
abstract symbols. Similar planning penalties for asymmetric bimanual reaches are not observed when reach location is cued directly. It has been suggested that deficits in asymmetric reaching are not found in direct cueing conditions where the stimulus maps directly onto target location because such movements are planned by a rapid automatic response selection system that can generate bimanual reach trajectories in parallel. In contrast, bimanual reaches planned off abstract symbols necessitate use of a slower and serial response selection processes to translate the stimulus to the correct response, which introduces a potent source of interference\textsuperscript{20}. This notion is bolstered by neuroimaging evidence showing that directly cuing reaching is associated with bilateral brain activation in the parietal cortices while reaching based off abstract symbolic cues is associated with laterialized activation within frontal premotor regions\textsuperscript{21}. The improvement of rhythmic bimanual movement stability with the use of stimuli which enable perception-action coupling therefore may be similarly driven by the capacity to use a neuro-cognitive system that is more efficient at generating asymmetric spatial codes.

1.2.5 Modifying the perceptual outcomes of rhythmic bimanual movements affects movement stability

Several studies have shown that modifying the perceptual outcomes of bimanual movements can also increase the capacity to perform coordination patterns that are otherwise difficult or impossible, including asymmetric rhythmic bimanual movements. For example, in a rhythmic bimanual circle drawing task, participants were shown to be capable of circling their hands at a temporal ratio of 4:3 when their hands were hidden from view and a special gear system was used to change the perception of hand speed to be equal between hands\textsuperscript{10}. This temporal ratio is nearly impossible to learn under normal conditions but was learned within 20 minutes of practice under the conditions described above. Similarly, repeated asymmetric index finger oscillations are as
stable as symmetric oscillations when one wrist was supinated such that the perceptual outcome of the movements was symmetric\textsuperscript{10}. Other researchers have shown that asymmetric bimanual rhythmic line drawing movements are stabilized when the visual feedback for asymmetric patterns is transformed to show symmetric outcomes\textsuperscript{38}. The capacity to learn asymmetric bimanual circle drawing movements is significantly improved when participants are given concurrent enhanced online visual feedback that communicates the displacement angles between hands. Learning of these tasks also successfully transfers to conditions with no online feedback\textsuperscript{17}.

\subsection*{1.2.6 Increasing cognitive load through additional task demands reduces rhythmic bimanual stability}

If performing asymmetric bimanual movements incurs an increased cost of cognitive resources, then the addition of task demands that tax cognitive resources would be expected to have a greater detrimental effect on asymmetric compared to symmetric movements. This has been demonstrated by requiring participants to engaging in counting memorization task while engaging in bimanual circle drawing in time with an auditory metronome. Errors on the counting task were significantly higher during asymmetric movements compared to symmetric movements\textsuperscript{16}. Other work asked participants to perform an RT task while concurrently performing symmetric and asymmetric rhythmic bimanual movements. RT was found to be slowed when participants performed asymmetric compared to symmetric rhythmic bimanual movements\textsuperscript{39}. The finding that concurrently performing asymmetric but not symmetric rhythmic bimanual movements impairs performance on a variety of secondary tasks further suggests that general cognitive processes used across many task domains play an important role in maintaining the stability of asymmetric bimanual coordination patterns.
1.2.7 Event and emergent timing mechanisms in bimanual coordination

While I have outlined the importance of considering cognitive-perceptual factors that shift the representation of task goals when studying principles of coordination of rhythmic bimanual movements, there is also work showing that the kinematic quality of the required bimanual movements can dramatically shift the governing principles. Specifically, research in healthy participants and patient populations indicates that whether repetitive bimanual movements are discontinuous (i.e., repeated discrete movements separated by a pause) or continuous (i.e., continuous cycles of motion with no pauses) dictates the type of internal timing mechanisms used to time movement cycles and affects performance outcomes. Behavioural evidence for the existence of distinct timing systems originated from experiments that tested performance of rhythmic finger tapping and rhythmic circle drawing. Performance within subjects on these tasks was uncorrelated, suggesting that they are underpinned by distinct control systems\(^{40-44}\). This viewpoint is bolstered by studies with callosotomy patients, who had their corpus callosum surgically cut to reduce the symptoms of epilepsy. These individuals were unable to spatiotemporally couple hands during rhythmic bimanual circle drawing\(^45\) but were able to spatiotemporally couple fingers when tapping in rhythm at regular frequencies\(^{46-48}\). However, this latter capacity was abolished when patients were instructed to perform smooth continuous finger oscillations instead of a series of discrete taps separated by a pause\(^9,45\). Interestingly, patients with cerebellum damage show the opposite pattern of deficits\(^{49,50}\).

Taken together, these results suggest that discrete rhythmic bimanual movements rely on “event timing” mechanisms, whereby temporal control is achieved via a representation of the time intervals between movements. Since deficits in this type of behaviour are observed in those with cerebellar damage, event timing representations are thought to be computed by the cerebellum\(^{49,50}\).
In contrast, continuous rhythmic bimanual movements rely on “emergent timing” mechanisms, whereby spatiotemporal coupling emerges via the control of higher order kinematic parameters such as speed or angular velocity\(^9,45,49\). Since deficits in this type of behaviour are observed in those with callosectomy, this process is thought to be mediated by transcallosal cortical networks. Therefore, whether task demands necessitate event or emergent timing mechanisms likely has the capacity to shift the coordination landscape and emergent constraints.

### 1.2.8 Summary: a coalition of constraints

The study of bimanual coordination has been dominated by the study of repetitive, rhythmic bimanual movements and the identification of associated coordination constraints. Opinion has differed on the appropriate theoretical framework for conceptualizing these limitations, but there is a general consensus that bimanual coordination is governed by a “coalition of constraints”\(^51–53\). While movement asymmetry has been shown to impose a strong constraint on bimanual coordination, changing how task goals are represented (by altering cognitive-perceptual task elements) can significantly reduce the strength of the constraint. Similarly, whether discontinuous or continuous rhythmic bimanual movements are required can further alter the emergence of coordination constraints. **Thus, when investigating principles of bimanual coordination and the neural implementation of these principles, it is essential to consider the impact of cognitive-perceptual factors on coordination constraints and also appreciate how task demands impact movement quality.**

### 1.3 The dorsal premotor cortex: structural and functional connectivity with the primary motor cortex

PMd is thought to be an important brain region for the neural implementation of the bimanual coordination principles outlined in section 1.2. Before reviewing evidence linking PMd activity
with bimanual behaviour, I will first review the basic anatomy and neurophysiology of PMd. The premotor cortex is a region on the superolateral surface of the brain encompassing the anterior lip of the precentral gyrus, the posterior portion of the middle frontal gyrus, and superior frontal gyrus, corresponding to part of Brodmann’s cytoarchitectonic area 6\textsuperscript{54}. The premotor cortex is typically separated into 4 subsections; PMd, the ventral premotor cortex (PMv), the supplementary motor area (SMA), and the cingulate motor area (CMA). These distinctions are based on anatomical\textsuperscript{55,56}, histochemical\textsuperscript{57}, cytoarchitectural\textsuperscript{58,59} and neurophysiological\textsuperscript{56,60,61} features.

### 1.3.1 Structural connectivity between PMd and other motor regions

Non-human primate tracer studies have allowed researchers to investigate to what degree neurons in PMd are connected with neurons in M1 and other premotor regions. One study identified the precise location of forelimb representations in M1 and PMd by applying intracortical microstimulation and measuring responses in forelimb musculature\textsuperscript{62}. Retrograde tracers were then injected at each of these locations to establish anatomical connectivity between the two regions. Approximately 50,000 cells per subject in premotor and parietal regions were labeled by the tracers. Both M1 and PMd were shown to send and receive dense projections to and from one another (23-28% of labelled cells). M1 also received dense input from PMv (20% of labelled cells). Input to M1 from SMA was moderately dense (9.5% of labelled cells). PMd received moderately dense input from PMv (18.1% of labelled cells) and dense input from SMA (21.3% of labelled cells). Notably, PMv received moderately dense inputs from prefrontal regions of the cortex (13.5% of labelled cells), whereas PMd and M1 received almost none (0.8% of labelled cells). A later study largely replicated these findings\textsuperscript{63}.

PMd is also interconnected with its counterpart in the opposite hemisphere, via the corpus callosum, although the number of cells in the contralateral hemisphere labeled by tracers is
generally much lower than in the ipsilateral hemisphere [~50,000 vs. ~2,500]\textsuperscript{62,64}. Tracer studies in macaque monkeys show that the hand area of M1 receives dense monosynaptic input from contralateral M1 [38\% of labelled cells] and contralateral PMd [~48\% of labelled cells]\textsuperscript{65}. Similar to M1, the hand area of PMd receives the most dense transcallosal input from its contralateral counterpart [48\% of labelled cells]\textsuperscript{66}. PMd also receives some monosynaptic input from contralateral M1 [4\% of labelled cells]\textsuperscript{64}. Structural magnetic resonance imaging (MRI) approaches to modelling transcallosal pathways in humans largely mirror what is found in non-human primate tracer studies, with PMd exhibiting dense homologous projections, as well as widespread projections to other premotor and primary motor regions in the contralateral hemisphere\textsuperscript{67} (Figure 1.1).
Figure 1.1. Structural connectivity of the hand region of M1 and PMd.

The thickness of the black lines represents the density of connections between each region based on anatomical tracer studies in non-human primates and structural magnetic resonance imaging studies in humans. Ipsilateral connections between PMd and M1 are most dense, followed by transcallosal projections connecting homogenous regions, followed by transcallosal projections connecting heterogenous regions.
1.3.2 **Functional connectivity between premotor and primary motor regions in non-human primates**

Invasive intracortical microstimulation studies in unconscious non-human primates have provided a rich source of data to investigate cortico-cortical connections between premotor regions and M1. A detailed analysis of the functional connectivity of three distinct premotor regions (PMd, PMv, SMA monkey analogues) with M1 demonstrated that PMd has the capacity to excite and inhibit neurons across multiple layers of M1. Activity in arrays of 105 neurons located within M1 of 2 awake macaque monkeys was recorded in response to stimulation of PMd, PMv, and SMA in the ipsilateral hemisphere. Neuronal activity was recorded in both corticospinal tract neurons located in layer V of M1 (33 total) and in non-corticospinal tract neurons located in layers II/III of M1 (72 total). Stimulation of premotor areas was shown to bring about either: (1) brief excitation of corticospinal tract neurons followed by a longer period of inhibition, or (2) pure inhibition of corticospinal tract neurons. Zero corticospinal tract neurons showed pure excitation in response to stimulation of premotor areas. For non-corticospinal tract neurons, 44% showed pure excitatory responses, with 56% showing no response. The latency of responses to stimulation in M1 neurons was varied (range 1-13 ms), with excitatory responses tending to lie within the faster end of the range of latencies. Inhibition lasted 1 ms to 200 ms in some corticospinal tract neurons. The vast majority of neurons in M1 showed response to stimulation in at least 2 of the 3 examined premotor regions. Strikingly, no M1 neurons tested showed exclusive response to PMd stimulation. This convergence of stimulation effects points to the functional complexity, interconnectedness, and lack of a clear hierarchical structure between premotor regions. Overall, this work indicates that PMd has the capacity to influence M1 activity via a rich network of inhibitory and excitatory neuronal connections.
1.3.3 Functional connectivity between PMd and M1 in humans

The investigation of PMd-M1 connectivity in humans has relied on use of non-invasive brain stimulation technologies including transcranial magnetic stimulation (TMS). In particular, dual coil TMS has been valuable for assessing cortico-cortical connections between non primary motor cortical regions and M1.

1.3.3.1 Dual coil transcranial magnetic stimulation

TMS is a non-invasive electrophysiological technique that enables stimulation of cortical tissue through the intact scalp of conscious participants. A short latency electrical pulse (~200 us) of up to 5 kA is induced in a stimulation coil positioned over the scalp of a participant. This pulse generates a magnetic field perpendicular to the direction of the coil, which penetrates the scalp and induces an electrical current in the cortex perpendicular to the magnetic field.

When applied over M1, TMS can elicit descending volleys of activity within corticospinal projection neurons, which activate spinal motoneurons predominantly contralateral to the site of stimulation. The activation of spinal motoneurons can evoke a twitch in a targeted muscle or group of muscles, which can be recorded using electromyography (EMG) in the form of a “motor evoked potential” (MEP). MEPs typically have a bipolar shape, and the amplitude, duration, and latency of MEPs are used to index corticospinal output.

Dual coil TMS paradigms are used to isolate the influence of a specific cortical or subcortical region on corticospinal output. With this approach two consecutive stimuli are administered either through two TMS coils positioned over different regions to determine the effect of the first conditioning stimulus on the readout of the second test stimulus. Typically, a conditioning TMS stimulus is delivered 1-100ms before a test TMS stimulus. By expressing the amplitude of
conditioned MEPs as a percentage of the amplitude of MEPs generated by a test TMS stimulus alone, it is possible to assess whether the distal cortical region of interest has an inhibitory or facilitatory influence on corticospinal output. When the conditioning stimulus is delivered over a cortical region contralateral to the M1 test stimulus (e.g. conditioning stimulus over contralateral M1 or contralateral PMd) and results in a reduced MEP amplitude relative to the M1 test stimulus alone, the effects are referred to as interhemispheric inhibition (IHI)\textsuperscript{69}. Conversely, an increase in MEP amplitude resulting from similar bilateral positioning of TMS coils is referred to as interhemispheric facilitation (IHF)\textsuperscript{70}. Adjusting stimulation parameters such as conditioning stimulus intensity and interstimulus interval can preferentially target inhibitory or facilitatory transcortical circuits. Conditioning effects observed with short interstimulus intervals (~1-10ms) are typically considered to be due to direct connections between the secondary cortical region and M1, while conditioning effects observed with longer interstimulus intervals (>10ms) can be due to multiple connected sites and recurrent or indirect loop activations, making the locus of effect more difficult to interpret\textsuperscript{71}.

Dual coil TMS protocols can also be used to assess the engagement of inhibitory or facilitatory mechanisms in behavioural tasks, by programming TMS stimuli to arrive at specific points within a task and examining changes in the resulting MEP amplitudes relative to rest or across task conditions\textsuperscript{72} (Figure 1.2).
Figure 1.2. Dual coil transcranial magnetic stimulation during behaviour: from motor evoked potentials to inhibition ratios.

(A) Typical experimental setup for studies combining dual coil TMS with behavioural tasks. Motor evoked potentials (MEPs) elicited by TMS over M1 are captured by continuous EMG recording of the effector(s) of interest. Dual coil TMS parameters can be varied to target specific inhibitory and facilitatory neural circuits. MEPs can be sampled at rest (1) or at different points within a trial of a behavioural task (B). For example, MEPs could be collected prior to the onset of an imperative “GO” stimulus (2), or after the onset of an imperative stimulus but before the onset of reaction time related EMG activity (3), (4). (C) Example MEP traces across each of the 4 time points outlined in (B). The relationship between MEPs elicited by single and dual coil TMS can vary across different timepoints in a given behavioural task. (D) The amplitude of MEPs displayed in (C). (E) Expressing the amplitude of double stimulation MEPs (conditioning stimulus + test stimulus, CS + TS) as a fraction of single stimulation MEPs (test stimulus alone, TS alone) allows experimenters to track net inhibitory/facilitatory output of PMd on M1 across time in the behavioural task of interest. *RMT: resting motor threshold.*
1.3.3.2 PMd-M1 intercortical circuits as assessed by dual coil TMS

Similar to what has been observed in non-human primate research, TMS studies in conscious, at-rest humans indicate that PMd has the functional capacity to inhibit and facilitate corticospinal output from M1 at a range of latencies from 2 to 100 ms. Within the same hemisphere, TMS pulses over PMd inhibit MEPs when delivered 4-6 ms prior to TMS pulses over M1. TMS pulses over PMd facilitate MEPs when delivered 15 ms prior to and 2 ms after TMS pulses over M1. Between the hemispheres, TMS pulses over PMd inhibit MEPs when delivered 6-10 ms and 40-60 ms prior to TMS pulses over M1. When a lower conditioning stimulus intensity is used, TMS pulses over PMd facilitate MEPs when delivered 8 ms and 80 ms prior to TMS pulses over M1. Available evidence suggests that short latency and long latency interhemispheric interactions are underpinned by distinct anatomical pathways, with short latency interactions seemingly arising from monosynaptic connections between PMd and contralateral PMd and M1 and long latency interactions arising from monosynaptic relays with ipsilateral M1.

1.3.4 Summary

There is extensive structural connectivity between PMd and M1, both within and between hemispheres. These structural connections allow PMd to influence corticospinal output via inhibitory and excitatory interactions. Studies with dual coil TMS in at rest humans have uncovered specific stimulation parameters required to probe specific inhibitory and excitatory circuits between PMd and M1 in a non-invasive manner. This knowledge provides a platform to investigate how PMd-M1 connectivity changes across different behavioural states, including during the preparation of bimanual behaviours.
1.4 The functional role of the dorsal premotor cortex in behaviour

PMD is implicated in a wide array of motor behaviours, including action preparation, motor sequencing, internally generated movements, motor imagery, and more. The activation of PMD across such an array of tasks with wildly varying cognitive demands and movement kinematics, along with broad effects of damage to PMD on motor function, suggests that PMD performs universal computations which underpin many types of voluntary action. Considering the possibility of a universal role for PMD across such a wide array of behaviours may be useful when interpreting observed relationships between PMD activity and bimanual control.

1.4.1 PMD activity during reach planning in non-human primates

The functional role of PMD in behaviour has most frequently been studied in non-human primates using a center-out instructed delay reaching task, in which subjects are trained to hold a manipulandum within a central target, and then move to a second target at a peripheral location after a set delay period. A cardinal finding from such experiments is that activity in certain PMD neurons correlates with kinematic aspects of the planned movement, including direction, trajectory, amplitude and speed. This pattern of results has resulted in a general consensus that PMD plays an important role in motor planning.

1.4.2 The representational perspective on preparatory PMD activity

While there is general consensus that PMD is involved in planning motor output, there have been differing theoretical perspectives on specifically how preparatory activity is transformed to motor output. Early theories seeking to explain this phenomenon proposed that tuned activity during preparation represents a subthreshold and/or gated version of the planned movement that is
prevented from early onset by inhibitory mechanisms. Inhibition of the preparatory activity prevents early movement onset, and the release of inhibition results in movement initiation.

There are several theoretical issues with this representational perspective. First, intracranial recordings in macaques\textsuperscript{95–97}, rabbits\textsuperscript{98}, and rats\textsuperscript{99} during action preparation does not support the existence of an inhibitory gate. These studies showed no evidence for the specific recruitment of intracortical inhibitory interneurons during the preparatory period. In fact, inhibitory interneurons were typically more active during movement. Second, the representational perspective fails to explain single neuron tuning could be non-correlated tuning between motor preparation and motor execution, as is often the case\textsuperscript{12}. Third, the alteration of specific movement parameters such as movement speed can alter single neuron tuning curves\textsuperscript{11,100}. These properties should not be dynamic if neurons have fixed representations of movement parameters.

1.4.3 The dynamical systems perspective on preparatory PMd activity

Modelling work suggests that preparatory activity can be viewed as a dynamical system, with population level neuronal activity in PMd reflecting a temporary state of the motor system before it is “put in motion”\textsuperscript{12,101–104} and generates movement. The dynamical systems framework eschews a focus on correlating single neuron activity with movement parameters in favour of examining how neural population states evolve over time to produce different movements\textsuperscript{12,102}. This is achieved by applying dimensionality reduction techniques to simultaneous recordings of 100s of neurons and modelling the dynamics of the emergent latent activity components. This approach demonstrates that during the preparatory period, variance in PMd population activity is best described by neural state trajectories that converge on discrete locations in activity space, in a manner consistent with point attractor dynamics\textsuperscript{11,12,105,106}. The attractor at which population activity settles during the preparatory period predicts future movement kinematics. In contrast,
during the movement period, variance in PMd population activity is best described by rotational
dynamics\textsuperscript{104,107}. These rotational dynamics resemble activity observed in muscles during
movement and are thought to control ongoing voluntary movement\textsuperscript{104,108,109}. Critically, switches
between orthogonal preparatory and execution neural states is thought to be brought about by the
onset of a “condition invariant signal”: a ramping signal which accounts for the majority of
variance in PMd population activity in the period after the onset of the imperative cue\textsuperscript{106,110}. The
timing of each condition invariant signal onset correlates highly with movement onset on a trial-
to-trial basis\textsuperscript{110}.

1.4.4 PMd: an integrator within a distributed motor system?

The dynamical systems framework offers an eloquent solution to many of the challenges faced by
representational perspectives on motor preparation, by reframing preparation and movement
related activity as related neural states with distinct dynamics. While activity in PMd during center
out reaching is explained by orthogonal preparatory and execution related components, other work
shows that manipulating behavioural urgency between trials in a decision-making paradigm is
associated with the emergence of an additional latent pattern of population activity, which track
urgency within and across trials\textsuperscript{13}. Therefore, activity of neurons within PMd seems to be multi-
faceted and track a wide variety of task elements. It is also notable that the rich heterogeneity
observed in PMd activity seems to be anatomically specific. For example, neurons in the
dorsolateral prefrontal cortex contribute weakly to timing but strongly to the movement direction
signals. An opposite pattern is observed for neurons located in the internal and external globus
pallidus of the basal ganglia, which instead emphasize the urgency and timing of a response but
not the planned movement direction\textsuperscript{13}. This suggests that PMd may have a special role in
integrating signals within a larger distributed system which enables behaviour. Such a universal 
function may explain why PMd is activated across such a diverse range of behavioural contexts.

1.4.5 Summary

Single neuron recordings from non-human primates during various reaching paradigms provide a 
rich source of data to build theories regarding the functional role of PMd in behaviour. While it 
should be stressed that more corroborative evidence is needed to clarify whether these proposed 
mechanisms generalize from non-human primates to humans, they nonetheless provide a useful 
resource for theorizing the functional role of PMd in human behaviour. The activity of some PMd 
neurons during preparation correlates with specific kinematic parameters of the planned 
movement. Similarly, the application of dimensionality reduction methods to large populations of 
PMd neurons reveals latent components which correlate with kinematic parameters. Ultimately, 
activity within PMd during behaviour appears to be relatively high dimensional compared to other 
brain regions such as the dorsolateral prefrontal cortex or the global pallidus. This feature points 
to a general integrative role for PMd in motor control, which is important to consider when 
interpreting observed relationships between PMd activity and bimanual behaviours.

1.5 Associations between dorsal premotor cortex activity and rhythmic bimanual 
coordination

Interest in understanding the neural implementation of principles of bimanual coordination has 
motivated studies which combine bimanual behavioural tasks with neuroimaging and/or brain 
stimulation methods. These studies have found associations between PMd activity and bimanual 
control, particularly asymmetric rhythmic bimanual control.
1.5.1 Functional neuroimaging and rhythmic bimanual coordination

Positron emission tomography (PET) imaging has shown that auditory-cued asymmetric bimanual rhythmic index finger movements are associated with a significant increase in regional cerebral blood flow in right PMd and right SMA when compared to symmetric or unimanual rhythmic movements. Such an effect was found when participants repeated index finger movements at a frequency of 1 Hz and 2 Hz, and when participants performed flexion-extension and abduction-adduction movements. Functional MRI shows that performance of auditory-cued asymmetric rhythmic index and index/middle finger movements are associated with an increase in blood-oxygen-level-dependent (BOLD) signal over symmetric movements in a distributed network of brain regions including right PMd and right SMA, but also encompassing bilateral M1, bilateral PMv, right insula, bilateral putamen, bilateral thalamus, bilateral cerebellum, left inferior parietal lobule, and right inferior frontal gyrus\textsuperscript{4,5}. Furthermore, around the time of spontaneous transitions from asymmetric to symmetric movements, BOLD signal is significantly increased in a right lateralized cortical network encompassing right PMd, right PMv, right pre-SMA, right inferior frontal gyrus, right middle frontal gyrus, and bilateral cingulate cortex\textsuperscript{5}. In other work that required participants to perform repetitive bimanual wrist movements along different directional axes, directional incompatibility was associated with an increase in BOLD signal in a right lateralized cortical network (encompassing right PMd and numerous right parietal cortex regions)\textsuperscript{3}. In summary, rhythmic bimanual movements are associated with activation in a distributed network of cortical regions, with transitions from asymmetric to symmetric rhythmic bimanual movements specifically associated with an increase in a right lateralized network of premotor-parietal cortical regions.
1.5.2  TMS induced perturbations of PMd during rhythmic bimanual coordination

While I have outlined the capacity for TMS to index brain activity at rest and during behaviour, TMS is also capable of focally modulating cortical activity. This facilitates the transient perturbation of a cortical region of interest, which enables “knock-out” investigations into the specific contributions of that cortical region to a behaviour of interest. Brain activity can be transiently modulated by applying TMS “offline” or “online”. For “offline” protocols, repetitive trains of TMS are applied while participants are at rest. This modulation transiently inhibits\textsuperscript{111–116} or excites\textsuperscript{116,117} the targeted region for up to 60 minutes, depending on the precise stimulation parameters selected. This experimental approach allows experimenters to test the effect of inhibition or excitation of the targeted cortical region on behaviour, by comparing performance on a task of interest versus a condition in which sham stimulation is applied. Alternatively, single TMS pulses or short trains of TMS pulses can be applied over the targeted region “online” while participants perform a trial or block of a task\textsuperscript{4,6,118,119}. If this transient perturbation of the targeted region by TMS results in a change in task performance metrics versus a sham stimulation condition, the targeted region is interpreted as playing a functional role in the studied behaviour.

To investigate which cortical region(s) are specifically responsible for maintaining asymmetric rhythmic bimanual movements and preventing transitions to symmetric movements, researchers applied single pulse TMS “online” during an auditory-cued asymmetric bimanual finger tapping task. TMS was applied over several candidate cortical regions which were active during an associated PET imaging experiment\textsuperscript{4}. The application of single pulses of TMS over right PMd elicited switching from asymmetric to symmetric bimanual rhythmic 2-finger tapping patterns at a significantly higher rate than other cortical regions, including left PMd, bilateral SMA, and bilateral somatosensory cortex (S1)\textsuperscript{4}. This effect was successfully replicated in a 4-finger bimanual
rhythmic tapping paradigm, with significant rates of switching from asymmetric to symmetric patterns observed after rapid double pulses of TMS over left and right PMd, with the percentage of induced switches slightly higher in right PMd⁶. Switching effects were not found when participants performed a 4-finger asymmetric, rhythmic unimanual task. The finding that switches from asymmetric to symmetric patterns were stronger after right PMd perturbation and was specific to bimanual action compliments evidence from functional neuroimaging studies that right PMd may be specialized for asymmetric rhythmic bimanual control.

1.5.3 Associations between PMd-M1 interhemispheric inhibition and rhythmic bimanual control

Studies applying dual coil TMS to index PMd-M1 IHI during motor preparation have also shown a relationship between the degree of change in IHI during preparation and the capacity to perform asymmetric bimanual movements accurately. In one such study, right-handed participants performed circular rhythmic movements using both of their index fingers to move a dot along a path on a computer monitor. Task conditions required that participants rotated their index fingers at different velocity ratios (3:1, 1:1, 1:3) to control a dot on a screen. The path for participants to trace with the dot was presented as a preparatory cue for 2s followed by the presentation of an imperative stimulus, i.e., a white dot moving along the target path. PMd-M1 IHI was sampled at the presentation of the path cue and 50ms prior to the onset of the imperative stimulus. The direction of modulation of left PMd-right M1 IHI depended on bimanual movement symmetry⁷. Specifically, when the left index finger was required to rotate 3 times faster than the right index finger, left PMd-right M1 interactions became more facilitatory 50 ms prior to the imperative stimulus. Conversely, when the left hand was required to rotate 3 times slower than the right hand, left PMd–right M1 interactions became more inhibitory. In contrast, no change in the influence of
PMD on M1 was observed when both index fingers were required to move symmetrically at the same angular velocity regardless of hemisphere. Furthermore, the degree of modulation of left PMD–right M1 IHI correlated with lower target tracking errors in the behavioural task when asymmetric hand rotations were required. This correlation suggests that PMD-M1 interhemispheric circuits may be important for the preparation of asymmetric rhythmic bimanual circling movements.

Other researchers have measured PMD-M1 IHI during preparation of discrete unimanual index finger movements and correlated these measures against performance in a 4-finger bimanual asymmetric rhythmic tapping task. Participants performed a visually cued simple RT task with their right hand. Right PMD-left M1 IHI was measured after the onset of the imperative stimulus at 20%, 50%, 80%, and 95% of each participants mean reaction time (RT). In line with other studies, PMD-M1 IHI was found to be released at 20% of mean RT, much earlier than what was observed for M1-M1 IHI, which was released at 90% of mean RT. The finding that PMD-M1 IHI is released early in the RT period relative to M1-M1 IHI suggests that PMD-M1 interhemispheric circuits begin to sculpt corticospinal output rapidly in response to incoming novel sensory information. Importantly, the degree of release of PMD-M1 IHI at 20% of mean RT positively correlated with performance on a secondary 4-finger bimanual rhythmic tapping task. Individuals who showed a greater release of PMD-M1 IHI tended to maintain asymmetric tapping patterns at higher frequencies. This correlation suggests that the capacity to rapidly modulate PMD-M1 IHI during preparation of simple unimanual movements may be important for the performance of complex asymmetric bimanual movements.
1.5.4 Summary

Attempts to identify neural correlates of rhythmic bimanual control using functional neuroimaging has indicated that a distributed network of cortical and subcortical regions are activated during the performance of asymmetric bimanual movements. Transitions from asymmetric to symmetric modes of coordination are associated with increased activation in a right lateralized premotor-parietal cortical network. Follow up TMS studies show that perturbation of PMd (in particular right PMd) during performance of asymmetric bimanual finger tapping movements is associated with induced switches from asymmetric to symmetric movement patterns. Complementary work employing dual coil TMS also shows that the capacity to rapidly modulate the excitability of M1 by contralateral PMd via transcallosal connections is associated with increased ability to perform difficult asymmetric movements with accuracy. **Thus, PMd appears to play an important role in rhythmic bimanual control, in particular in the control of asymmetric rhythmic bimanual movements. PMd-M1 interhemispheric circuits may be a particularly important neurophysiological pathway through which PMd controls descending motor output during asymmetric rhythmic bimanual control.**

1.6 Thesis overview

A review of the literature demonstrates a relationship between PMd activity, in particular right PMd, and the control of asymmetric rhythmic bimanual movements. TMS experiments that transiently disrupt PMd during performance of rhythmic bimanual movements have helped to establish this association. Other TMS work has indicted that PMd-M1 interhemispheric circuits may be a critical pathway through which PMd exerts control on motor output during asymmetric rhythmic bimanual movements. Yet there are still outstanding questions regarding the nature of this brain-behaviour relationship. One reason the relationship between PMd and asymmetric
bimanual control is unclear is that it is not well understood whether PMd-M1 interhemispheric circuits are actively involved in planning of bimanual movements. A greater release of IHI from right PMd to left M1 during movement preparation correlates with an increased capacity to maintain asymmetric bimanual movements at high frequencies, suggesting that transcallosal connections from right PMd to left M1 may underpin asymmetric rhythmic bimanual control. However, since the PMd-M1 IHI changes were assessed in a unimanual RT paradigm, it remains unclear whether PMd-M1 circuits are actually involved in bimanual movement preparation. Therefore, the observed relationship may not be causal. It also remains unclear whether similar patterns in IHI change are observed for left PMd–right M1 circuits.

A second reason why the relationship between PMd and asymmetric bimanual control is unclear is that cognitive load has not been systematically controlled in existing investigations. The majority of existing research studies in this area have assessed symmetric and asymmetric rhythmic bimanual movements in a single cueing condition, without manipulation of cognitive-perceptual factors that could general cognitive load. The lack of control of cognitive load is important because asymmetric rhythmic bimanual tasks incurs a higher tax on cognitive resources compared to symmetric rhythmic bimanual tasks. Therefore, any change in PMd activity between symmetric and asymmetric movement conditions may simply reflect a general increase in cognitive load and not be specific to response asymmetry.

In order to effectively probe the role of cognitive load in the relationship between PMd and rhythmic bimanual control, additional task manipulations that systematically increase and decrease cognitive load beyond changing response requirements from symmetric to asymmetric are needed. Previous work indicates that altering the perceptual quality of the visual stimulus used to cue the target movement frequency of rhythmic movements from stimuli that are spatially congruent to
stimuli that are symbolic can decrease the stability of rhythmic movements by increasing the amount of cognitive resources required to successfully decode the stimuli. These task manipulations could provide a platform to probe the potential mediating role of cognitive load in the relationship between PMd activity and asymmetric bimanual control. However, the majority of existing work has investigated the effect of spatial congruence on movement stability during the performance of rhythmic circling movements, and it is not well understood if these mechanisms generalize to bimanual rhythmic finger tapping movements. A lack of clear understanding if spatially congruent stimuli stabilize bimanual rhythmic finger tapping movements is important, since bimanual finger tapping movements have largely formed the basis of the proposed relationship between PMd activity and asymmetric bimanual control.

A third reason why the relationship between PMd and asymmetric bimanual control is unclear is that studies supporting a link between PMd and asymmetric rhythmic bimanual control have not explicitly considered how movement quality may change across task conditions. The lack of appreciation for changes in movement quality is important since behavioural and neuropsychological evidence indicates that discontinuous and continuous repetitive movements are controlled by distinct neuro-cognitive systems. Therefore, the lack of stability observed with increases in movement frequency and between symmetric and asymmetric movement patterns could be partly driven by differences in the quality of movement between these conditions. Gaining an appreciation of how the quality of movement changes across conditions in bimanual rhythmic finger tapping tasks within and between participants is important to furthering understanding the relationship between PMd and asymmetric rhythmic bimanual control, since these tasks can be successfully performed with discontinuous repetitive movements or with smooth continuous movement strategies.
In light of these outstanding questions, the overall objective of my thesis was to improve understanding of the functional role of PMd in asymmetric bimanual control. Specifically, I aimed to better characterise the functional role of PMd-M1 interhemispheric circuits in bimanual control and explicitly test the potential moderating role of cognitive load in the putative relationship between PMd and asymmetric rhythmic bimanual control. These objectives were achieved by: (1) characterizing how PMd-M1 interhemispheric communication differs between preparation of simple unimanual and bimanual movements, (2) defining the degree to which spatially congruent cueing stimuli affect the stability of symmetric and asymmetric rhythmic bimanual finger tapping movements versus abstract stimuli, (3) assessing how underlying movement kinematics impact the stability of rhythmic bimanual movements, and (4) determining whether TMS-induced transient inhibition of right PMd specifically reduces stability of asymmetric rhythmic tapping or generally reduces stability in rhythmic tapping with high cognitive load.

1.6.1 Specific research aims and hypotheses

Chapter 2 Aims and Hypotheses

Aim 1: To determine whether preparation related changes in PMd-M1 interhemispheric inhibition are different for unimanual and bimanual movements.

- Hypothesis 1A: PMd-M1 interhemispheric inhibition will be released during preparation when unimanual responses are performed.

- Hypothesis 1B: PMd-M1 interhemispheric inhibition will be unchanged during preparation when bimanual responses are performed.

Aim 2: To determine whether motor preparation related changes in PMd-M1 interhemispheric inhibition are different between hemispheres.
• Hypothesis 2: Patterns of change in PMd-M1 interhemispheric inhibition will be similar across hemispheres.

Chapter 3 Aims and Hypotheses

Aim 1: To determine whether spatially congruent cues increase the speed of planning of discrete symmetric and asymmetric bimanual finger movements compared to abstract symbolic cues.

• Hypothesis 1: Bimanual choice reaction time will be significantly slower for asymmetric responses compared to symmetric responses, but only when movement is cued with abstract symbolic stimuli and not spatially congruent stimuli.

Aim 2: To determine whether spatially congruent movement frequency cues increase the stability of rhythmic symmetric and asymmetric bimanual finger movements compared to abstract symbolic movement frequency cues.

• Hypothesis 2: Cueing movement frequency with spatially congruent stimuli will significantly improve the stability of asymmetric versus symmetric 4-finger rhythmic tapping, compared to conditions in which movement frequency is cued with abstract symbolic stimuli.

Aim 3: To determine if movement strategies change with increasing movement frequency in symmetric and asymmetric 4-finger rhythmic tapping tasks.

• Hypothesis 3: Increases in the movement frequency of 4-finger rhythmic tapping will be associated with a change in movement kinematics from repeated discrete movements separated by long pauses to continuous movement with no pauses.
Chapter 4 Aims and Hypotheses

Aim 1: To test whether right PMd is specifically involved in programming rhythmic asymmetric bimanual movements or generally involved in managing cognitive load.

- Hypothesis 1: The stability of 4-finger bimanual rhythmic tapping will be decreased after real 1 hz rTMS over right PMd compared to sham 1 hz rTMS over right PMd. Any detriments to stability will scale with the degree of cognitive load engendered by specific combinations of response type (i.e., symmetric vs. asymmetric) and stimulus type (i.e., spatial vs. symbolic cue) task conditions.
2 Interhemispheric inhibition between dorsal premotor and primary motor cortices is released during preparation of unimanual but not bimanual movements.

2.1 Introduction

The dorsal premotor cortex (PMd) is a brain region critical for the translation of external information into voluntary action\textsuperscript{84,122}. Evidence from non-human primate studies shows that PMd single-neuron firing rates during reach preparation correlate to kinematic features of the upcoming movement, including direction\textsuperscript{80,88–90}, trajectory\textsuperscript{91}, amplitude\textsuperscript{89} and speed\textsuperscript{11}. Furthermore, the application of dimensionality reduction methods to concurrent recordings of hundreds of PMd neurons has revealed distinct latent neural dynamics during reach preparation and execution, with population firing rates that are best modelled with point attractor dynamics during preparation and with rotational dynamics during execution\textsuperscript{12,101,104}. Notably, transitions between these preparation-related and execution-related “neural states” occurs in a temporally predictable manner that correlates highly with the onset of movement\textsuperscript{13,110}. The finding that the activity of PMd neurons reflects kinematic features of movement at both the single-cell and population level suggests that this brain region is critical for selecting, planning, and initiating movements\textsuperscript{118,122}.

PMd presumably influences descending motor activity through a rich network of inhibitory and facilitatory intercortical connections with the primary motor cortex (M1)\textsuperscript{68}. Dual coil transcranial magnetic stimulation (TMS) can be used to non-invasively study the excitatory and inhibitory influence of PMd on M1 during motor preparation in humans at short time intervals (6-10ms) by comparing the size of motor evoked potentials (MEPs) in targeted effectors in response to TMS over M1 alone (i.e., unconditioned MEP) to TMS over M1 preceded by TMS over PMd (i.e.,
conditioned MEP)\textsuperscript{71,76}. During reaction time (RT) tasks, TMS pulses can be applied at specific
timepoints before movement onset to track whether PMd increases or decreases excitability in
contralateral M1 across motor preparation. During unimanual movement preparation, PMd
transiently alters inhibition on cortical effector representations in contralateral M1 40-150 ms after
the presentation of an imperative stimulus (i.e., the stimulus which communicates that the
participant should move)\textsuperscript{8,77,120,121,123}. Importantly, PMd-M1 interhemispheric inhibition (IHI) is
transiently \textit{released} if the targeted effector is selected for movement; the net effect is to decrease
inhibition\textsuperscript{8,120,121,123}. In contrast, when the target effector is not selected for movement PMd-M1
IHI is transiently \textit{increased}\textsuperscript{77}. Furthermore, the timing of transient changes in PMd-M1 IHI
depends on the selection demands of the task. In simple RT tasks transient changes in PMd-M1
IHI occur \textasciitilde{}40-50 ms after the imperative cue\textsuperscript{8,120,121}. During choice RT and Go/NoGo RT tasks
changes in PMd-M1 IHI occur \textasciitilde{}75-150 ms after the imperative cue\textsuperscript{77,121,123}. These findings have
led to speculation that PMd contralateral to active M1 is actively involved in shaping corticospinal
output to control the selection and timing of movements during unimanual RT tasks\textsuperscript{118,122}.

While the modulation of PMd-M1 interhemispheric circuits prior to movement onset has been well
defined for unimanual movements, bimanual movements have received less attention. In one study,
right-handed participants rotated circular manipulanda using both of their index fingers to move a
dot along a path on a computer monitor. Modulation of left PMd-right M1 IHI depended on
bimanual movement symmetry\textsuperscript{7}. Specifically, when the left index finger was required to rotate 3
times faster than the right index finger, left PMd-right M1 interactions became more facilitatory
50 ms prior to the imperative stimulus. Conversely, when the left hand was required to rotate 3
times slower than the right hand, left PMd–right M1 interactions became more inhibitory.
Furthermore, the degree of modulation of left PMd–right M1 IHI compared to rest correlated with
lower target tracking errors in the behavioural task when asymmetric hand rotations were required. In contrast, no change in the influence of PMd on M1 was observed when both index fingers were required to move symmetrically at the same angular velocity regardless of hemisphere. This suggests that PMd-M1 interhemispheric circuits may be important for asymmetric but not symmetric bimanual control.

How PMd-M1 interhemispheric inhibitory circuits are modulated during the pre-movement period (i.e., after the imperative stimulus but before movement onset) of bimanual RT tasks is not well understood. One study measured PMd-M1 IHI in right-handed individuals in both hemispheres during the pre-movement period and found that inhibition ratios were similar across unimanual and symmetric bimanual simple RT trials. However, PMd-M1 IHI was assessed only at 75% of mean RT (~110-180 ms), much later than the range in which transient switches in inhibition are observed for unimanual simple RT tasks (20% of mean RT; ~30-40 ms).

The current study aimed to assess how PMd-M1 IHI is modulated across early and late stages of the pre-movement period of a simple RT task, and if patterns of PMd-M1 circuit modulation depend on whether the planned response is unimanual or symmetrically bimanual. In line with previous research, I predicted that PMd-M1 IHI would be more facilitatory at 50 ms (early) compared to 100 ms (late) post imperative signal when unimanual responses are performed (Hypothesis 1A). Since evidence suggests that the preparation of symmetrical bimanual movements is not associated with significant modulation in PMd-M1 IHI 50 ms prior to the onset of the imperative cue or ~40 ms prior to the onset of movement during simple RT trials, I predicted that PMd-M1 IHI would remain unchanged across the pre-movement period when symmetrical bimanual responses are performed (Hypothesis 1B). PMd-M1 IHI was assessed in both hemispheres to determine whether preparation related changes in PMd-M1 IHI display
hemispheric asymmetry. Since evidence suggests inhibitory output of left PMd and right PMd on contralateral M1 is similar during preparation of unimanual\textsuperscript{77,121} and symmetric bimanual movements\textsuperscript{7}, I further predicted that patterns of PMd-M1 IHI modulation would be similar across hemispheres (\textbf{Hypothesis 2}).

2.2 Methods

2.2.1 Participants

Eighteen healthy individuals recruited from British Columbia completed the study. One participant was removed due to technical issues during TMS data collection. Therefore, 17 individuals (5 male, 12 female, 36.9 ± 14.5 years old) were included in the final analyses. All participants were right-handed according to the Edinburgh Handedness Inventory\textsuperscript{125}. Participant characteristics are presented in \textbf{Table 2.1}. All protocols were approved by Clinical Research Ethics Board at the University of British Columbia. Prior to participating, individuals were screened for contraindications to TMS and provided written informed consent in accordance with the Declaration of Helsinki.

2.2.2 Experimental design

Using a within-subjects design, each participant completed two experimental sessions separated by at least 24 hours to determine whether PMd-M1 IHI is modulated across the pre-movement period of a simple RT task. Sessions differed only in terms of which hemispheres were assessed (right PMd-left M1/left PMd-right M1). The order in which hemispheres were assessed was randomized and counter-balanced between participants. During each session, PMd-M1 IHI were assessed at 50 ms and at 100 ms post an imperative visual stimulus, and also while the participant was at rest and not performing a task. During each session, participants performed 2 versions of
the simple RT task: one which required unimanual responses and one which required bimanual responses (Figure 2.1A). Both versions of the task were performed in discrete blocks of 80 trials (160 total), with the order of task version counterbalanced between participants but held constant between experimental sessions. PMd-M1 IHI was measured using established dual coil TMS methods.\textsuperscript{71,76}

<table>
<thead>
<tr>
<th>Participant</th>
<th>Sex</th>
<th>Age</th>
<th>Right M1 PMd Coil RMT</th>
<th>Right M1 M1 Coil RMT</th>
<th>Right M1 M1 Coil TPT</th>
<th>Left M1 PMd Coil RMT</th>
<th>Left M1 M1 Coil RMT</th>
<th>Left M1 M1 Coil TPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>24</td>
<td>61</td>
<td>55</td>
<td>85</td>
<td>68</td>
<td>51</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>32</td>
<td>52</td>
<td>49</td>
<td>56</td>
<td>54</td>
<td>56</td>
<td>68</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>54</td>
<td>54</td>
<td>52</td>
<td>65</td>
<td>53</td>
<td>48</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>30</td>
<td>44</td>
<td>41</td>
<td>47</td>
<td>41</td>
<td>40</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>21</td>
<td>69</td>
<td>60</td>
<td>85</td>
<td>72</td>
<td>58</td>
<td>85</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>37</td>
<td>53</td>
<td>48</td>
<td>55</td>
<td>51</td>
<td>46</td>
<td>53</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>25</td>
<td>33</td>
<td>31</td>
<td>36</td>
<td>39</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>31</td>
<td>54</td>
<td>52</td>
<td>64</td>
<td>60</td>
<td>49</td>
<td>65</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>34</td>
<td>59</td>
<td>46</td>
<td>71</td>
<td>55</td>
<td>49</td>
<td>85</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>40</td>
<td>59</td>
<td>59</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>77</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>59</td>
<td>53</td>
<td>53</td>
<td>75</td>
<td>53</td>
<td>45</td>
<td>66</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>66</td>
<td>34</td>
<td>40</td>
<td>40</td>
<td>38</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>62</td>
<td>44</td>
<td>44</td>
<td>49</td>
<td>42</td>
<td>38</td>
<td>48</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>30</td>
<td>48</td>
<td>56</td>
<td>56</td>
<td>59</td>
<td>52</td>
<td>60</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>27</td>
<td>48</td>
<td>46</td>
<td>45</td>
<td>44</td>
<td>40</td>
<td>47</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>22</td>
<td>60</td>
<td>60</td>
<td>75</td>
<td>64</td>
<td>58</td>
<td>73</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>29</td>
<td>57</td>
<td>64</td>
<td>85</td>
<td>59</td>
<td>60</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 2.1. Participant characteristics.

M1 – primary motor cortex; PMd – dorsal premotor cortex; RMT – resting motor threshold (percent maximum stimulator output); TPT – test pulse threshold (percent maximum stimulator output); PMd Coil refers to the 5-cm diameter figure-of-8 coil used to stimulate PMd, M1 Coil refers to the 7-cm diameter figure-of-8 coil used to stimulate M1.
2.2.3 Procedure

2.2.3.1 Experimental setup

Participants were seated in front of a computer monitor (BenQ, CA, USA; 144 Hz refresh rate) with both hands placed palm-down on the surface of a table (Figure 2.1A). USB-interfaced response buttons (Makey Makey, CA, USA) were fixed to two sides of a wooden box such that button presses could be executed starting from a resting hand position. Visual stimulus presentation was controlled by Psychtoolbox 3.0 \(^{126}\) in MATLAB (MA, USA); EMG recording and the timing of TMS stimuli was controlled using the VETA MATLAB toolbox \(^ {127}\).

2.2.3.2 Magnetic resonance imaging

A 3D T1-weighted turbo field echo magnetic resonance imaging (MRI) image was used in conjunction with neuronavigation software to ensure consistent targeting of PMd and M1 by TMS across participants (repetition time = 7.7 milliseconds, time to echo = 3.5 milliseconds, flip angle \(\theta = 8^\circ\), field of view = 256 \(\times\) 256 mm, 170 slices, 1 mm slice thickness, scan time = 6.6 minutes; Phillips, MA, USA). A Montreal Neurosciences Institute (MNI) space region of interest mask for left and right PMd was created based on established MNI coordinates for PMd (right PMd: X=25, Y=3, Z=71; left PMd: X=-25, Y=3, Z=71) \(^ {118}\). T1-weighted images were non-linearly transformed into MNI space using FSL FNIRT \(^ {128}\). The PMd region of interest mask was then transformed from MNI space to native space using the inverse of the non-linear transformation warp created by FNIRT. Each individualized region of interest mask was overlayed on each T1-weighted image in FSLview, and the native space coordinates for left and right PMd were visually inspected by an experimenter. Native space coordinates were later used to target PMd with TMS using Brainsight neuronavigation software (Rogue Research Inc, QC, Canada; see Transcranial Magnetic Stimulation).
(A) Participants completed 2 experimental sessions separated by at least 24 hours. Sessions differed only in the hemispheric direction of PMd-M1 IHI assessed. Participants completed 80 unimanual simple reaction time (RT) trials and 80 bimanual simple RT trials during each session. Electromyography (EMG) was recorded from bilateral flexor dorsal interossei to acquire motor evoked potentials (MEPs) and infer reaction time (RT) based on EMG “bursts” on each trial. (B) Transcranial magnetic stimulation (TMS)/EMG measures were acquired at rest and during the pre-movement period of an SRT paradigm (lightning bolts). MEPs were elicited at either 50ms or 100ms into the pre-movement period on discrete trials. (C) Example EMG traces from unimanual and bimanual trials for both TMS targeted and non-TMS targeted hands for the 50ms condition. MEP amplitude and EMG RT burst onset were the primary EMG events of interest.
2.2.3.3 Electromyography

Surface electromyography (EMG) was recorded using bipolar electrodes placed over both first dorsal interosseous (FDI) muscles. A ground electrode was placed over the ulnar styloid process of the right arm. EMG was sampled at 5,000 hertz (Hz), amplified x1,000, and bandpass filtered (50–450 Hz; Delsys, MA, USA).

2.2.3.4 Simple reaction time task

Participants completed two versions of a simple RT task; one in which unimanual responses were required and one where bimanual responses were required (Figure 2.1A). On each trial, participants were shown a fixation cue (black square) followed by an imperative cue (white circle) centrally presented on a monitor. The fixation cue was presented with a 2000–4000 ms jitter on each trial to prevent anticipatory responses (Figure 2.1B). The imperative cue disappeared after a button press or after 2000 ms if no response was registered. Participants were instructed to press the response button(s) as fast as possible following the presentation of the imperative cue and to keep their hands relaxed between button presses. EMG from left and right FDI was visually checked by the experimenters throughout each session on a second monitor situated outside of the participants’ field of vision. If resting background EMG activity was observed outside of button presses, participants were instructed to relax their hands. During each session, participants performed a block of 80 unimanual trials and a block of 80 bimanual trials. The order of blocks was counterbalanced between participants. An opportunity for a break was provided after every 20 trials.

2.2.3.5 Transcranial magnetic stimulation

Setup and thresholding
TMS was administered using a Magstim BiStim-200<sup>2</sup> stimulator (Carmarthenshire, UK). The TMS coil used to stimulate M1 was a 7-cm diameter figure-of-8 coil, while the TMS coil used to stimulate PMd was a 5-cm diameter figure-of-8 “branding iron” coil. Brainsight neuronavigation (Rogue Research Inc, QC, Canada) was used to ensure coil positioning was accurate and consistent across sessions. Two experimenters stood adjacent to the participant and each held one TMS coil over each region of interest. Each coil had an infrared tracker attached which allowed experimenters to track the position of the coil relative the head in real time in Brainsight throughout each session and outside of the participant’s field of vision.

For TMS over M1, the center of the coil was positioned in a posterior-to-anterior orientation 45-degrees to the mid-sagittal plane over the left/right M1 (Figure 2.1A). The “hotspot” for the FDI representation was found by probing different locations in the M1 “hand knob” area. The location at which TMS most consistently resulted in MEPs was defined as the “hotspot”. Once the M1 “hotspot” was determined, the PMd coil was placed in position over contralateral PMd, to ensure that both coils would be able to fit and sit flush on the participants head during dual-coil assessments. Small adjustments were made to the position of the M1 coil if necessary. At the “hotspot”, the resting motor threshold, defined as the lowest TMS pulse intensity that elicited five out of ten MEPs greater than or equal to a peak-to-peak amplitude of 0.05 mV, was determined. After establishing resting motor threshold, a test pulse threshold, defined as the lowest TMS pulse intensity that elicited five out of 10 MEPs greater than or equal to a peak-to-peak amplitude of 1 mV, was determined.

For TMS over PMd, the center of the coil was positioned in a lateral-to-medial orientation 90-degrees to the mid-sagittal plane (Figure 2.1B). The coordinates for PMd stimulation were defined by a standardised FSL analysis procedure applied to each participant’s T1-weighted MRI image.
(see Magnetic Resonance Imaging). Following standard procedure, PMd stimulation intensity was based off the resting motor threshold of M1 ipsilateral to PMd\textsuperscript{71,76}. Therefore, the hotspot and resting motor threshold of M1 ipsilateral to the site of PMd stimulation were determined with the 5-cm PMd coil, with the coil held in posterior-to-anterior orientation 45-degrees to the mid-sagittal plane. During dual coil TMS assessments, PMd TMS intensity was set to 110\% of ipsilateral M1 resting motor threshold.

To ensure M1 was not stimulated by the PMd coil during PMd-M1 IHI dual coil TMS assessments, 5 single pulses of TMS were applied over the PMd target at 110\% of resting motor threshold to test whether any MEPs were elicited. If an MEP was observed, the PMd target was moved anterior by 5mm. If MEPs were still observed in 5 further trials of TMS over this adjusted target, intensity was lowered by 1\% maximum stimulator output until 5 consecutive trials without MEPs were observed. PMd target/intensity adjustments were made in 4 of 17 participants, and all intensities remained suprathreshold. M1 TMS pulse intensity was set to the test pulse threshold (see Table 2.1 for individual resting motor threshold and test pulse threshold values).

\textit{Dual coil transcranial magnetic stimulation}

Dual coil TMS was used to non-invasively study the excitatory and inhibitory influence of PMd on M1. The amplitude of MEP evoked by a single pulse of TMS over M1 (i.e., unconditioned pulse) were compared to the amplitude of MEPs evoked by a pulse of TMS over M1 preceded shortly by a conditioning pulse over PMd (i.e., conditioned pulse)\textsuperscript{71,73,76}. If conditioned TMS pulses resulted in MEPs that had larger average peak-to-peak amplitude compared to MEPs elicited by unconditioned TMS pulses, then PMd had an inhibitory influence over M1. Alternatively, if conditioned TMS pulses resulted in MEPs that had larger average peak-to-peak
amplitude than MEPs elicited by unconditioned pulses, then PMd had a facilitatory influence over M1.

In the current study, unconditioned MEPs were collected by delivering a test pulse (TP) over M1 alone, with stimulator intensity set to the test pulse threshold. Conditioned MEPs were collected by delivering a conditioning pulse (CP) over PMd with stimulator intensity set 110% of resting motor threshold, followed by a TP over M1, with stimulator intensity set to the test pulse threshold. The interpulse interval was set to 8 ms. These stimulation parameters have been shown to consistently elicit PMd-M1 IHI when stimulation is delivered at rest, i.e., conditioned pulses elicit MEPs of smaller amplitude than unconditioned pulses.⁷¹,⁷⁶,⁷⁷

Before performing the simple RT task, 20 unconditioned MEPs and 20 conditioned MEPs were collected in pseudorandomised order while participants were at rest. During these TMS trials, the participant was instructed to stare at a white fixation square and to keep their hands relaxed. These MEPs were collected to assess how PMd-M1 IHI evolved during motor preparation compared to a resting baseline.

TMS was delivered on every trial of the simple RT task, either at 50 ms (50% of trials) or 100 ms (50% of trials) after the imperative cue. Equal numbers of unconditioned and conditioned MEPs were elicited at each timepoint. The timing of TMS pulses on trials was pseudorandomized and random jitter of 2000-4000 ms was inserted between trials so that participants could not predict the timing of TMS pulses.

2.2.4 Data processing

Offline analysis of EMG data was performed using the VETA toolbox and custom-automated procedures within MATLAB. EMG variables of interest included MEP peak-to-peak amplitude.
and EMG RT burst onset (Figure 2.1C). The ‘findEMG.m’ function was used to automatically identify EMG events. All automatically identified EMG events were visually inspected by a human rater using the ‘visualizeEMG.m’ function. Adjustments were made to MEP and EMG RT burst onset and offset when necessary, using the ‘visualizeEMG.m’ function in the VETA toolbox. Any trials that contained >0.05 mV of background muscle activity in either hand 50 ms prior to the TMS artifact were removed from further analysis. Any trial in which EMG RT bursts overlapped with MEPs or vice versa were excluded from further analysis. On average, 10.4 ± 1.2% of trials were excluded per participant.

2.2.5 Dependent variables

**PMd-M1 Interhemispheric Inhibition** – To measure PMd-M1 IHI, the mean peak-to-peak amplitude of 20 unconditioned MEPs (TP alone) and the mean peak-to-peak amplitude of 20 conditioned MEPs (CP + TP) were calculated for each experimental condition. The mean peak-to-peak amplitude of conditioned MEPs was then divided by the mean peak-to-peak amplitude of unconditioned MEPs to generate an PMd-M1 inhibition ratio value for each participant within each experimental condition.

**Corticospinal Excitability** – To measure corticospinal excitability, the mean peak-to-peak amplitude of 20 unconditioned MEPs (TP alone) were calculated for each participant within each experimental condition.

**Reaction Time** – To measure RT, the time difference between imperative cue onset and the onset of EMG bursts was calculated for each RT trial. The mean RT of 20 trials was then calculated for each participant within each experimental condition. For the bimanual version of the simple RT task, separate averages were calculated for each hand (the hand contralateral to M1 stimulation
(i.e., TMS targeted), and the hand ipsilateral to M1 stimulation (i.e., non-TMS targeted). Separate values were calculated because the onset of an MEP in the TMS targeted hand during the pre-movement RT period typically results in a delayed RT EMG burst compared to non-TMS targeted effectors, presumably due to the onset of involuntary muscle activity and/or mechanical perturbation of the TMS targeted effector\textsuperscript{129,130}. This makes it difficult to directly compare RT values across TMS targeted and non-TMS targeted hands.

### 2.2.6 Statistical analysis

All statistical analyses were carried out in RStudio (MA, USA). Post hoc analyses were performed using Tukey’s honestly significant difference or the false discovery rate correction for multiple comparisons where appropriate.

#### 2.2.6.1 Primary analyses

*PMd-M1 Interhemispheric Inhibition* – PMd-M1 IHI values had homogeneity of variance across conditions of interest according to the outcome of a Levene’s test. However, a Shapiro’s test for normality found PMd-M1 IHI values had significant positive skew in 2 of 10 tested conditions. Therefore, to test if PMd-M1 IHI changes differently across time during preparation of unimanual and bimanual movements (*Hypothesis 1*), and whether changes in PMd-M1 IHI depend on the hemispheres assessed (*Hypothesis 2*), a generalized linear mixed effects (GLME) model was performed. PMd-M1 IHI data were fit to a gamma distribution using an identity link function. Fitting data to a gamma distribution using a GLME is an appropriate alternative to traditional *F*-test statistics when data is continuous, non-negative, and positively skewed\textsuperscript{131,132}, all of which was the case with collected PMd-M1 IHI values. Furthermore, GLME models can account for random differences in measurements between subjects, by modelling subject ID as a random effect, rather than a single mean intercept\textsuperscript{133–135}. STIMULUS ONSET ASYNCHRONY (50 ms, 100 ms), TASK
VERSION (UNIMANUAL, BIMANUAL), and HEMISPHERE (RIGHT PMd/LEFT M1, LEFT PMd/RIGHT M1) were included as fixed effects in the GLME. SUBJECT was included as a random effect. Measures of PMd-M1 IHI recorded at rest were included in post hoc analyses to assess how PMd-M1 IHI values changed during motor preparation compared to a resting baseline.

2.2.6.2 Supplementary analyses

Corticospinal Excitability – Corticospinal excitability values had homogeneity of variance across conditions of interest according to the outcome of a Levene’s test. However, a Shapiro’s test for normality found corticospinal excitability values had significant positive skew in 1 of 10 tested conditions. Therefore, to assess whether hypothesized changes in PMd-M1 IHI described by Hypotheses 1 and 2 were driven by changes in corticospinal excitability, a GLME model was performed with fixed effects of STIMULUS ONSET ASYNCHRONY (50 ms, 100 ms), TASK VERSION (UNIMANUAL, BIMANUAL), and HEMISPHERE (LEFT M1, RIGHT M1). SUBJECT was included as a random effect. Data were fit to a gamma distribution using an identity link function. Measures of corticospinal excitability recorded at rest were included in post hoc analyses to assess how corticospinal excitability values changed during motor preparation compared to a resting baseline.

Reaction time – Reaction time values in both the TMS targeted and non-TMS targeted hands were found to have homogeneity of variance according to Levene’s test. Non-TMS targeted RT values were normally distributed across all 8 conditions of interest. TMS targeted RT values were significantly skewed in 1 of 16 conditions of interest. Given that skewness in RT values was constrained to a small percentage of the total conditions, I decided to precede with parametric statistics despite the violation to the assumption of normality. To assess whether hypothesized changes in PMd-M1 IHI described by Hypotheses 1 and 2 were driven by differences in RT across
conditions of interest, two repeated measures analyses of variance (rmANOVAs) were performed for TMS targeted and non-TMS targeted hands. For the TMS targeted hand, a four-way RM-ANOVA was performed using within-subjects factors STIMULUS ONSET ASYNCHRONY (50 ms, 100 ms), TASK VERSION (UNIMANUAL, BIMANUAL), HEMISPHERE (LEFT M1, RIGHT M1), and TMS TYPE (UNCONDITIONED, CONDITIONED). For the non-TMS targeted hand, a three-way RM-ANOVA was performed using within-subjects factors STIMULUS ONSET ASYNCHRONY (50 ms, 100 ms), HEMISPHERE (LEFT M1, RIGHT M1), and TMS TYPE (UNCONDITIONED, CONDITIONED).

2.2.6.3 Exploratory analyses

Associations between PMd-M1 Interhemispheric Inhibition and Reaction Time – Based on results from a priori planned RM-ANOVAs to test Hypothesis 1 and 2, an exploratory a posteriori analysis was performed to examine the effect of individual differences in RT on PMd-M1 IHI across stimulus onset asynchronies, task version, and hemisphere conditions. The purpose of this analysis was to assess the extent to which individual differences in mean RT were driving the modulation of PMd-M1 IHI across preparation. This was motivated by comparing results from our primary analysis to those reported in a previous study, which showed relatively larger changes in PMd-M1 IHI across preparation when individualizing timing of TMS pulses to the average RT of participants\textsuperscript{8,120} rather than employing fixed TMS pulse timings between subjects as was the case in the current study.

Since no true “non-TMS” RT trials were collected in this experiment, the mean of all non-TMS targeted hand RT values recorded on bimanual trials was used as a proxy for mean RT\textsuperscript{129,130}. To test for the possible effect of MEAN RT on PMd-M1 IHI across factors of interest, a GLME model was performed with fixed effects of STIMULUS ONSET ASYNCHRONY (50 ms, 100 ms),
TASK VERSION (UNIMANUAL, BIMANUAL), HEMISPHERE (LEFT M1, RIGHT M1), and MEAN RT. SUBJECT was included as a random effect. Data was fit to a gamma distribution using an identity link function. Exploratory post hoc bivariate Pearson correlations were performed to assess the direction and strength of the relationship between PMd-M1-IHI and MEAN RT across significant within-subject factors. MEAN RT was expressed as a percentage of STIMULUS ONSET ASYNCHRONY when assessing relationships between MEAN RT and PMd-M1 IHI across levels of STIMULUS ONSET ASYNCHRONY to aid with interpretation versus previous studies. Exploratory post hoc bivariate Pearson correlations were corrected for multiple comparisons using the false discovery rate correction.

2.3 Results

2.3.1 PMd-M1 interhemispheric inhibition

*PMd-M1 interhemispheric inhibition* – For PMd-M1 IHI, a significant STIMULUS ONSET ASYNCHRONY x TASK VERSION interaction effect was found ($\beta = -0.22$, 95% confidence interval [CI; -0.37 -0.06], $p = 0.006$), indicating that the difference in IHI ratios was reduced at 100ms compared to 50ms for the unimanual task condition compared to the bimanual task condition. To further interrogate the interaction effect and assess how PMd-M1 IHI values compared with values collected at rest, the data was split by TASK VERSION and 2 follow-up GLME models with fixed effects of STIMULUS ONSET ASYNCHRONY and HEMISPHERE and random effects of SUBJECT were performed. PMd-M1 IHI measures collected at rest were included as an additional level of STIMULUS ONSET ASYNCHRONY. No significant effects for STIMULUS ONSET ASYNCHRONY or HEMISPHERE were found for the bimanual task condition, although a near significant HEMISPHERE x STIMULUS ONSET ASYNCHRONY effect was observed ($\beta = -0.15$, 95% CI [-0.31 -0.01], $p = 0.061$). For the unimanual task
condition, a significant main effect of STIMULUS ONSET ASYNCHRONY was found between measures collected at rest and 50 ms (β = -0.15, 95% CI [-0.27 -0.03], p = 0.017). To further understand the simple main effect of STIMULUS ONSET ASYNCHRONY for the unimanual task condition, estimated marginal means were calculated with data pooled across HEMISPHERE. PMd-M1 IHI was significantly greater at 50 ms compared to rest (μ = 0.1 ± 0.04, p = 0.002, Cohen’s d = 0.75), and significantly greater at 50 ms compared to 100 ms, (μ = 0.12 ± 0.04, p = 0.015, d = 0.59) (Figure 2.2). There was no significant difference in PMd-M1 IHI at 100ms compared to rest (μ = 0.02 ± 0.03, p = 0.82, d = 0.06).
Dorsal premotor cortex (PMd) - primary motor cortex (M1) interhemispheric inhibition as assessed by dual coil transcranial magnetic stimulation at rest and across preparation of unimanual and bimanual movements. Data are pooled across hemisphere. PMd-M1 interhemispheric inhibition ratios switched from an inhibition resting state to facilitation 50ms post imperative stimulus and back to inhibition at 100ms post imperative stimulus during unimanual trials. PMd-M1 interhemispheric inhibition ratios remained inhibitory during bimanual trials. Black horizontal lines represent the mean. For unimanual task comparisons, *p < 0.05, **p < 0.005.
2.3.2 Corticospinal excitability

For corticospinal excitability, a main effect of HEMISPHERE was found ($\beta = 0.62$, 95% CI [0.19 1.05], $p = 0.005$), indicating that corticospinal excitability was greater during motor preparation in left M1 compared to right M1. To assess how corticospinal excitability values changed in preparation compared to rest for right and left M1, data was split by HEMISPHERE and pooled across TASK VERSION, and follow up GLMEs were performed with fixed effects of STIMULUS ONSET ASYNCRONY. Measures of corticospinal excitability recorded at rest were included as an additional level of STIMULUS ONSET ASYNCRONY. Main effects of STIMULUS ONSET ASYNCRONY were found in both follow up GLMEs. Estimated marginal means indicted that for left M1, corticospinal excitability was significantly greater at 100 ms compared to 50 ms ($\mu = 0.35 \pm 0.01$, $p < 0.0001$, $d = 0.86$). Left M1 corticospinal excitability was also significantly greater at 50ms ($\mu = 1.4 \pm 0.01$, $p < 0.0001$, $d = 1.51$) and at 100 ms ($\mu = 1.8 \pm 0.01$, $p < 0.0001$, $d = 1.75$) compared to rest (Figure 2.3A). For right M1, there was no significant difference at 100 ms compared to 50 ms ($\mu = 0.1 \pm 0.03$, $p = 0.92$, $d = 0.35$). However, right M1 corticospinal excitability was significantly greater at 50 ms compared to rest ($\mu = 0.83 \pm 0.23$, $p = 0.0008$, $d = 1.11$), and at 100ms compared to rest ($\mu = 0.92 \pm 0.24$, $p = 0.0004$, $d = 1.08$; Figure 2.3B). In summary, corticospinal excitability increased significantly during preparation of unimanual and bimanual responses compared to rest for left and right M1, and was significantly greater at 100ms compared to 50ms stimulus onset asynchrony for left M1.
Figure 2.3. Corticospinal excitability across preparation.

Corticospinal excitability as measured by peak-to-peak motor evoked potentials (MEPs) assessed by single pulse transcranial magnetic stimulation in left (A) and right (B) primary motor cortex (M1). Both left and right M1 showed significant increases in corticospinal excitability when recorded at 50 and 100ms post imperative stimulus compared to at rest. Only left M1 showed an increase in corticospinal excitability at 100ms compared to 50ms post imperative stimulus. Changes in corticospinal excitability were similar across unimanual and bimanual trials. Black horizontal lines represent the mean, and dots represent individual participants. *p < 0.001, **p < 0.0005, ***p < 0.0001.
2.3.3 Reaction time

For TMS targeted hand RT, main effects of STIMULUS ONSET ASYNCHRONY ($F_{1,16} = 368.76$, $p < 0.0001$, $\eta^2_p = 0.96$; 50 ms trials: 213 ± 29 ms, 100ms trials: 249 ± 34 ms; Figure 2.4A), TMS TYPE ($F_{1,16} = 28.2$, $p < 0.0001$, $\eta^2_p = 0.64$; conditioned TMS trials: 228 ± 37 ms, unconditioned TMS trials: 235 ± 35 ms), and TASK VERSION ($F_{1,16} = 4.6$, $p = 0.048$, $\eta^2_p = 0.22$; unimanual trials: 229 ± 37 ms, bimanual trials: 234 ± 35 ms) were found. TMS TYPE and TASK VERSION main effects should be interpreted in the context of a significant HEMISPHERE x TMS TYPE x TASK VERSION interaction effect ($F_{1,16} = 11.54$, $p = 0.004$, $\eta^2_p = 0.42$). To further assess this three-way interaction, data were pooled across stimulus onset asynchronies and split by task version. Two separate two-way rmANOVAs were then performed using within subject-factors HEMISPHERE, and TMS TYPE. For unimanual trials, a main effect of TMS TYPE ($F_{1,16} = 17.2$, $p = 0.0008$, $\eta^2_p = 0.52$; conditioned TMS trials: 225 ± 38 ms, unconditioned TMS trials: 231 ± 36 ms) was found (Figure 2.4B). For bimanual trials, a main effect of TMS TYPE ($F_{1,16} = 15.13$, $p = 0.001$, $\eta^2_p = 0.49$, conditioned TMS trials: 230 ± 29 ms, unconditioned TMS trials: 237 ± 29 ms) was also found, as well as a HEMISPHERE x TMS TYPE interaction effect ($F_{1,16} = 20.89$, $p = 0.0003$, $\eta^2_p = 0.57$). To assess this interaction effect, bimanual task data were split by HEMISPHERE, and two pairwise t-tests were performed with within-subject factor TMS TYPE. For bimanual trials in which left M1 was stimulated, there was a no significant difference in RT across unconditioned TMS and conditioned TMS trials ($t(16) = 1.29$, $p = 0.21$, $d = 0.31$; conditioned TMS trials: 234 ± 34 ms, unconditioned TMS trials: 237 ± 32 ms). For bimanual trials in which right M1 was stimulated, RT was significantly lower on conditioned TMS bimanual trials compared to unconditioned TMS bimanual trials ($t(16) = 5.35$, $p < 0.0001$, $d = 1.3$; conditioned TMS trials: 226 ± 25 ms, unconditioned TMS trials: 238 ± 26 ms) (Figure 2.4B).
For non-TMS targeted hand RT, a main effect of STIMULUS ONSET ASYNCHRONY ($F_{1, 16} = 448.32, p < 0.0001, \eta_p^2 = 0.97$; 50ms trials: $180 \pm 29$ ms, 100 ms trials: $208 \pm 26$ ms; Figure 2.4A) and a HEMISPHERE x TMS TYPE interaction ($F_{1, 16} = 8.9, p = 0.009, \eta_p^2 = 0.36$) were found. To further assess the interaction effect, data were pooled across STIMULUS ONSET ASYNCHRONY and split by HEMISPHERE. Follow-up pairwise t-tests for each hemisphere found a non-significant trend for faster RT on conditioned TMS bimanual trials compared to unconditioned TMS bimanual trials in which right M1 was stimulated ($t(16) = 2.03, p = 0.059, d = 1.3$; conditioned TMS trials: $187 \pm 23$ ms, unconditioned TMS trials: $193 \pm 30$ ms) but not for trials in which left M1 was stimulated ($t(16) = 0.21, p = 0.84, d = 0.31$; conditioned TMS trials: $197 \pm 31$ ms, unconditioned TMS trials: $197 \pm 31$ ms) (Figure 2.4B).
Figure 2.4. Reaction time.

(A) Reaction time (RT) for each stimulus onset asynchrony for TMS targeted (left) and non-TMS targeted (right; bimanual trials only) hands. Data pooled over other within subject factors of hemisphere, TMS pulse type and task version (unimanual trials only – non-TMS targeted hand RT was not collected on unimanual trials). (B) RT for left and right hands and single and double TMS pulse types, shown for unimanual trials (left), the TMS targeted hand on bimanual trials (middle), and the non-TMS targeted hand on bimanual trials (right). RT was defined as the time between the onset of the imperative stimulus and the onset of EMG burst activity. *p<0.001, **p<0.0001.
2.3.4 Associations between PMd-M1 interhemispheric inhibition and reaction time

The GLME model conducted to explore the relationship between RT and PMd-M1 IHI found a main effect of STIMULUS ONSET ASYNCHRONY was found ($\beta = -0.99$, 95% CI [-1.8 -0.17], $p = 0.018$). Furthermore, STIMULUS ONSET ASYNCHRONY x MEAN RT ($\beta = 5.83$, 95% CI [1.57 10.09], $p = 0.008$), STIMULUS ONSET ASYNCHRONY x TASK VERSION ($\beta = 1.15$, 95% CI [0.01 2.29], $p = 0.049$), and STIMULUS ONSET ASYNCHRONY x TASK VERSION x MEAN RT ($\beta = -6.46$, 95% CI [-12.42 -0.49], $p = 0.034$) interaction effects were also found. To further unpack the STIMULUS ONSET ASYNCHRONY x TASK VERSION X MEAN RT interaction effect, the data were split by TASK VERSION and two follow up GLMEs with fixed effects of STIMULUS ONSET ASYNCHRONY, HEMISPHERE, and MEAN RT and random effect of SUBJECT were run. For the unimanual task version, a main effect of STIMULUS ONSET ASYNCHRONY ($\beta = -0.96$, 95% CI [-1.8 -0.12], $p = 0.026$) and a STIMULUS ONSET ASYNCHRONY x MEAN RT ($\beta = 5.7$, 95% CI [1.29 10.11], $p = 0.012$) interaction effect were found. There were no significant main effects of STIMULUS ONSET ASYNCHRONY ($p = 0.72$) or a STIMULUS ONSET ASYNCHRONY x MEAN RT interaction effect ($p = 0.79$) for the bimanual task version.

To further interrogate the effect of stimulus onset asynchrony on the strength and direction of the relationship between PMd-M1 IHI and mean RT in the unimanual task condition, Pearson bivariate correlations were performed between measures of PMd-M1 IHI collected at each STIMULUS ONSET ASYNCHRONY and MEAN RT. STIMULUS ONSET ASYNCHRONY was expressed as a percentage of MEAN RT to aid with interpretation. PMd-M1 IHI was shown to significantly correlate with MEAN RT when measured at 50 ms post imperative stimulus ($\rho = -0.4$, $p = 0.038$; Figure 2.5) but not at 100 ms post imperative stimulus ($\rho = 0.23$, $p = 0.23$). This indicates that
participants with slower MEAN RT showed greater disinhibition of PMd-M1 IHI at 50 ms post imperative RT stimulus.

Figure 2.5. PMd-M1 interhemispheric and mean RT correlation.

Correlation between mean RT and PMd-M1 IHI recorded during unimanual trials at a stimulus onset of asynchrony of 50ms. Facilitation tended to be observed when 50ms represented 20-28% of an individual’s mean RT, while inhibition was more likely when 50ms represented >28% of an individual’s mean RT.
2.4 Discussion

In the current study, I investigated the modulation of PMd-M1 IHI across two timepoints within the pre-movement period (i.e., the period of time between the onset of the imperative stimulus and the onset of movement) of a visually cued unimanual and symmetrically bimanual simple RT task. For unimanual movements, I found that PMd-M1 interhemispheric interactions transiently switched from inhibitory to facilitatory early (50 ms) and back to inhibitory later (100 ms) within the pre-movement period (Figure 2.2). Additionally, the strength of PMd-M1 IHI at 50 ms correlated with mean RT (Figure 2.5). In contrast, PMd-M1 interhemispheric interactions remained inhibitory throughout the pre-movement period when bimanual movements were required (Figure 2.2), and were unrelated to RT. Taken together, these results suggest that discrete unimanual and bimanual movements may be preceded by different patterns of excitability in PMd-M1 interhemispheric circuits.

2.4.1 PMd-M1 interhemispheric interactions differ for unimanual and bimanual preparation

During the pre-movement period of unimanual movements, PMd-M1 IHI shifted to interhemispheric facilitation 50 ms post imperative stimulus before returning to IHI 100 ms post imperative stimulus across both hemispheres (Figure 2.2). These findings are consistent with previous investigations that showed PMd-M1 interhemispheric circuits in both hemispheres are involved early in the pre-movement period of unimanual movements 8,77,120,121,123. Similar effects are noted across a variety of externally cued RT tasks, including simple RT 8,120,121, choice RT 77,121, and Go/NoGo RT 123 tasks. Importantly, these previous experiments have demonstrated that switches from inhibition to facilitation are not sustained throughout the pre-movement period but are transient, occurring in a time window of ~25 ms 77,121. Furthermore, the timing of transient
switches in PMd-M1 IHI within the pre-movement period depends on the selection demands of the task, with transient switches occurring ~40-50 ms after the imperative cue in simple RT tasks with fast mean RTs (~200 ms) and at ~75-150 ms after the imperative cue in choice RT tasks with relatively slower mean RTs (~350 ms). The timing of switches from inhibition towards facilitation in PMd-M1 circuits is different to those observed within M1-M1 circuits, which occur much later in the pre-movement period, at ~30 ms before voluntary EMG activity.

While there was a significant effect of stimulus onset asynchrony on PMd-M1 IHI ratios for unimanual movements, our mean IHI ratio of 1.05 at 50 ms was lower relative to other studies using a similar paradigm, where mean ratios of 1.15-1.25 were reported. Past work individualized the timing of TMS stimuli to 20% of each participants’ mean RT rather than using a fixed stimulus onset asynchrony (as was the case in the current study) and showed a larger average PMd-M1 IHI ratio of 1.25 at this timepoint. To assess whether individual differences in average RT were driving the evolution of PMd-M1 IHI across unimanual preparation, I performed an additional analysis with the same within-subjects factors but included mean RT as a covariate. I observed that the strength of PMd-M1 facilitation at 50 ms post imperative stimulus correlated negatively with RT when 50 ms was expressed as a percentage of individual mean RT (Figure 5). When the 50 ms fixed stimulus onset asynchrony equated to 20-28% of the participants mean RT, IHI ratios tended to be facilitatory; however, when 50 ms equated to >28% of mean RT, IHI ratios tended to be inhibitory. This percentage of RT may represent a rough temporal threshold that demarcates “early” vs “late” phases of the pre-movement period, after which PMd facilitatory input to the selected effector representation in contralateral M1 is diminished. Given that 50 ms represented >28% of mean RT for a subset of participants in our study (6/17), the use of fixed TMS pulse timing of 50 ms may not have occurred early enough within the pre-movement period to
identify a switch from inhibition towards facilitation in these participants. This may explain the discrepancy between the mean PMd-M1 IHI ratio reported in the current study and previous investigations, and further supports the notion that switches excitation within in PMd-M1 interhemispheric circuits are transient and important for the timing of unimanual discrete movements\textsuperscript{121}.

PMd-M1 IHI remained unchanged throughout the pre-movement period for bimanual movements. The absence of an effect of stimulus onset asynchrony did not appear to be due to individual differences in mean RT, since mean RT was not a significant factor for bimanual trials in the exploratory analysis. This result is in line with previous work showing that PMd-M1 IHI does not differ from baseline levels during motor preparation 50 ms before the presentation of an imperative stimulus of a bimanual task where symmetrical circular finger movements are required\textsuperscript{7}. It also aligns with other work showing PMd-M1 interhemispheric interactions do not change significantly from baseline values during late stages of the pre-movement period of a simple RT task (75\% of mean RT)\textsuperscript{124}. The current study adds to this body of literature, demonstrating that PMd-M1 interactions remain inhibitory early (50 ms) in the pre-movement period of simple bimanual RT trials. Interestingly, previous studies of M1-M1 IHI showed disinhibition at 75\% of mean RT in a simple RT task regardless as to whether a unimanual or bimanual response was required, suggesting that the differential effect of unimanual versus bimanual response requirements on patterns of IHI change during preparation does not generalize from PMd-M1 to M1-M1 interhemispheric circuits. However, further studies which assess the evolution of M1-M1 IHI across at multiple timepoints in the premovement period of a bimanual RT task are needed for greater clarity on this question. Taken together, these findings suggests that even simple discrete
symmetrical bimanual finger movements may be preceded by fundamentally different patterns of activity between PMd and M1 in the contralateral hemisphere.

2.4.2 Changes in corticospinal excitability are similar for unimanual and bimanual preparation

Unlike PMd-M1 interhemispheric interactions, modulation of corticospinal excitability was similar across preparation of bimanual and unimanual movements. For both task versions, there was a large increase in corticospinal excitability in the pre-movement period relative to rest. Corticospinal excitability was slightly greater in left M1 compared to right M1 during preparation. This effect was partially driven by hemispheric differences in the modulation of corticospinal excitability across the pre-movement period, with significantly larger MEP amplitudes observed at 100 ms post imperative stimulus compared to 50 ms post imperative stimulus when left M1 was stimulated but not when right M1 was stimulated (Figure 2.3). Such an effect of hemisphere on corticospinal excitability was not anticipated, and given the relatively small effect size ($d = 0.35$) difference may have simply been due to random noise within MEP measurements. Alternatively, this finding could be attributed to differences in average RT between dominant and non-dominant hands. While I did not find a significant difference in RT between right and left index fingers on unimanual RT trials, the onset of MEPs instituted a broad slowing of RTs compared to non-TMS targeted hand RTs (Figure 2.4), making RT data difficult to interpret. It is well established that RT is slower and more variable in the non-dominant hand versus the dominant hand\textsuperscript{138–140}. Previous investigations showed that corticospinal excitability tends to remain stable or become reduced through the early stages of the pre-movement period and begin to rapidly increase ~60 ms prior to movement onset\textsuperscript{141–144}. Given that all participants were right hand dominant, the stimulus onset asynchrony of 100 ms was likely closer to the onset of voluntary muscle activity in the right index
finger when left M1 was stimulated, which might explain why corticospinal excitability increased between 50 ms and 100 ms stimulus onset asynchronies in left M1.

The difference between rest and pre-movement period corticospinal excitability may have been context dependent. Resting single pulse MEPs were collected with the participant staring at a fixation square and not planning to make any movements. Previous research shows that there is a broad increase in corticospinal excitability of the selected effector during simple RT tasks when participants are certain of the identity of an upcoming movement, possibly due to modulation of attentional resources. Therefore, as participants prepared to perform RT trials in the current study, there may have been an attentional shift which increased baseline corticospinal excitability. Future studies should account for this general increase in corticospinal excitability by thresholding M1 TMS test pulse intensity during performance of RT trials and by collecting MEPs during task performance outside of the pre-movement period, such as when the preparatory signal is first displayed. Alternatively, a delayed response task paradigm with a pre-cued preparation period could be implemented to limit attention to a particular response during inter trial rest periods. Overall, the corticospinal excitability results suggest that the task specific effects I describe for PMd-M1 IHI are constrained to differences in activity in PMd-M1 interhemispheric circuits and not driven by differences in corticospinal excitability.

2.4.3 The functional role of PMd-M1 interhemispheric circuits

Although counterintuitive, electrocorticography, single neuron recording, and functional MRI studies provide evidence that the PMd ipsilateral to the moving hand plays a role in unimanual movements. Furthermore, patients with unilateral M1 lesions exhibit motor control deficits in the less affected hand and skilled unimanual sequence production is impaired in
healthy individuals after inhibiting the ipsilateral M1 with repetitive TMS (rTMS)\textsuperscript{153}. Thus, it appears that the ipsilateral cortex plays a role in shaping unimanual movement.

In dual coil TMS studies, the timing of inhibitory and facilitatory switches in PMd-M1 IHI correlate with the onset of voluntary muscle activity\textsuperscript{121}, and the direction of inhibition (i.e., increasing or releasing) covaries with whether an effector is selected for an upcoming movement\textsuperscript{77}. Intracranial recordings suggest this is not an inhibitory gating mechanism (for review see\textsuperscript{122}). Perhaps the transient alterations in PMd-M1 IHI during preparation instead reflect the transition of neural population responses from output-null to output-potent “neural spaces” necessary for movement execution according to the dynamical systems framework for movement preparation\textsuperscript{101,108}. Previous work demonstrates that the timing of such neural space transitions correlates with movement onset on a trial-to-trial basis and occur \textasciitilde 150 ms before movement onset\textsuperscript{110}. In the current study, transient switches in PMd-M1 IHI during unimanual movements were stronger when sampled \textasciitilde 150 ms prior to movement onset (Figure 2.5), and previous studies further suggest the timing of transient switches in PMd-M1 interactions in the pre-movement period may correlate with RT\textsuperscript{122}. Work that combines TMS with single neuron recordings may be required to clarify this question. However, the overall evidence suggests that activity in PMd-M1 IHI circuits may be important for accurate timing of unimanual movements.

If contralateral PMd is necessary for accurate timing of unimanual movements, then how might symmetrical bimanual movements be initiated in the absence of this input? One possibility is that cortical activity is separated during symmetrical bimanual movements but not during unimanual movements. In this scenario, PMd of each hemisphere interacts with ipsilateral but not contralateral M1. Previous TMS research has shown that the balance of inhibition within PMd-M1 intrahemispheric circuits is modulated in a similar way to PMd-M1 interhemispheric circuits.
during a unimanual choice RT task\textsuperscript{74,75}. It may be the case that PMd-M1 intrahemispheric circuits show a similar pattern of modulation during the initiation of discrete symmetrical bimanual movements, providing a mechanism for movement initiation in the absence of input from contralateral PMd. Another possibility is that other cortical regions are selectively engaged during planning and execution of bimanual movements. The supplementary motor area (SMA) has been hypothesized to play a specialized role in bimanual behaviours\textsuperscript{154–156}, and dual-coil TMS protocols have been used to measure the inhibitory influence of SMA on M1\textsuperscript{157,158}. Indeed, it has been shown that larger SMA-M1 inhibition ratios recorded at rest correlate with greater bimanual but not unimanual task performance\textsuperscript{159}, suggesting a potential specialized role for this circuit in bimanual control. The application of dual coil TMS methods at different stimulus onset asynchronies during performance of bimanual RT trials could help elucidate whether modulation of inhibition through SMA-M1 circuits during preparation shows a similar pattern to that observed in PMd-M1 interhemispheric circuits for unimanual trials.

While the current study focused solely on PMd-M1 interactions during preparation, and I have discussed the possibility of SMA-M1 circuits as an important region for the preparation of bimanual movements, it is also worth interrogating the assumption that a single cortical region should be responsible for preparing unimanual or bimanual movements. It may be more fruitful to eschew single region approaches and consider how such responsibilities are shared among a network of distributed brain regions. Indeed, TMS studies which probe interactions between cortical and subcortical regions other than PMd hint that transient switches in inhibition during motor preparation may be a feature shared among many intercortical circuits. For example, the cerebellum releases inhibition on M1 about 40\% of the way through the pre-movement period of unimanual RT trials for the selected effector\textsuperscript{160}. Similarly, the right posterior parietal cortex (PPC)
transiently increases facilitation to ipsilateral M1 50ms after the imperative cue when unimanual reaches to the left but not right direction are required\textsuperscript{161}. Left PPC-left M1 interactions also become more facilitatory 120ms after the imperative cue during Go trials but not NoGo trials during a unimanual Go/NoGo task\textsuperscript{162}. Furthermore, distinct subregions of the left PPC (superior parietal occipital cortex and anterior region of the intraparietal sulcus) selectively increase facilitation of M1 during preparation depending on whether arm transport or gripping movements, respectively\textsuperscript{163,164}. Similarly, the primary visual cortex increases facilitation of M1 50 ms after the onset of the imperative stimulus in a simple visual RT task\textsuperscript{165}. The similarities in the modulation of multiple non-PMd-M1 circuits during motor preparation with the pattern of change observed in PMd-M1 IHI during unimanual preparation in the current study raises the possibility that preparatory processes which transform sensory signals into movement may be implemented concurrently by distributed cortical and subcortical regions and not just PMd. This could help explain why temporary disruption of PMd by rTMS does not result in any change in RT in a unimanual choice RT task, even when concurrent changes in PMd-M1 interhemispheric connectivity\textsuperscript{118} and PMd activation as assessed by functional MRI\textsuperscript{166} are observed. Therefore, future studies may need to investigate the contributions of multiple intercortical circuits to motor preparation within the same participant sample, to decompose their relative contributions and account for the possibility that unimanual and bimanual preparatory processes are implemented by a cortical network rather than any one specific region.

### 2.4.4 Limitations

The current study is not without limitations. As discussed, non-TMS RT trials were not collected, which meant that RT in the non-TMS targeted hand on bimanual trials had to be used as a proxy for non-TMS RT in the exploratory analysis assessing the relationship between individual
differences in RT and PMd-M1 IHI. While this method has been used previously in other studies\textsuperscript{129,130}, the sound and physical sensation of the TMS pulses as well as indirect effects of stimulation of the cortex may have affected RT in the non-TMS targeted hand, (e.g., via cross-modal facilitation\textsuperscript{144}), even in the absence of direct stimulation of the associated effector representation in M1. This could have produced RT values that differ from trials where no TMS is applied. Future studies should collect non-TMS RT in a separate block of trials or interleave non-TMS trials throughout the task blocks. Relatedly, the lack of non-TMS trials may have prompted participants to anticipate TMS pulse timing and subsequently affected behaviour in unintended ways, especially for 100 ms stimulus onset asynchrony trials where timing would be certain after the 50 ms mark. However, given that an identical process was used to randomize TMS pulse timing across unimanual and bimanual task blocks, any potential confounding effects on changes in PMd-M1 IHI across preparation caused by the lack of non-TMS trials should have been constant across task versions and hemispheres, minimizing their effect on key results. Another potential consequence of a lack of non-TMS trials is that it may have shifted attentional focus away from the visual imperative RT stimulus and prompted participants to use the sound of the TMS pulse as a movement trigger. However, given that the mean difference in RT between 50 ms and 100 ms stimulus onset asynchrony trials was just 28 ms, it appears that on average participants did not use the sound of the TMS pulse as the imperative cue. Nevertheless, future investigations should ensure to interleave some non-TMS trials with TMS trials within task blocks to avoid these potential confounds.

2.4.5 Conclusion

I observed that PMd-M1 IHI shows a different pattern of change during preparation for unimanual vs bimanual movements. Our data suggest that even simple unimanual and bimanual actions are
preceded by distinct patterns of change in excitability within human interhemispheric circuits. Previous work has hinted at hemispheric asymmetry in the modulation of PMd-M1 interhemispheric circuits during action preparation\textsuperscript{77,121}. However, I found no significant difference between hemispheres across unimanual or bimanual action preparation. The current study may have clinical relevance, in particular for individuals who have experienced a stroke. MRI studies demonstrate that the structural integrity of interhemispheric pathways connecting premotor and prefrontal cortices correlates with motor impairment in chronic stroke\textsuperscript{167} and that the functional connectivity between contralesional PMd and ipsilesional M1 correlates with motor recovery after stroke\textsuperscript{168}. Furthermore, recent non-human primate research demonstrate that the blockage of contralesional PMd with chemogenetics impairs dexterous hand movements in the acute recovery stage after stroke\textsuperscript{169}. Therefore, applying the TMS techniques used in the current study to investigate PMd-M1 circuits across movement preparation in stroke populations may be a fruitful avenue to further inform basic questions about the role of PMd in motor control and also improve understanding of the neural basis of recovery after stroke.

2.5 Bridging statement

Recruitment of right PMd-left M1 interhemispheric circuits has been proposed as a mechanism through which right PMd exerts control on bilateral descending motor activity during asymmetric rhythmic bimanual behaviour\textsuperscript{4,8}. This view is supported by findings that the degree of release of right PMd–left M1 IHI during unimanual preparation correlates with the capacity to maintain symmetric finger tapping patterns at high frequencies\textsuperscript{8}. Results from the current study call into question a direct relationship between right PMd-left M1 interhemispheric circuits and asymmetric rhythmic bimanual control, since PMd-M1 interhemispheric circuits were not found to be recruited during bimanual preparation and patterns of change in PMd-M1 IHI were similar
for both hemispheres. Previous studies indicate that the timing and strength of release of PMd-M1 IHI during unimanual preparation depends on task complexity. Our finding that the strength of PMd-M1 IHI release correlated with RT further supports this view. Therefore, perhaps the degree of release of PMd-M1 IHI during unimanual preparation is a marker of an individual’s capacity to process task-relevant information in a fast and efficient manner, i.e., cognitive efficiency. Since asymmetric rhythmic bimanual behaviours are known to tax cognitive processes more than symmetric behaviours, this could explain the relationship between PMd-M1 IHI release during unimanual preparation and asymmetric bimanual rhythmic control. Those who are able to efficiently process task-relevant information in a unimanual RT task are able to leverage this efficiency in a second unfamiliar asymmetric bimanual rhythmic tapping task. In line with this idea, in the next chapter I will further assess the extent to which cognitive factors affect the stability of asymmetric rhythmic bimanual tapping behaviours.
3 Movement quality moderates the effect of spatially congruent cues on the stability of symmetric and asymmetric rhythmic bimanual finger movements.

3.1 Introduction

Deriving principles of bimanual coordination has most frequently been pursued through the study of rhythmic bimanual movements. A cardinal finding from this paradigm is that mirror-symmetric bimanual movements are executed with greater stability than asymmetric movements (for reviews, see1,2,30,31). Less stability in asymmetric movements may be due to “crosstalk” between the neural processes responsible for translating task goals and external stimuli into rhythmic action32–35,170. According to this view, stabilization of asymmetric bimanual movement patterns requires that sources of interference are successfully suppressed.

There are competing perspectives regarding the dominant source of neural crosstalk driving the instability of asymmetric rhythmic bimanual movements. The “motor outflow hypothesis” argues that interference within motor programming processes are the dominant source of neural crosstalk driving asymmetric instability30,171,172. This view is supported by transcranial magnetic stimulation (TMS) studies, which show that during ongoing performance of unimanual and bimanual rhythmic movements, corticospinal excitability is modulated in a phasic manner correlating with the rhythmic phase of the moving limb(s)173. According to the motor outflow hypothesis, limitations in the control of asymmetrical movements can be attributed to “hard-wired” connections between motor areas of the left and right hemispheres. Mediated by the corpus callosum, asymmetric patterns of excitability of effector representations within bilateral primary motor cortices (M1) destabilize asymmetric bimanual behaviour46,174,175.
In contrast, according to the “central processing hypothesis”, cognitive-perceptual processes upstream of motor programming, are the dominant source of neural interference driving the instability of asymmetric bimanual movements. This view is supported by evidence from discrete bimanual reaching paradigms, in which participants were required to quickly perform asymmetric or symmetric reaches after the onset of a visual cue. The target bimanual reaches were cued either symbolically by letters or cued in a spatially congruent manner through illumination of the endpoint locations. Bimanual reaches were slower in the symbolic cue condition. Importantly, symbolic cues were also associated with an increase in reaction time (RT) for asymmetric compared to symmetric reaches, while there was no difference in RT between symmetric and asymmetric reaches when spatially congruent cues were used\textsuperscript{18–21}. These results suggest that constraints on planning discrete asymmetric bimanual movements are primarily driven by interference within central cognitive processes responsible for translating task-relevant information from sensory representations to action plans. Complementary neuroimaging evidence suggests that spatially congruent cue conditions facilitate use of a rapid automatic response selection system, enabling the planning of two reach trajectories in parallel. In contrast, abstract symbols force the use of a slower and serial response selection process, which introduces a potent source of interference\textsuperscript{21}.

Cognitive-perceptual factors have been shown to affect rhythmic bimanual control. For example, asymmetric index finger flexion-extension oscillations are performed with similar accuracy to symmetric oscillations if the wrist position of one hand is rotated such that the perceptual consequences of asymmetric movements are made symmetrical\textsuperscript{10}. Furthermore, asymmetric bimanual rhythmic finger circling is more stable when cued with flashing light emitting diodes (LEDs) that are spatially congruent with the direction of motion\textsuperscript{14,37}, while asymmetric rhythmic
hand circling is more easily learned and retained when participants are provided with enhanced online visual feedback of the position of their hands. Similarly, switching from symmetric to asymmetric rhythmic circling patterns results in fewer errors when the timing of switches are cued with spatially congruent stimuli cues.

Existing investigations into the effect of spatially congruent cues on rhythmic bimanual movements primarily focus on rhythmic circling movements, whereas repetitive finger tapping movements have received less attention. This is an important distinction because rhythmic bimanual tapping and circling may be fundamentally different classes of movement with distinct coordination principles. For example, performance on rhythmic finger tapping and rhythmic circle drawing tasks does not correlate within subjects. Furthermore, callosotomy patients cannot spatiotemporally couple hands during rhythmic bimanual circle drawing but can spatiotemporally couple fingers when tapping in rhythm at regular frequencies. However, this latter capacity is abolished when patients are instructed to perform smooth continuous finger oscillations instead of a series of discrete taps separated by a pause. Interestingly, patients with cerebellum damage show the opposite pattern of deficits. Taken together, these results suggest that rhythmic bimanual movements that are discrete rely on event timing mechanisms mediated by the cerebellum. In contrast, rhythmic bimanual movements that are continuous rely on “emergent timing” mechanisms mediated by transcallosal cortical networks with spatiotemporal coupling emerging via the control of higher order kinematic parameters such as speed.

In the current study, across two experiments I tested (1) whether cueing discrete bimanual key presses with spatially congruent visual stimuli versus abstract symbolic visual stimuli decreases choice RT (Experiment 1), (2) whether cueing the movement frequency of asymmetric bimanual rhythmic finger tapping movements with spatially congruent visual stimuli versus abstract
symbolic visual stimuli influences the stability of bimanual spatiotemporal coupling (Experiment 1), and (3) whether the movement strategy used to perform rhythmic bimanual key presses changes with increasing movement frequency (Experiment 2). In Experiment 1, I assessed whether the benefits of spatially congruent cues versus abstract symbolic cues are shown when performing discrete bimanual finger movements. Participants completed two versions of a bimanual 4-choice RT task, which differed in terms of whether choices were triggered by spatially congruent or abstract symbolic visual stimuli (Figure 3.1). Reaction times for symmetric and asymmetric bimanual finger movements were recorded. Based on findings from bimanual reaching paradigms\textsuperscript{18,21}, I predicted that RT would be faster when movement was cued with spatially congruent stimuli (Hypothesis 1A), that RT would be faster when symmetric responses were required (Hypothesis 1B), and that the cost to RT for asymmetric responses would be significantly reduced when movement was cued with spatially congruent stimuli (Hypothesis 1C).

In Experiment 1, I also assessed whether spatially congruent and abstract symbolic cues have an effect on the accuracy and stability of rhythmic bimanual finger tapping movements. Participants performed four versions of a 4-finger bimanual rhythmic tapping task. The task versions differed in terms of whether repeated symmetric or asymmetric responses were required and whether movement frequency was cued by repeating spatially congruent or symbolic visual stimuli (Figure 3.1). Rhythmic tapping was assessed across a range of movement frequencies. It was predicted that the accuracy and stability of rhythmic tapping across all task versions would decrease with increasing movement frequency (Hypothesis 2A). It was further predicted that symmetric movement patterns would be more stable compared to asymmetric movement patterns (Hypothesis 2B) and that movement patterns cued with spatially congruent cues would be more stable compared to those cued with abstract symbolic cues (Hypothesis 2C). I also hypothesized that the
cost of asymmetric patterns on stability would be greater when movement patterns were cued with abstract symbolic stimuli compared to spatially congruent stimuli (Hypothesis 2D).

Whether rhythmic bimanual movements are discrete or continuous can dramatically alter the coordination principles at play. Since measures of task performance were derived entirely from key press timing data in Experiment 1, it was not possible to interrogate potential shifts in movement strategy from repeated discrete movements to continuous movement with data from Experiment 1. Therefore, to better understand how changes in movement strategy across movement frequencies might influence results from Experiment 1, a follow up Experiment 2 was conducted. Participants performed the same 4-finger bimanual rhythmic tapping tasks from Experiment 1 in a laboratory setting, but with concurrent video recording of their fingers during task performance. Recordings of finger movements were analyzed with deep learning algorithms to capture 2-dimensional spatiotemporal kinematics of the fingers during task performance. The mean relative speed and acceleration of finger trajectories in a prescribed time window around the time of key presses was used as a proxy measure of movement smoothness. It was predicted that mean relative speed would increase while mean relative acceleration would decrease as movement frequency was increased, in line with a change in movement quality from rapid discrete movements separated by long pauses to smoother continuous movement with no pauses (Hypothesis 4A). It was further predicted that changes in mean relative speed and acceleration would be similar across task conditions (Hypothesis 4B).
3.2 Methods

3.2.1 Experiment 1

3.2.1.1 Participants

Individuals were automatically excluded if they were not aged 18-65 yr, were not fluent English speakers, or had a history of brain injury/disease. A total of 250 participants completed Experiment 1. The majority of participants were recruited via a University of British Columbia undergraduate human research participants pool and received academic credit for participating. The remaining participants were recruited through personal relationships. All participants had the option to enter their name into a lottery for a retail store gift voucher award. Of the 250 participants, 9 did not perform key presses as required on certain task blocks and their data were excluded from the final analyses, leaving a total of 241 participants (22.1 ± 6.2 years old; 186 female, 55 male; 225 right-handed, 16 left-handed). All protocols were approved by Behavioural Research Ethics’ Board at the University of British Columbia. Before participating, individuals provided written informed consent in accordance with the Declaration of Helsinki.

3.2.1.2 Experimental design

Using a within-subjects design, all participants first performed two versions of a 4-finger bimanual choice RT task: one in which responses were cued with abstract symbolic stimuli (i.e., words which described the identity of the requisite bimanual finger pair), and another in which responses were cued with spatial stimuli (i.e., boxes which denoted the relative spatial location of the requisite bimanual finger pair; Figure 3.1). Next, participants performed four versions of a 4-finger bimanual rhythmic tapping task. Task versions differed in terms of whether symmetric or asymmetric tapping patterns were required and whether movement timing was cued with abstract symbolic or spatially congruent stimuli (Figure 3.1). The order of task stimulus type
(symbolic/spatial) was counterbalanced between subjects for both the choice RT tasks and the rhythmic tapping task.
In both tasks, movement timing was either cued with spatially congruent visual stimuli, where red boxes directly denoted the relative spatial location of the fingers required to move, or abstract symbolic stimuli where words abstractly denoted the identity of the fingers required to move. Required responses were either symmetric or asymmetric. In the bimanual 4-finger rhythmic tapping task, participants performed 4 versions of the task, in which movement frequency was cued with either spatially congruent or abstract symbolic visual cues, and the required response pattern was either symmetric or asymmetric. (B; left) Participants performed 12 blocks of 32 bimanual taps for each task condition. Movement frequency started at 1 hertz (Hz) and increased by 0.2 Hz after each block. (B; right) Participants performed 2 versions of the bimanual 4-choice RT task, one in which the visual cues used to cue movement were spatially congruent and one in which the visual cues used to cue movement were symbols. Participants performed 60 RT trials each for the spatial and symbolic versions of the task.

Figure 3.1. Stimulus-response mappings and tasks outline.

(A) In both tasks, movement-response timing was either cued with spatially congruent visual stimuli, where red boxes directly denoted the relative spatial location of the fingers required to move, or abstract symbolic stimuli where words abstractly denoted the identity of the fingers required to move. Required responses were either symmetric or asymmetric. In the bimanual 4-finger rhythmic tapping task, participants performed 4 versions of the task, in which movement frequency was cued with either spatially congruent or abstract symbolic visual cues, and the required response pattern was either symmetric or asymmetric. (B; left) Participants performed 12 blocks of 32 bimanual taps for each task condition. Movement frequency started at 1 hertz (Hz) and increased by 0.2 Hz after each block. (B; right) Participants performed 2 versions of the bimanual 4-choice RT task, one in which the visual cues used to cue movement were spatially congruent and one in which the visual cues used to cue movement were symbols. Participants performed 60 RT trials each for the spatial and symbolic versions of the task.
3.2.1.3 Procedure

The experiment was delivered via online platforms, with participants using a web browser on their personal desktop or laptop computer to complete the required tasks. Participants accessed the study via Qualtrics, a secure online survey tool (qualtrics.com), where they first indicated they met the study criteria, digitally signed a consent form, and provided demographic information. Participants were then redirected to the Gorilla Experiment Builder (www.gorilla.sc) to enter the online experiment environment. Participants could not progress to Gorilla unless they were using a desktop or laptop computer with a keyboard.

In Gorilla, participants were given instructions on how to correctly perform trials of the 4-finger bimanual choice RT task. Participants performed the 2 versions of the task (symbolic stimuli/spatial stimuli) in consecutive blocks, with the order of task version counterbalanced between participants. There were 4 stimulus-response mappings for the symbolic and spatial versions of the task (Figure 3.1A). Before starting each block of trials, participants were given an opportunity to practice the task, to ensure they understood the instructions and to build familiarity with the stimulus-response mappings. Participants had to demonstrate that they could complete 6 consecutive practice trials without making an error before they were allowed to advance to the experiment. For each task version, participants performed 4 blocks of 15 task trials (15 per each 4 stimulus-response mappings) with an opportunity for a break every block. The order of the 4 choice RT stimuli was randomized between participants and between task versions. A random intertrial interval of 2-4 s was used so that participants could not anticipate stimulus onset timing (Figure 3.1B). Reaction time and the identity of keys pressed were recorded on each trial.

After completion of the bimanual RT task, participants began the 4-finger bimanual rhythmic tapping task. Participants were first shown instructions which described the types of movements...
that would be required. Instructions were accompanied by videos demonstrating the difference between symmetric and asymmetric tapping movements. After viewing the instructions, participants were subject to an attention check to ensure the instructions were properly read. They were asked how many taps they will perform per block. Participants that failed the attention check were looped back to the instruction screen and asked to read the instructions again. Participants were given no instructions on what type of movement strategy to use.

Upon passing the attention check, participants performed 4 versions of the 4-finger bimanual rhythmic tapping task (spatial-symmetric/spatial asymmetric/symbolic symmetric/symbolic asymmetric). Before each task version, participants practiced the task before beginning testing. During practice, cartoon hands were added to the task stimuli to explicitly demonstrate which fingers should respond to each stimulus. Participants were not allowed to progress to the first block of the task until they demonstrated they were capable of performing an abbreviated block of tapping (6 taps total) at a movement frequency of 1 hertz (Hz) for the given tapping condition. For each task version, participants performed 12 blocks of 32 taps (Figure 3.1B). Before the start of each block, a fixation cross was displayed with a clock graphic counting down from 3 seconds to allow participants to anticipate the start of the block. There was an opportunity for a break after every block. The movement frequency of rhythmic tapping started at 1 Hz and increased by 0.2 Hz each block, finishing at 3.2 Hz. For each stimulus type condition, participants always performed the symmetric version of the task first directly followed by the asymmetric version of the task. The order of the stimulus type condition was counterbalanced between participants and mirrored the order used in the 4-finger bimanual RT task. Key press timing and the identity of key presses were recorded in each task block.
3.2.1.4 Data processing

Custom MATLAB scripts were used to process data downloaded from Gorilla. For the 4-finger bimanual choice RT task, the RT for each responding finger was processed for each trial, and the mean of these 2 values was calculated to create a bimanual RT value for each trial. For each task condition, RT values greater than three scaled median absolute deviations were defined as outliers and removed from further analysis to prevent positive RT distribution skew from affecting mean and standard deviation RT calculations\textsuperscript{178}. Trials were designated as an error if the wrong pair of keys were pressed, if no keys were pressed, if only one key was pressed, or if more than 2 keys were pressed. These trials were not included in mean and standard deviation RT calculations.

For the 4-finger bimanual rhythmic tapping task, an array of key press timing values was filtered from the raw data file for each finger and for each block. These arrays were used to calculate a relative phase value for each key press made. Relative phase values were calculated according to the formula:

\[
\phi = \frac{t_{\text{tar},i} - t_{\text{ref},i}}{t_{\text{ref},i+1} - t_{\text{ref},i}} \times 360 \ \text{[deg]}
\]

Here \( \phi \) equals relative phase, \( t_{\text{tar},i} \) equals the time of the \( i \)th tap of the target digit, \( t_{\text{ref},i} \) equals the time of the \( i \)th reference tap, and \( t_{\text{ref},i+1} \) equals the time of the \( i \)th +1 reference tap\textsuperscript{179,180} (see Figure 3.2 for a graphical example). For symmetric blocks, the reference finger was the same effector on the opposite hand, while for asymmetric trials, the reference finger was the opposite finger of the opposite hand. The first 4 key presses were excluded from relative phase calculations, to allow participants time to get in rhythm.
3.2.1.5 Dependent variables

4-finger bimanual choice reaction time task

- **Reaction time** – mean RT for symmetric and asymmetric trials was calculated for each stimulus type condition to provide a measure of RT magnitude. Error trials were not included in mean calculations.

- **Reaction time variability** – the standard deviation (SD) of RT for symmetric and asymmetric trials was calculated for each stimulus type condition. Error trials were not included in SD calculations.
• **Error rate** – the percentage of symmetric and asymmetric trials that were errors was calculated for each stimulus type condition.

• **Temporal coupling** – the mean absolute time between bimanual key presses for symmetric and asymmetric trials was calculated for each stimulus type condition.

**4-finger bimanual rhythmic tapping task**

• **Tapping timing accuracy** – The mean absolute timing error was calculated from relative phase to provide a measure of tapping accuracy. Absolute timing error was calculated for each key press by subtracting the relative phase of each key press from ideal relative phase (0°) and calculating the absolute value of the resultant integer. The mean of absolute timing error was then calculated for each block.

• **Tapping stability** – The SD of absolute error was calculated for each block to provide a measure of tapping stability.

• **Transition occurrence** – Blocks in which a transition key press occurred (i.e., a key press with absolute relative phase greater than 180°) were marked as having a transition occurrence (1), whereas blocks in which no transition key press occurred were marked as having no transition occurrence (0).

**3.2.1.6 Statistical analysis**

Statistical analyses were carried out in RStudio. Post hoc analyses were performed using Bonferroni’s correction for multiple comparisons where appropriate.

*Quality control*
Since the experimental tasks was delivered online and there was no experimenter present to supervise performance, it is possible that participants did not properly understand the tasks or were not engaging in the task in the desired manner (e.g., making minimal effort, not paying attention, or performing the tasks incorrectly). To account for this possibility, quality control criteria were applied to the data before statistical analysis. For the 4-finger bimanual choice RT task, participants were removed from analysis if they produced errors at a rate of 3 SDs above the group mean error rate. This led to the removal of 7 participants from the dataset (n = 234). For the 4-finger bimanual rhythmic tapping task, participants were removed from analysis if their observed movement frequency was 20% above or below the desired movement frequency of 32 taps per block on at least 12 out of 48 task blocks, or if the grand mean of their mean absolute timing error scores was above 60°. This led to the removal of 49 participants from the dataset (n = 192).

4-finger bimanual choice reaction time task

Before performing inferential statistics, mean RT, SD of RT, error rate, and temporal coupling values were assessed for skewness using the Shapiro-Wilks test. Results from these tests indicated that all variables were significantly positively skewed. To reduce the potential impact of skewness biasing results, linear mixed effect regression (LMER) analysis, rather than traditional F-tests, were used. LMER is robust against violations to distributional assumptions required by parametric tests. LMER also accounts for random variation due to participants, through modeling of multiple intercepts for each participant as a random effect, rather than a single mean intercept.

To test the effect of symbolic and spatial cueing stimuli on symmetric and asymmetric bimanual choice RT (Hypothesis 1), an LMER with fixed effects of STIMULUS TYPE (symbolic/spatial) and RESPONSE TYPE (symmetric/asymmetric) was used. Participant ID was included as a
random effect. Separate LMERs were performed for mean RT, SD of RT, error rate, and mean temporal coupling.

4-finger bimanual rhythmic tapping task

Before performing inferential statistics, mean absolute timing error and SD of absolute error values were assessed for skewness using the Shapiro-Wilks test. Results from these tests indicated that both variables were significantly positively skewed. To reduce the risk of data skewness biasing statistical models, observations that were deemed extreme outliers (i.e., 3 SDs above or below the mean) were removed from the dataset. The potential impact of skewness was also decreased by the decision to use LMER.

LMERs were performed to establish the moderating effects of within-subject fixed factors STIMULUS TYPE (symbolic/spatial) and RESPONSE PATTERN (symmetric/asymmetric) on the relationship between within-subject fixed continuous variable MOVEMENT FREQUENCY (1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2 Hz) and mean absolute error and the SD of absolute error (Hypothesis 2, Hypothesis 3). Participant ID was included as a random effect.

Visual inspection of group means indicated the potential for a non-linear exponential relationship between MOVEMENT FREQUENCY and both mean absolute error and the SD of absolute error. Therefore, for these dependent variables, 2 LMERs were performed, one in which dependent variable data were untransformed and a second in which dependent variable data were log transformed to capture potential exponential components within the data. Models were compared using the Akaike information criterion (AIC) to assess whether a linear or exponential fit was more appropriate for each dependent variable.
Given that transition occurrence was a dichotomous variable (0 or 1), logistic regression was used to model the probability of transition occurrence across the continuous factor of MOVEMENT FREQUENCY and across categorical factors STIMULUS TYPE and RESPONSE PATTERN. Logistic regression is a statistical technique used to model dichotomous outcome variables\(^\text{183}\). This is achieved by modeling the log odds of the outcome as a linear combination of the predictor variables.

3.2.2 Experiment 2

3.2.2.1 Participants

A total of 20 participants completed Experiment 2 (35.2 ± 11.3 years old; 14 female, 6 male; 16 right-handed, 4 left-handed). Twelve of these participants had previously participated in Experiment 1. The same inclusion criteria were applied as those used in Experiment 1. All protocols were approved by Behavioural Research Ethics’ Board at the University of British Columbia. Before participating, individuals provided written informed consent in accordance with the Declaration of Helsinki.

3.2.2.2 Experimental Design

The design of Experiment 2 was identical to Experiment 1, except participants only performed the 4-finger bimanual rhythmic tapping task in a laboratory setting. In addition, video of the participants hands was captured during task performance.

3.2.2.3 Procedure

The 4-finger bimanual rhythmic tapping task was performed as described in Experiment 1 on a desktop computer (iMac; Apple, CA) with a wired keyboard. Before participants began the task, a GoPro Hero 9 video camera (GoPro, CA) was placed below the computer monitor facing the
participant to record finger movements from a frontal perspective. The camera lens was set to “narrow mode”, video resolution was set to 1080p, and video frame rate was set to 120 frames per second.

### 3.2.2.4 Data processing

Task data downloaded from Gorilla was processed as described in experiment 1. An open source deep learning toolbox (DeepLabCut\(^{176}\)) was used to estimate the 2D coordinate of index and middle fingers on every frame of the collected videos. DeepLabCut facilitates the training of a deep neural network to identify features of interest within every frame of a video. Labels were placed at the midpoint on the edge of the fingernails of the index and middle fingers of each video frame selected for neural network training. Labelled video frame data from every participant were included in the training dataset. Each participant contributed 60 video frames: 20 from a slow pace block, 20 from a medium pace block, and 20 from a fast pace block. Training frames were selected using a k-mean clustering approach that automatically identified representative frames. A single human rater labelled each training frame. ResNet-50 – a pretrained Convolutional Neural Network for image classification – was used as the initial weights of the neural network. The deep neural network underwent 600,000 iterations of training. Every frame of collected video was then processed through the trained network, producing 2D coordinate data for each finger on every frame of video, as well as a probability value indicating the network’s confidence in the coordinate data for each frame. 2D coordinate data was smoothed across time with a gaussian kernel with a width of 3 frames.

Each participant’s 2D coordinate data was cut into task blocks using timestamp data acquired from Gorilla. Kinematic analyses focused on motion in the Y-plane of the 2D video, given that the majority of motion occurred in this dimension. To examine the effect of task conditions on relative
kinematics, Y-plane coordinate data from each block was normalized to the time of the 1 Hz task blocks, i.e., 32 s or 3840 frames. This was achieved by interpolating Y-coordinate data for each block into the length of the first task block using the “interp2” function in MATLAB. A linear function was used to join interpolated data points. After interpolation, the “findpeaks” function in MATLAB was used to locate the relative timepoints in each block when peaks in the Y-coordinates occurred for each finger (i.e., the relative timepoints when key presses occurred). Using these indices, an average shape of finger trajectories adjacent to key presses was calculated for each finger and for each block, by calculating the mean of Y-coordinate data in a 120 frame window around the peak indices (see Figure 3.6A for an example). Peaks in which there were at least 12 frames where the DeepLabCut probability tracking rating were below 0.95 were not included in mean calculations, to reduce the effect of inconsistent tracking on trajectory shape.

3.2.2.5 Dependent variables

The dependent variables used to assess performance on the 4-finger bimanual rhythmic tapping task were the same as those described in Experiment 1. In addition, the following dependent variables were derived from mean peak shapes to test for changes in relative kinematics across task conditions:

- **Mean relative speed**: the relative speed of each finger in a time window of 120 frames around each peak was calculated by differentiating Y-coordinates with respect to time using the “diff” function in MATLAB. The mean of the resulting array of speed values was calculated for each tracked finger. The mean of these four finger values was calculated to give a single mean relative speed value for each task block.
• **Mean relative acceleration**: the relative acceleration of each finger in a time window of 120 frames around each peak was calculated by differentiating peak speed values with respect to time using the “diff” function in MATLAB. The mean of the resulting array of acceleration values was calculated for each tracked finger. The mean of these 4 values was calculated to give a single mean relative acceleration value for each task block.

### 3.2.2.6 Statistical analysis

Statistical analyses were carried out in RStudio. Statistical approaches to analyzing finger bimanual rhythmic tapping task performance data were the same as those described for experiment 1, except that LMERs were run only on log transformed mean absolute timing error and SD of absolute timing error values.

LMERs were performed to establish the effects of within-subject fixed factors **STIMULUS TYPE** (symbolic/spatial) and **RESPONSE PATTERN** (symmetric/asymmetric) and within-subject fixed continuous variable **MOVEMENT FREQUENCY** (1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2 Hz) on relative range of motion, relative speed, and relative acceleration (Hypothesis 4). Participant ID was included as a random effect.

Visual inspection of group means indicated the potential for a non-linear exponential relationship between **MOVEMENT FREQUENCY** and relative acceleration. Therefore, for relative acceleration two LMERs were performed, one in which dependent variable data were untransformed and a second in which dependent variable data were log transformed to capture potential exponential components within the data. Models were compared using the AIC to assess whether a linear or exponential fit was more appropriate for each dependent variable.
3.3 Results

3.3.1 Experiment 1

3.3.1.1 4-finger bimanual choice reaction time task

Mean reaction time – significant main effects of STIMULUS TYPE ($\beta = 188$, 95% confidence interval [CI; 173 202], $p < 0.001$, Cohen’s $d = 1.95$) and RESPONSE TYPE ($\beta = 47$, 95% CI [36 58], $p < 0.001$, $d = 0.83$) were found, as well as a significant STIMULUS TYPE x RESPONSE TYPE interaction ($\beta = 59$, 95% CI [45 71], $p < 0.001$; Figure 3.3). Estimated marginal means were calculated to further interrogate the interaction effect. A significant difference in mean RT was found for trials cued with symbolic stimuli and spatial stimuli, but the difference in mean RT was greater when trials were symbolically cued ($\mu = 105 \pm 6$, $p < 0.0001$, $d = 1$) compared to those that were spatially cued ($\mu = 47 \pm 6$, $p < 0.0001$, $d = 0.75$).

Standard deviation of reaction time – significant main effects of STIMULUS TYPE ($\beta = 60$, 95% CI [51 68], $p < 0.001$, $d = 1.15$) and RESPONSE TYPE ($\beta = 15$, 95% CI [8 21], $p < 0.001$, $d = 0.57$) were again shown, as well as a significant STIMULUS TYPE x RESPONSE TYPE interaction ($\beta = 31$, 95% CI [23 40], $p < 0.001$, Figure 3.3). Estimated marginal means were calculated to further interrogate the interaction effect. A significant difference in SD of mean RT was found for trials cued with symbolic stimuli and spatial stimuli, but the difference in mean RT was greater when trials were symbolically cued ($\mu = 47 \pm 3$, $p < 0.0001$, $d = 0.74$) compared to those that were spatially cued ($\mu = 15 \pm 3$, $p < 0.0001$, $d = 0.41$).

Error rate – Higher error rates for asymmetric movements were demonstrated by a significant main effect for and RESPONSE TYPE ($\beta = 1$, 95% CI [1 2], $p < 0.001$, $d = 0.33$). There was no
significant main effect of STIMULUS TYPE or a significant STIMULUS TYPE x RESPONSE TYPE interaction effect for error rate.

*Temporal coupling* – Asymmetric responses were associated with a decrease in temporal coupling, as evidenced by significant main effect of RESPONSE TYPE ($\beta = 3$, 95% CI [2 3], $p < 0.001$, $d = 0.31$). There was no significant main effect of STIMULUS TYPE or STIMULUS TYPE x RESPONSE TYPE interaction effect for temporal coupling.

![Figure 3.3. Bimanual 4-choice reaction time task results.](image)

Mean (left) and standard deviation (right) of reaction time (RT) values for symmetric and asymmetric response types collected in the spatially cued and symbolically cued bimanual 4-choice reaction time tasks. RT was significantly faster when movements were cued with spatially congruent cues and when movements were asymmetric. Furthermore, the difference in symmetric and asymmetric movements for the magnitude and variability of RT was significantly greater when movement was cued with abstract symbolic cues.
3.3.1.2 4-finger bimanual rhythmic tapping task

*Mean absolute timing error* – Because the LMER fit to log transformed data produced a model with lower AIC (6368) compared to the LMER fit to untransformed data (72534), suggesting an exponential fit, I report results based on these transformed data. Mean absolute error increased exponentially with increasing movement frequency for all task conditions, as was evidenced by a main effect of MOVEMENT FREQUENCY ($\beta = 0.74$, 95% CI [0.71 0.78], $p < 0.001$), as shown in Figure 3.4. The detrimental effects of symbolic cues compared to spatial cues on mean absolute error increased exponentially with movement frequency, as was evidenced by a significant MOVEMENT FREQUENCY x STIMULUS TYPE interaction ($\beta = 0.19$, 95% CI [0.15 0.24], $p < 0.001$). Similarly, the detrimental effects of asymmetric patterns compared to symmetric patterns on mean absolute error increased exponentially with movement frequency, as was evidenced by a significant MOVEMENT FREQUENCY x RESPONSE PATTERN interaction ($\beta = 0.12$, 95% CI [0.08 0.17], $p < 0.001$).

*Standard deviation of absolute timing error* - Based on the LMER fit to log transformed data; the SD of absolute timing error increased exponentially with increasing movement frequency for all task conditions, as was evidenced by a main effect of MOVEMENT FREQUENCY ($\beta = 0.96$, 95% CI [0.91 1.01], $p < 0.001$). The detrimental effects of symbolic cues compared to spatial cues on the SD of absolute error increased exponentially with movement frequency, as was evidenced by a significant MOVEMENT FREQUENCY x STIMULUS TYPE interaction ($\beta = 0.22$, 95% CI [0.15 0.29], $p < 0.001$). Similarly, the detrimental effects of asymmetric patterns compared to symmetric patterns on the SD of absolute error increased with movement frequency, as was evidenced by a significant MOVEMENT FREQUENCY x RESPONSE PATTERN interaction ($\beta = 0.16$, 95% CI [0.09 0.23], $p < 0.001$). The relative detrimental effects of asymmetric responses
patterns compared to symmetric response patterns was greater for spatially cued movements compared to symbolically cued movements, demonstrated by a STIMULUS TYPE x RESPONSE PATTERN interaction ($\beta = 0.4$, 95% CI [0.18 0.62], $p < 0.001$). All 2-way interaction effects should be considered in light of a 3-way MOVEMENT FREQUENCY x STIMULUS TYPE x RESPONSE PATTERN interaction, which indicated that the relative detrimental effects of asymmetric patterns compared to symmetric patterns on the SD of absolute timing error with increasing frequency was greater for spatially cued movements than for symbolically cued movements ($\beta = -0.25$, 95% CI [-0.35 -0.15], $p < 0.001$) (Figure 3.4).

Transition occurrence – Logistic regression analysis revealed that the probability of a transition occurrence increased with increases in MOVEMENT FREQUENCY, as demonstrated by a significant effect of MOVEMENT TYPE ($\beta = 1.12$, 95% CI [0.95 1.28], $p < 0.001$). Symbolically cued movements were associated with a higher probability of transition occurrences than spatially cued movements, as demonstrated by a main effect of STIMULUS TYPE ($\beta = 0.77$, 95% CI [0.25 1.29], $p <0.005$). Similarly, symmetric patterns were associated with a lower probability of transition occurrences than asymmetric movement patterns, as demonstrated by a main effect of RESPONSE PATTERN ($\beta = -0.94$, 95% CI [-1.61 -0.27], $p <0.01$) (Figure 3.4).
Figure 3.4. Bimanual 4-finger rhythmic tapping task results.

Predicted task performance values in the rhythmic bimanual tapping task derived from linear mixed effect and logistic regressions. Lines represent predicted values and shaded regions represent 95% confidence intervals. An exponential increase in the mean absolute error of relative phase (left) and standard deviation of absolute error of relative phase (middle) was found. Effects of cue type (spatial vs. symbolic) and response type (symmetric vs. asymmetric) were low at slower movement frequencies and high at faster movement frequencies. The probability of transition out of the required response pattern also increased with movement frequency (right), but the effects of response type and cue type were consistent across movement frequencies.
3.3.2 Experiment 2

Analysis of key press data in Experiment 2 largely replicated those found in Experiment 1. There were some exceptions, likely driven by decreased statistical power.

Mean absolute timing error – As shown in Figure 3.5, results from Experiment 1 were replicated. A main effect of MOVEMENT FREQUENCY (β = 0.74, 95% CI [0.71 0.78], \( p < 0.001 \)), as well as significant MOVEMENT FREQUENCY x STIMULUS TYPE (β = 0.19, 95% CI [0.15 0.24], \( p < 0.001 \)) and MOVEMENT FREQUENCY x RESPONSE PATTERN (β = 0.12, 95% CI [0.08 0.17], \( p < 0.001 \)) interactions were found. However, the 3-way MOVEMENT FREQUENCY x STIMULUS TYPE x RESPONSE PATTERN interaction effect was not significant (β = -0.14, 95% CI [-0.31 0.03], \( p = 0.12 \)).

SD of absolute timing error – Results from experiment 1 were replicated. A significant main effect of MOVEMENT FREQUENCY (β = 0.76, 95% CI [0.58 0.93], \( p < 0.001 \)) as well as significant MOVEMENT FREQUENCY x RESPONSE PATTERN interaction (β = 0.25, 95% CI [0.03 0.48], \( p < 0.001 \)) were found. However, the MOVEMENT FREQUENCY x STIMULUS TYPE interaction was not significant (β = 0.22, 95% CI [-0.01 0.44], \( p = 0.059 \)). Furthermore, the 3-way MOVEMENT FREQUENCY x STIMULUS TYPE x RESPONSE PATTERN interaction was not significant (β = -0.2, 95% CI [-0.51 0.12], \( p = 0.22 \)).

Transition occurrence – Results from experiment 1 were replicated with one notable exception. Significant main effects of MOVEMENT FREQUENCY (β = 1.37, 95% CI [0.87 1.91], \( p < 0.001 \)) and STIMULUS TYPE (β = 1.71, 95% CI [0.15 3.33], \( p < 0.05 \)) were found. However, the main effect of RESPONSE PATTERN was not significant, although it did approach significance (β = -0.75, 95% CI [-3.04 1.37], \( p = 0.11 \)).
**Relative speed** – As shown in Figure 3.6B the relative speed of fingers increased with increasing movement frequency, \( \beta = 0.18, 95\% \ CI [0.15 \ 0.22], p<0.001. \)

**Relative acceleration** – The LMER fit to untransformed data produced a model with lower AIC (-3881) compared to the LMER fit to log transformed data (98), suggesting a linear fit. Therefore, I report results from the untransformed model. Relative acceleration of fingers decreased with increasing movement frequency as was evidenced by a main effect of MOVEMENT FREQUENCY \( \beta = -0.6, 95\% \ CI [-0.6 \ -0.5], p<0.001. \) Relative acceleration decreased at a slightly lower rate with increasing movement frequencies for symbolically cued blocks compared to spatially cued blocks, as was evidenced by a significant MOVEMENT FREQUENCY x STIMULUS TYPE interaction \( \beta = 0.1, 95\% \ CI [0.1], p<0.05 \) (Figure 3.6B).
Figure 3.6. Increasing movement frequency was associated with a shift in movement quality.

(A) Example of the average trajectory of the left middle finger in time windows around key presses in a single participant across movement frequency conditions. Trajectories were normalized by time to enable comparison of relative kinematics. (B) Mean relative speed (left) and mean relative acceleration (right) of all finger trajectories across movement frequency conditions. The mean relative speed of finger trajectories linearly increased with increasing movement frequency, while the mean relative acceleration linearly decreased with increasing movement frequency. Movements cued with spatially congruent stimuli showed a greater decrease in relative acceleration with increasing movement frequency. This indicates that the quality of finger kinematics transitioned from repeated discrete movements to smooth continuous movement as movement frequency increased.
3.4 Discussion

The current study demonstrated that the perceptual quality of visual stimuli used to cue movement timing has powerful effects on the control of discrete and rhythmic bimanual finger movements. In a bimanual 4-choice RT paradigm, actions cued with spatially congruent stimuli resulted in faster and less variable RTs compared to actions cued with abstract symbolic stimuli. Furthermore, the difference in RT between asymmetric responses versus symmetric responses was significantly reduced in the spatially congruent condition (Figure 3.3). These results largely mirror what has been found in bimanual reaching paradigms\textsuperscript{18,19,21}, providing evidence that crosstalk within response selection processes is the dominant source of interference constraining the planning of discrete asymmetric bimanual movements. As predicted, rhythmic bimanual movements became less accurate and less stable with increasing movement frequency; this relationship was best modeled as a non-linear exponential curve (Figure 3.4). Asymmetric movement patterns were less accurate and less stable than symmetric patterns. Similarly, when rhythmic movement frequency was cued with spatially congruent stimuli, accuracy and stability increased compared to conditions in which movement frequency was cued with abstract symbolic stimuli. The magnitude of these effects was minimal at lower frequencies but large at higher frequencies. Surprisingly, asymmetry effects were consistent across cueing conditions, suggesting that spatially congruent stimuli do not have the capacity to reduce asymmetry costs to temporal coupling of rhythmic bimanual movements. Increases in movement frequency were associated with increased mean relative speed and a decreased mean relative acceleration of finger trajectories, suggesting a shift in movement quality (Figure 3.5). A shift in movement quality might explain the non-linear fit between movement frequency and rhythmic task performance as well as the increase in magnitude of effect of response pattern type and movement cueing type across movement frequency.
3.4.1 Spatially congruent cues reduce planning costs for asymmetric bimanual finger movements

As predicted, in a bimanual 4-choice RT task, responses cued with spatially congruent stimuli were faster and less variable than those cued by abstract symbolic stimuli, while symmetric responses were faster and less variable that asymmetric responses. Furthermore, the difference in RT from symmetric to asymmetric responses was greater for movements cued with abstract symbolic stimuli (Figure 3.3). These results extend findings from bimanual reaching paradigms and broadly supports the central processing hypothesis, which proposes that constraints in the production of discrete asymmetric bimanual movements stem from interference within central response selection processes that translate task relevant stimuli into action18-21. However, our results were not in complete agreement with bimanual reaching paradigms as there were significant differences between symmetric and asymmetric RTs in the spatially congruent cue condition. This discrepancy is most likely due to the increased statistical power in the current study, which had a much larger number of participants than previous bimanual reaching experiments and therefore better positioned to detect a small effect. The discrepancy with bimanual reaching experiments could also be due to differences in movement time between finger press and reaching movements. In reaching, movement time is on the scale of 100s of milliseconds, which might allow participants to extend motor planning into movement execution and make corrections online. Such an outcome is not possible in the task used in this study, because participants had to keep their fingers resting over the relevant keys during preparation which kept movement times very short. Another possible driver of discrepancy with bimanual reaching experiments is that the perceptual quality of stimuli contributed to discrepancies between studies using finger press vs. reaching movements. The spatially congruent stimuli used in the current study were presented at a ~90-degree angle on a
computer monitor facing the participant. In previous bimanual reaching studies, LEDs within the reaching space directly defined the required bimanual movement. It is possible that presenting spatially congruent cues at a ~90-degree angle may have necessitated a mental rotation process which introduced a source interference that slowed down asymmetric responses in the spatially congruent condition.

3.4.2 Increasing movement frequency drives a change in rhythmic movement quality from a series of discrete movements to continuous movement

When assessing how various task manipulations affect rhythmic bimanual task performance, it is important to concurrently track how the movement quality changes with movement frequency, because changes in movement quality can alter coordination principles\(^9\). When the kinematics of rhythmic bimanual movements comprise a series of discrete movements, temporal control is achieved through “event timing” representations\(^{40–43}\). In this mode, spatiotemporal coupling is achieved by repeating movements at a set time interval, likely computed by the cerebellum\(^{49,50}\). In contrast, when kinematics comprise smooth continuous movement, temporal control is achieved via “emergent timing” representations, where spatiotemporal coupling emerges via the control of higher order kinematic parameter such as speed, likely computed by transcallosal cortical networks\(^{40–43,45,48}\).

I predicted that increasing movement frequency would necessitate a qualitative shift in kinematics from repeated discrete movements to smooth continuous movement. To investigate this hypothesis, I captured full spatiotemporal kinematics of task-relevant fingers during bimanual rhythmic task performance by applying deep learning algorithms to video recordings of finger movements. To quantify changes in kinematic quality, the mean relative speed and acceleration of finger trajectories during each task block was calculated. This analysis showed that the relative
speed and relative acceleration of finger trajectories changed across movement frequency in a manner consistent with a shift from repeated discrete to continuous movement (Figure 3.5). Mean relative speed linearly increased with movement frequency, as the relative amount of time fingers spent resting on keys decreased, while mean relative acceleration linearly decreased with increasing movement frequency, as finger trajectories became smoother and more continuous with less dramatic shifts in relative speed. This change in movement quality provides important context with which to interpret temporal coupling results.

3.4.3 Accuracy and stability of bimanual rhythmic finger tapping decreased exponentially with increasing movement frequency

As predicted, in Experiment 1 the accuracy and stability of bimanual tapping and the probability of transition out of the desired movement pattern increased with movement frequency across all task conditions. The relationship between movement frequency and task performance variables were best modelled as exponential curves, with increases in movement frequency having a far greater effect on task performance variables at higher frequencies compared to lower frequencies (Figure 3.4). Given the finding that the quality of movement changed with increasing movement frequency, it seems likely that the exponential component within the data may have been driven by a change in coordination mode. At lower frequencies, participants likely favoured a strategy of rapid discrete bimanual movements separated by pauses, with pause length defined according to an event timing representation. Since results from the bimanual 4-choice RT task demonstrated that temporal coupling of discrete bimanual movements was high (~11 ms on average), it follows that accuracy and stability of temporal coupling of bimanual rhythmic movements would be high when using a repeated discrete movement strategy. The use of a repeated discrete movement strategy could also explain how increases in movement frequency between low frequencies had a
minimal effect on task performance. All that needed to be updated between blocks when using a repeated discrete movement strategy was the magnitude of the interval within the event timing representation, which is easily achieved by healthy participants when the intervals are regular\textsuperscript{184,185}.

The exponential decrease in accuracy and stability shown in predicted values after approximately 2 Hz could be explained by a shift in strategy by participants towards smooth continuous movement. Within this coordination mode, instead of controlling movements according to an event timing representation, participants likely had to rely on a dynamic representation of speed derived from the flickering stimulus that denoted movement frequency. Given the relatively large decreases in task accuracy after this point, it appears that such a method of control was less effective for accurately coupling bimanual movements, and less capable of managing increases in target speed; increases in movement frequency was associated with large detriments to task performance.

3.4.4 Effects of response symmetry and spatial congruence on rhythmic bimanual tapping stability emerge at higher movement frequencies

Changing the required response patterns from symmetric to asymmetric had the hypothesized effect on task performance; with symmetric patterns reducing the slope defining the relationship between movement frequency and tapping accuracy and stability, compared to asymmetric patterns. Whether the frequency of rhythmic movements was cued with repeating spatially congruent stimuli or abstract symbolic stimuli had a near identical effect on task performance. Asymmetric response patterns and abstract symbolic cues were consistently associated with a greater probability of transition out of the desired response pattern across all movement frequencies. In contrast, the magnitude of the effects of response pattern type and timing cue
stimulus type on bimanual coupling accuracy and stability was much greater at higher frequencies compared to lower frequencies.

Unexpectedly, the impact of response pattern symmetry on task performance for rhythmic movements was similar across cue types. Based on the central processing hypothesis, I predicted that spatially congruent cues would reduce the impact of asymmetric relative to symmetric response requirements on spatiotemporal coupling during the performance of rhythmic bimanual finger movements. However, asymmetric response patterns were associated with similar detriments to performance across spatially congruent and abstract symbolic cueing conditions. One exception to this was in measures of stability of bimanual tapping. Here, an interaction effect was found between response pattern type and cue type. However, the direction of this interaction effect was the opposite to what was predicted, with symmetric and asymmetric instability increasing at similar rate across movement frequency for abstract symbolically cued response patterns but not spatially congruent cued response patterns.

Given that increasing movement frequency likely induced a qualitative shift in movement kinematics from repeated discrete movements to smooth continuous movement, it seems important to consider the increasing magnitude of effect of response pattern type and cue type across movement frequency. Since participants may have adopted a strategy of rapid discrete bimanual movements separated by pauses at lower frequencies, the task may have effectively mirrored the bimanual 4-choice RT paradigm. This could explain why cue type and response type had a relatively low impact on task stability at slow frequencies; results from the 4-choice bimanual RT task indicate that discrete bimanual movements have a high degree of temporal coupling regardless of cue (no difference) or response type (~3 ms).
As movement frequency was gradually increased, at some point, participants changed movement strategy from repeated discrete bimanual movements to smooth continuous bimanual movements. Once a continuous movement strategy was selected, the basis for temporal control likely switched to from an internal representation of the time interval between events to an internal representation of speed⁹. Therefore, the capacity to accurately perceive speed from the flickering visual stimuli likely became a key component of task success.

Psychophysical studies of speed and flicker perception have demonstrated that both the contrast ratio¹⁸⁶–¹⁹¹ and spatial frequency¹⁹²–¹⁹⁵ of flickering visual stimuli can affect the accuracy of perceived speed estimates. While the quality of the visual stimuli used in such studies are difficult to directly compare with the stimuli used to cue movement timing in the current study, the spatially congruent and abstract symbolic stimuli used to cue rhythmic movements did differ in terms of their contrast ratio and spatial frequency. Given the increased stability found in spatially congruent conditions, I assume that the combination of perceptual qualities of the spatially congruent stimuli facilitated a more accurate representation of speed over the abstract symbolic stimuli. Other researchers have shown that increasing cognitive load decreases the capacity to accurately perceive the presence of a flickering visual stimulus¹⁹⁶. Therefore, it is possible that the greater cognitive load associated with the repeated translation of abstract symbolic stimuli had a compounding effect on the accuracy of perceived speed estimations in the abstract symbolic conditions, contributing to the decreased stability. Such a scenario could explain why stability decreases at a similar rate regardless of asymmetry in the abstract symbolic condition but not in the spatially congruent condition. The benefits of symmetric patterns on stability may have been overwhelmed by the compounding effects of increased cognitive load on an already noisier representation of speed. The compounding effect of cognitive load could also explain the ability to reduce relative acceleration
with increasing movement frequency in the spatially congruent cueing condition compared to the abstract symbolic condition. A more reliable representation of speed may have allowed participants to more effectively smoothen bimanual rhythmic movements in the spatially congruent condition.

The finding that response pattern symmetry had a greater effect on temporal coupling at higher movement frequencies implies that the degree of interference in processes responsible for implementing asymmetric finger movements were also much greater when the quality of movement was continuous as opposed to discrete. The idea the movement quality drives coordination principles is important because while symmetric rhythmic bimanual movements have long been established as more stable than asymmetric rhythmic bimanual movements\textsuperscript{1,2,30,31}, previous investigations linking brain activity to bimanual finger tapping behaviour have not always explicitly considered movement strategy in putative brain-behaviour relationships\textsuperscript{4,6,8}. Detriments to temporal coupling of continuous bimanual finger movements caused by response asymmetry is probably due to interference within motor processes responsible for implementing continuous asymmetric bimanual spatial trajectories. This form of interference seems to be independent to the interference caused by varying the perceptual qualities of movement timing cue, since performance detriments due to response pattern symmetry were mostly constant across spatially congruent and abstract symbolic cue types.

The neural source of response asymmetry interference may arise from transcallosal connections between the primary motor cortices, which have been shown to cause crossed facilitation between homologous effector representations under some movement conditions\textsuperscript{173}. In the case of asymmetric movements, where homologous muscles are active on asymmetric schedules, this could cause interference within effector representations which drives instability and inaccuracy in bimanual coupling. Another candidate is within systems responsible for defining spatial codes for
movement. When these spatial codes are not perceptually symmetrical between hands, it may lead to interference between codes that reduces accuracy and stability of bimanual coupling. Studies with callosectomy patients indicate that such interference is probably localized within transcallosal circuits connecting the parietal cortices, since the capacity to couple bimanual reach directions is abolished only after callosectomy to the posterior third of the corpus callosum. Our data is not capable of distinguishing between these candidate neural sources of interference, since the asymmetric tapping conditions were both perceptually asymmetric and physically asymmetric in terms of muscle activation patterns. However, in a previous bimanual finger tapping task, perceptually symmetric movements were consistently more accurate than perceptually asymmetric movements, even when physically they were asymmetric. The finding that perceptual symmetry and not physical symmetry was the driver of the effect suggests that interference within systems representing movement at the level of spatial codes is the dominant source of interference contributing to detriments in tapping performance.

3.4.5 Limitations

One limitation of the current study is that experiment 1 was conducted via online platforms, with participants performing the behavioural tasks at home on their own computers. Delivering the experiment via online platforms may have introduced uncontrolled environments with distracting background elements for some participants, which could have affected their performance. In addition, without the active supervision of an experimenter, participants may not have been motivated to perform the tasks to their best ability. These factors likely introduced noise into the dataset. However, the use of online platforms may have concurrently helped to mitigate these issues, since it facilitated the collection of a large sample size which reduced the impact of noise on results. The finding that results from Experiment 1 were largely replicated in Experiment 2,
which took place in a controlled laboratory setting with a supervising experimenter, helps to further assuage fears on the validity of behavioural data collected via online platforms. Another limitation of the current study is that concurrent measures of unimanual task performance were not collected. This was driven by a desire to constrain the experiment time limit to 30 minutes to ensure participants gave full effort throughout the online session. The lack of a unimanual choice RT task or unimanual rhythmic tapping tasks limits our ability to assert whether the cognitive-perceptual constraints identified in this study generalize to unimanual movements or are specific to bimanual control. Future investigations could address this by additionally collecting unimanual versions of the described tasks. Finally, while I have discussed the likelihood that a change in the quality of movement (from discrete to continuous), alters the effect of stimulus type and response type on the stability of rhythmic bimanual finger movements, I cannot claim this relationship is causal, based on the data collected in this study alone. Future studies could better parse out causality by explicitly asking participants to adopt a repeated discrete movement strategy or a continuous movement strategy and examining the effect of these strategies on temporal coupling across a range of movement frequencies.

3.4.6 Conclusion

Bimanual coordination is a complex phenomenon. Previous attempts to unify behavioural findings under a singular set of principles or map bimanual control to a specific set of brain regions have not fully appreciated the full diversity of the human bimanual repertoire and how small changes in the demands of tasks used to measure bimanual coordination can radically alter the underlying coordination principles at play. Therefore, calls to conceive of bimanual coordination as a phenomenon governed by a “coalition of constraints”\(^51-53\), with task demands modulating the relative contributions of given constraints and how they interact, seems to be a reasonable
perspective. Such a view underscores that coordination principles will not always generalize between different movement types. This is highlighted in the current set of experiments, where I showed that increasing movement frequency can change the movement strategy selected by participants, which in turn appeared to transform the effect of response pattern asymmetry, as well as the effects of stimulus cue type on movement timing. Consideration of how changes in task demands prompt changes in movement strategies may be key to future efforts at uncovering principles of bimanual coordination and associated attempts at localizing the neural substrate which underpins their implementation.

3.5 Bridging statement

The current study demonstrates that the perceptual quality of the stimulus used to cue movement frequency and whether response patterns are symmetric or asymmetric have equal effects on the stability of rhythmic finger tapping behaviours. This creates a platform to directly test whether right PMd is specifically involved in controlling asymmetric rhythmic bimanual finger movements, or more generally involved in managing cognitive load during rhythmic task performance, because it provides symmetric and asymmetric tapping conditions which engender varying degrees of cognitive load. In the next chapter, I will directly test these competing hypotheses by delivering the described tasks after disruption of right PMd with repetitive TMS.
4 Disruption of right PMd activity with repetitive transcranial magnetic stimulation has no effect on the stability of rhythmic bimanual finger movements.

4.1 Introduction

Previous research employing transcranial magnetic stimulation (TMS) suggests that the dorsal premotor cortex (PMd) plays an important role in the control of rhythmic asymmetric bimanual movements. The application of single pulses of TMS over right PMd elicits greater rates of switching from asymmetric to symmetric bimanual rhythmic 2-finger tapping patterns as compared to stimulation over other cortical regions, including left PMd, bilateral supplementary motor area (SMA), and bilateral somatosensory cortex (S1). This effect has also been shown in a 4-finger bimanual rhythmic tapping paradigm, with significant rates of switching from asymmetric to symmetric patterns observed after single pulse TMS over left and right PMd compared to a sham stimulation condition. Studies employing dual coil TMS to index interhemispheric inhibition (IHI) between PMd and the primary motor cortex (M1) further demonstrate that PMd-M1 IHI is modulated during preparation of asymmetric but not symmetric bimanual movements. Furthermore, individual differences in the capacity to maintain asymmetric bimanual rhythmic finger tapping patterns and asymmetric bimanual finger circling movements correlates with the degree of modulation of PMd-M1 IHI during movement preparation. Taken together, these studies suggest that PMd may play a specialized role in asymmetric bimanual control, possibly via direct intercortical connections with corticospinal neurons in M1.
Despite the links between PMd and asymmetric rhythmic bimanual control, the specific function of PMd in these behavioural contexts remains elusive. While it is tempting to speculate that PMd directly programs asymmetric bimanual movements, the potential mediating role of cognitive load must also be considered. Besides the difference in kinematics, asymmetric task demands incur an increased cognitive load compared to symmetric task demands. For example, asymmetric bimanual circling movements are more destabilized compared to symmetric movements when individuals are required to simultaneously perform a secondary task that taxes general cognitive resources\(^{16,39}\). Similarly, the stability of asymmetric bimanual circling movements is significantly increased when movement frequency is communicated with spatially congruent visual stimuli compared to spatially incongruent stimuli\(^{14}\). Spatial congruency is associated with decreased cognitive load in reaction time (RT) paradigms\(^{18–21}\). Findings from Chapter 3 also indicate that the beneficial effect of spatially congruent stimuli on task accuracy generalises to bimanual rhythmic finger tapping movements. Relatedly, when switching from symmetric to asymmetric bimanual circling patterns, error rates are decreased when the stimuli used to cue switches are spatially congruent\(^{15}\).

This evidence clearly indicates that across many task contexts, asymmetric task demands incur an increased demand on cognitive resources compared to symmetric task demands. This raises the possibility that the relationship between PMd and asymmetric bimanual control emerges not because PMd is specifically tasked with programming asymmetric bimanual movements, but because PMd has a general role in managing cognitive load, with downstream consequences for asymmetric bimanual control. Conceptualizing the function of PMd as a manager of cognitive load could help to explain why activity in the region increases across a broad range of behaviours and correlates with such a wide array of movement parameters\(^{11,13,54}\).
To effectively probe the role of increased cognitive load in the relationship between PMd and asymmetric bimanual control, additional experimental manipulations beyond switching the task demands from symmetric to asymmetric are required. In chapter 3, I demonstrated that performing rhythmic bimanual finger taps in time with spatially congruent stimuli significantly increases the accuracy and stability of temporal coupling when compared to abstract symbolic stimuli, for symmetric and asymmetric tapping patterns. Indeed, the degree of improvement at high frequencies (2-3.2 hertz [Hz]) was similar to that observed when changing the required response pattern from asymmetric to symmetric. Therefore, varying how the target movement frequency of bimanual rhythmic movements is communicated by different types of flickering stimuli and degrees of cognitive load may provide a platform to test whether PMd is: (1) specifically tasked with programming of asymmetric movements or (2) generally tasked with managing cognitive load (Figure 4.1). If model (1) is true, then TMS induced disruptions to PMd activity would be expected to result in detriments to performance exclusively in asymmetric tasks, regardless of the stimulus type used to cue movement frequency. If model (2) is true, then detriments to performance would be expected to scale with the degree of load engendered by the task conditions. Specifically, symbolically cued asymmetric movements (high load-high load) would be expected to be most affected, symbolically cued symmetric (high load-low load) and spatially cued asymmetric (low load-high load) movements to be second most affected, and spatially cued symmetric (low load-low load) movements to be least affected.
**Figure 4.1. Hypotheses representation.**

(A) Changes in response type from symmetric to asymmetric and changes in stimulus type from spatial to symbolic are associated with increases in cognitive load during rhythmic bimanual finger tapping. These factors linearly combine to create 4 task conditions with varying degrees of cognitive load. Expressing task conditions in cognitive load space demonstrates the capacity to disentangle response asymmetry from general increases in cognitive load across response types. (B) Hypothesized outcomes of application of 1 hertz rTMS to rhythmic bimanual finger tapping stability if right PMd is responsible for programming asymmetric response patterns (top), or if right PMd is responsible for managing cognitive load (bottom).
Another possible model to be considered is that (3) PMd is one node in a broader cortical network responsible for managing cognitive load, with the network capable of compensating for neuronal challenge to any single region. Support for this notion comes from a study which investigated the role of left PMd in decision making using functional magnetic resonance imaging (fMRI) and rTMS\textsuperscript{166}. It was found that 1 Hz repetitive TMS (rTMS) over left PMd had no effect on unimanual choice RT, but the delivery of rTMS was associated with an increase in blood-oxygen-level-dependent signal in a network of regions interconnected with left PMd, including right PMd, right M1, left SMA, and bilateral cingulate motor area (CMA). This suggests that although left PMd may play an important role in decision making processes, it does so as part of a broader network with built-in redundancies. More generally, it speaks against the notion of a regional focus when mapping brain function to behaviour. The capacity for compensation may have been masked in previous studies linking PMd to asymmetric bimanual rhythmic finger movements since perturbing PMd with single pulses of TMS on a trial-to-trial basis may not allow sufficient time for compensatory mechanisms to emerge, or because application of single pulses of TMS during behaviour transiently perturbs the broader cortical network, and not just PMd alone. In this scenario, TMS induced disruptions to PMd activity would be expected to result in no detriments to bimanual rhythmic finger tapping performance.

To test these competing predictions, I assessed performance on a 4-finger bimanual rhythmic finger tapping tasks after delivery of 1 Hz rTMS over right PMd (Figure 4.2). One Hz rTMS typically results in reduced excitability of the targeted cortical region\textsuperscript{111–114} and provides a platform to probe the specific contributions of the targeted region to a behaviour of interest. Task performance after 1 Hz rTMS was compared to task performance after sham stimulation, which mimicked the sound, sensation, and duration of 1 Hz rTMS. Right PMd was chosen as the rTMS target as detriments to
asymmetric bimanual rhythmic tapping have been shown to be stronger after disruption of right PMd compared to left PMd\(^4,6\), and increased brain activation has been shown in the right hemisphere during performance of asymmetric bimanual movements\(^5,197\). I predicted that the accuracy and stability of bimanual rhythmic tapping would be decreased after real rTMS compared to sham rTMS (Hypothesis 1A). I further predicted that results would follow the pattern described in model (2) (**Figure 4.1B**), with detriments to performance scaling with the degree of cognitive load engendered by specific task conditions (Hypothesis 1B). To assess the extent to which the hypothesized effect was constrained to rhythmic versus discrete movements, participants also performed a 4-choice bimanual RT task. Participants were required to perform symmetric or asymmetric responses in response to spatially congruent and abstract symbolic imperative cues. It was predicted that the hypothesized effects of rTMS over right PMd would be specific to rhythmic behaviours and therefore there would be no change in choice RT between sessions (Hypothesis 2)\(^166\). Simple RT was assessed before and after real/sham rTMS to assess how rTMS over right PMd affected motor execution processes. No change in simple RT was predicted (Hypothesis 3)\(^166\). Finally, corticospinal excitability was also assessed before and after sham/real rTMS to verify that rTMS over right PMd was not directly inhibiting right M1. No change in corticospinal excitability was predicted (Hypothesis 4).
Figure 4.2. Experimental overview.

Corticospinal excitability and simple reaction time were assessed directly before and after 1 hertz rTMS. All participants received real and sham rTMS across 2 sessions separated by at least 8 days. Session order was counter-balanced between participants. After TMS, participants completed 4 versions of a bimanual 4-finger rhythmic tapping task, followed by 2 versions of a bimanual 4-choice reaction time task. Task condition order was counterbalanced between subjects.
4.2 Methods

4.2.1 Participants

A total of 33 individuals were recruited from Vancouver, British Columbia to participate in the current study. All participants were right-handed according to the Edinburgh Handedness Inventory and had no history of neurological disease or injury. The majority of participants were recruited via a University of British Columbia undergraduate human research participants pool and received academic credit in exchange for participation. Other participants received financial remuneration for participation. Participation involved a short online introductory session as well as 2 in-person experimental sessions. Of the 33 recruited participants, 5 dropped out of the study after the first in-person session. These participants were excluded from final analyses, leaving a total of 28 participants (22.9 ± 7 years old; 15 female, 13 male). All protocols were approved by the Clinical Research Ethics Board at the University of British Columbia. Prior to participating, individuals provided written informed consent in accordance with the Declaration of Helsinki.

4.2.2 Experimental design

Using a within-subjects design, all participants completed 2 experimental sessions to examine the effect of rTMS induced inhibition of right PMd on the control of discrete and rhythmic bimanual movements (Figure 2). For each session, participants first underwent 1200 pulses of either sham or real 1 Hz rTMS delivered over right PMd. The intensity of rTMS over PMd was individualized to 110% of each participant’s resting motor threshold and single pulse motor evoked potentials (MEPs) were collected from the left first dorsal interosseous (FDI) muscle before and after rTMS to assess the effect of real and sham stimulation on corticospinal excitability. In addition, participants completed a simple RT task before and after sham and real rTMS to assess the effect of stimulation on motor execution processes. All participants underwent sham and real rTMS
across 2 sessions separated by at least 8 days. The order of sham/real rTMS sessions was counterbalanced between participants. After sham/real rTMS, participants performed 4 versions of a 4-finger bimanual rhythmic tapping task. Task versions differed in terms of whether symmetric or asymmetric tapping patterns were required, and whether movement frequency was cued with abstract symbolic stimuli or spatially congruent stimuli (Figure 4.3). Next, participants performed 2 versions of a 4-finger bimanual choice RT task: one in which responses were cued with abstract symbolic stimuli, and another in which responses were cued with spatial stimuli. For both task types, the order of task stimulus type (symbolic/spatial) was counterbalanced between participants.

4.2.3 Procedure

Before completing the in-person rTMS sessions, participants completed a 20-minute introductory practice session to ensure they understood the task requirements. The practice session was delivered via online platforms, with participants using a web browser on their personal desktop or laptop computer to complete the session. Participants accessed the practice session via a link to Qualtrics, a secure online survey tool (qualtrics.com), where they first indicated they met the study criteria, digitally signed a consent form, and provided demographic information. Participants were then redirected to the Gorilla Experiment Builder177 (www.gorilla.sc). Participants could not progress to Gorilla unless they were using a desktop or laptop computer with a keyboard. In Gorilla, participants were given instructions on how to correctly perform trials of the 4-finger bimanual choice RT task. In order to advance, participants had to demonstrate they understood the 4 stimulus-response mappings for the spatially cued and symbolically cued task versions. They also had to complete 6 practice trials of each task version without making an error. After successfully completing the 4-finger bimanual choice RT practice trials, participants were shown instructions for the 4-finger bimanual rhythmic tapping task, which described the types of
movements that would be required. Instructions were accompanied by videos demonstrating the difference between symmetric and asymmetric tapping movements. Participants were given no specific instructions on what movement strategy to use. Participants practiced 4 versions of the 4-finger bimanual rhythmic tapping task (spatial-symmetric/spatial asymmetric/symbolic symmetric/symbolic asymmetric). During practice, cartoon hands were added to the task stimuli to explicitly demonstrate which fingers should respond to each stimulus. Participants were not allowed to progress until they demonstrated they were capable of performing an abbreviated block of tapping (6 taps total) at a movement frequency of 1 Hz for the given tapping condition.

For the in-person rTMS sessions, participants first completed a TMS safety questionnaire to ensure they were safe to participate in the experiment. Participants then completed a short optional questionnaire to assess their daily caffeine, alcohol, and cannabis consumption habits. Before the first rTMS session, the experimenter verified that the participant had completed the online practice session and that they understood the task requirements. Participants had the opportunity to practice the tasks again under supervision of the experimenter.

Once they had indicated that they understood the task requirements, participants completed a simple RT task. Participants performed symbolically cued and spatially cued versions of the simple RT task on a desktop personal computer with a wired keyboard and a widescreen high-definition monitor (BenQ, CA, USA; 144 Hz refresh rate). Participants were shown a fixation stimulus followed by an imperative stimulus indicating they should respond as fast as possible. Participants were explicitly told which finger(s) to respond with before each block of trials. A 2-4 s inter-trial interval was randomly inserted between trials to prevent participants from anticipating the onset of the imperative stimulus. For each task version, participants completed a block of 6 unimanual
trials with their left index finger, a block of 6 unimanual trials with their right index fingers, and a block of 6 bimanual trials with their left and right index fingers (36 trials total).

Next, single pulse TMS was applied over M1 to establish resting motor threshold and corticospinal excitability. Surface electromyography (EMG) was recorded using bipolar electrodes placed over bilateral FDI muscles. A ground electrode was placed over the ulnar styloid process of the right arm. EMG was sampled at 5,000 Hz, amplified x1,000, and bandpass filtered (50–450 Hz; Delsys, MA, USA). EMG recording was controlled using the VETA MATLAB toolbox and displayed on a monitor outside of the participant’s field of vision. TMS was administered using a Magstim Rapid2 stimulator (Carmarthenshire, UK) with an AirFilm® Coil. Brainsight neuronavigation (Rogue Research Inc, QC, Canada) was used to ensure coil positioning was accurate and consistent across sessions. An infrared tracker was attached to the TMS coil which allowed experimenters to control coil position relative the head in real time throughout each session.

For TMS over M1, the center of the coil was positioned in a posterior-to-anterior orientation 45-degrees to the mid-sagittal plane over the right M1. The location at which TMS most consistently resulted in MEPs in the FDI was defined as the “hotspot”. Once the M1 “hotspot” was determined, the resting motor threshold, defined as the lowest TMS pulse intensity that elicited five out of ten MEPs greater than or equal to a peak-to-peak amplitude of 0.05 mV, was determined (see Table 4.1 for individual resting motor threshold values). After establishing resting motor threshold, corticospinal excitability was assessed by eliciting 10 MEPs over the M1 “hotspot” at an intensity of 120% of resting motor threshold.

For rTMS over right PMd, the center of the coil was positioned in a lateral-to-medial orientation 90-degrees to the mid-sagittal plane. The coordinates for PMd stimulation were established by moving the coil 7% of the distance from the nasion to the inion anterior from the “hotspot”, and
3.5% of the distance from the nasion to the inion medial to the “hotspot”\textsuperscript{121,166}. Stimulation intensity during rTMS over PMd was set to 110% of resting motor threshold. Before beginning rTMS 5 single pulses of TMS were applied over the PMd target at 110% of resting motor threshold to test whether any MEPs in M1 were elicited. If MEPs of any amplitude were observed, TMS intensity was iteratively lowered by 1% maximum stimulator output until 5 consecutive trials without MEPs were observed.

For sham rTMS sessions, the AirFilm® Coil was switched to a sham coil which looked and sounded like the AirFilm® Coil. For real rTMS sessions, the experimenter pretended to switch the AirFilm® Coil so that the sound of the coil restarting was constant between sessions. Participants wore ear plugs to protect their hearing during rTMS. rTMS took a total of 20 minutes, with 1200 pulses delivered at a rate of 1 Hz. Participants were instructed to avoid falling asleep, avoid moving their hands, and inform the experimenter if they experienced any pain or discomfort during rTMS delivery.

After rTMS was completed, corticospinal excitability assessments were repeated by delivering 10 single pulses of TMS over M1 at 120% of resting motor threshold. Next, participants repeated the simple RT task as described above. After these assessments, participants started the 4-finger bimanual rhythmic tapping task. For each task version, participants performed 3 blocks of 48 taps. Before the start of each block, a fixation cross was displayed with a clock graphic counting down from 3 seconds to allow participants to anticipate the start of the block. There was an opportunity for a break after every block. The movement frequency of rhythmic tapping started at 1.4 Hz and increased by 0.8 Hz each block, finishing at 3 Hz. All participants performed the asymmetric versions of the task first followed by the symmetric versions of the task. The order of the stimulus
type condition was counterbalanced between participants. Key press timing and the identity of all key presses were recorded in each task block.

Upon completion of the 4-finger bimanual rhythmic tapping task, participants began the 4-choice bimanual RT task. Participants performed 2 versions of the task (symbolic stimuli/spatial stimuli) in alternating blocks, with the order of task version counterbalanced. There were 4 stimulus-response mappings for the symbolic and spatial versions of the task (Figure 4.3). For each task version, participants performed 4 total blocks of 12 task trials each (12 per each 4 stimulus-response mappings) with an opportunity for a break every block. The task version was alternated every 2 blocks, and the order of the 4 choice RT stimuli was randomized between participants and between task versions. A random intertrial interval of 2-4 s was used so that participants could not anticipate stimulus onset timing. RT and the identity of keys pressed were recorded on each trial.

After their second rTMS session, participants completed an exit questionnaire in which they were asked if they used any explicit strategies during the 4-finger bimanual rhythmic tapping task, and whether they noticed any difference in the rTMS between sessions. Next, the experimenter informed the participant that one of the sessions had used real rTMS and the other had used sham rTMS. To assess explicit knowledge, participants were asked to guess which day was real rTMS.
In both tasks, movement timing was either cued with spatially congruent visual stimuli, where red boxes directly denoted the relative spatial location of the fingers required to move, or abstract symbolic stimuli where words abstractly denoted the identity of the fingers required to move. Required responses were either symmetric or asymmetric. In the bimanual 4-finger rhythmic tapping task, participants performed 4 versions of the task, in which movement frequency was cued with either spatially congruent or abstract symbolic visual cues, and the required response pattern was either symmetric or asymmetric.

**Figure 4.3. Stimulus-response mappings.**
<table>
<thead>
<tr>
<th>Participant</th>
<th>Sex</th>
<th>Age</th>
<th>RMT - SHAM</th>
<th>RMT - REAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>27</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>20</td>
<td>51</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>34</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>39</td>
<td>71</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>30</td>
<td>48</td>
<td>51</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>19</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>22</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>19</td>
<td>75</td>
<td>77</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>19</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>19</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>20</td>
<td>60</td>
<td>62</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>20</td>
<td>35</td>
<td>39</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>19</td>
<td>64</td>
<td>61</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>20</td>
<td>59</td>
<td>58</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>19</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>23</td>
<td>54</td>
<td>57</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>18</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>18</td>
<td>60</td>
<td>59</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>21</td>
<td>55</td>
<td>54</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>22</td>
<td>36</td>
<td>39</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>18</td>
<td>51</td>
<td>56</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>19</td>
<td>59</td>
<td>57</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>20</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>19</td>
<td>57</td>
<td>55</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>18</td>
<td>71</td>
<td>70</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>22</td>
<td>51</td>
<td>47</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>33</td>
<td>67</td>
<td>62</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>45</td>
<td>48</td>
<td>48</td>
</tr>
</tbody>
</table>

Table 4.1. Participant characteristics.

Participant sex, age, and resting motor threshold values.
4.2.4 Data processing

Custom MATLAB scripts were used to process data downloaded from Gorilla. For the simple and choice RT tasks, RT for each individual trial was filtered from the raw data produced by Gorilla. For bimanual trials, the mean of RT values for each responding finger was calculated to create a single bimanual RT value for each bimanual trial. For each condition of the choice RT task, RT values greater than three scaled median absolute deviations were defined as outliers and removed from further analysis to prevent positive RT distribution skew from affecting mean and standard deviation RT calculations. Choice RT trials were designated as an error if the wrong pair of keys were pressed, if no keys were pressed, if only one key was pressed, or if more than 2 keys were pressed.

For the 4-finger bimanual rhythmic tapping task, an array of key press timing values was filtered from the raw data file for each finger and for each block. These arrays were used to calculate a relative phase value for each key press made. Relative phase values were calculated according to the formula:

\[
\phi = \frac{t_{\text{Tar},i} - t_{\text{Ref},i}}{t_{\text{Ref},i+1} - t_{\text{Ref},i}} \times 360 \quad \text{[deg]}
\]

Here \( \phi \) equals relative phase, \( t_{\text{Tar},i} \) equals the time of the \( i \)th tap of the target digit, \( t_{\text{Ref},i} \) equals the time of the \( i \)th reference tap, and \( t_{\text{Ref},i+1} \) equals the time of the \( i \)th +1 reference tap (see Figure 4.4 for a graphical example). For symmetric blocks, the reference finger was the same effector on the opposite hand, while for asymmetric trials, the reference finger was the opposite
finger of the opposite hand. The first 4 key presses were excluded from relative phase calculations, to allow participants time to get in rhythm.

Offline analysis of TMS/EMG data was performed using the VETA toolbox and custom-automated procedures within MATLAB. MEP peak-to-peak amplitude was the sole EMG variable of interest. The ‘findEMG.m’ function was used to automatically identify EMG events. All automatically identified EMG events were visually inspected by a human rater using the ‘visualizeEMG.m’ function. Adjustments were made to MEP onset and offset when necessary, using the ‘visualizeEMG.m’ function in the VETA toolbox. Any trials that contained >0.05 mV of background muscle activity in either hand 50 ms prior to the TMS artifact were removed from further analysis (<0.5% of MEPs collected were removed).
Figure 4.4. Method to calculate relative phase of bimanual coupling from key press data.

An array of bimanual key press times from one task block is shown for target and reference fingers. The difference in time between each target finger key press and the temporally nearest reference key press is compared to the difference in time between that reference finger key press and the following reference finger key press. When bimanual temporal coupling is high, relative phase values are low, as is shown in example #1. When bimanual temporal coupling is low, relative phase values are high, as is shown in example #2.
4.2.5 Dependent variables

4-finger bimanual rhythmic tapping task

- **Tapping timing accuracy** – The mean absolute timing error was calculated from relative phase to provide a measure of tapping accuracy. Absolute timing error was calculated for each key press by subtracting the relative phase of each key press from ideal relative phase (0°) and calculating the absolute value of the resultant integer. The mean of absolute timing error was then calculated for each block.

- **Tapping stability** – The standard deviation (SD) of absolute error was calculated for each block to provide a measure of tapping stability.

- **Transition occurrence** – Blocks in which a transition key press occurred (i.e., a key press with absolute relative phase greater than 180°) were marked as having a transition occurrence (1), whereas blocks in which no transition key press occurred were marked as having no transition occurrence (0).

4-finger bimanual choice reaction time task

- **Reaction time** – mean RT for symmetric and asymmetric trials was calculated for each stimulus type condition to provide a measure of RT magnitude. Error trials were not included in mean calculations.

- **Reaction time variability** – the SD of RT for symmetric and asymmetric trials was calculated for each stimulus type condition. Error trials were not included in SD calculations.
Simple reaction time task

- Reaction time – mean RT for symmetric and asymmetric trials was calculated for each stimulus type and response condition to provide a measure of RT magnitude.

Single pulse transcranial magnetic stimulation

- Corticospinal excitability – the mean peak-to-peak amplitude of single pulse MEPs was calculated to provide a measure of corticospinal excitability.

4.2.6 Statistical analysis

Statistical analyses were carried out in RStudio. Post hoc analyses were performed using Tukey’s correction for multiple comparisons where appropriate.

Quality control

All data was visually checked to ensure that statistical models were based on high quality data. For the 4-finger bimanual rhythmic tapping task, one participant did not complete the bimanual tapping task practice in the online introductory session before their first in-person experimental session. Therefore, their data from this task was not included in analysis as the lack of practice may have introduced a confound due to the lack of task familiarity in their first session compared to their second session. A further 2 participants were not included as they were either pressing the wrong keys or holding down the keys on the majority of blocks meaning outcome measures could not be reliably calculated. Of the remaining 25 participants, 3 were found to be pressing the wrong keys during tapping on 1 or 2 blocks out of the total 24 blocks. Outcome measures could not be calculated for these blocks. However, since this error in task performance was not widespread, these participants were included in final analysis with partial datasets. Therefore, the sample size for the final analysis of 4-finger bimanual rhythmic tapping task was 25 participants.
For corticospinal excitability, technical issues with EMG data collection meant that MEP amplitude could not be calculated for 2 participants. Therefore, the sample size for the final analysis of corticospinal excitability was 26 participants.

### 4.2.6.1 Primary analyses

*4-finger bimanual rhythmic tapping task*

Before performing inferential statistics, mean absolute error and SD of absolute error values were assessed for skewness using the Shapiro-Wilks test. Results from these tests indicated that both variables were significantly positively skewed across conditions of interest. To reduce the risk of data skewness biasing statistical models, observations that were deemed extreme outliers (i.e., 3 SDs above or below the mean; 29 of 595 total observations) were removed from the dataset for each dependent measure. The potential impact of skewness was also decreased by the decision to use linear mixed effect regression (LMER) analysis, rather than traditional *F*-tests, as LMER is robust against violations to distributional assumptions required by parametric tests\textsuperscript{181}. LMER also accounts for random variation due to participants, through modeling of multiple intercepts for each participant as a random effect, rather than a single mean intercept\textsuperscript{133–135,182}. Use of LMERs also allows for the inclusion of partial data, meaning removal of extreme outlier observations did not necessitate the removal of the entire dataset of the associated participant from the analysis.

LMERs were performed to establish the effect of fixed effects of **STIMULATION CONDITION** (rTMS/sham), **STIMULUS TYPE** (symbolic/spatial), **RESPONSE PATTERN** (symmetric/asymmetric), and **MOVEMENT FREQUENCY** (1.4, 2.2, 3 Hz) on the mean absolute error and the SD of absolute error of relative phase (Hypothesis 1). Participant ID was included as a random effect.
Logistic regression is a statistical technique used to model dichotomous outcome variables. This is achieved by modeling the log odds of the outcome as a linear combination of the predictor variables. Given that transition occurrence was a dichotomous variable (0 or 1), logistic regression was used to model the probability of transition occurrence across categorical factors STIMULATION CONDITION, STIMULUS TYPE, RESPONSE PATTERN, and MOVEMENT FREQUENCY (Hypothesis 1).

4-finger bimanual choice reaction time task

For the 4-finger bimanual choice RT task, mean RT and SD of RT were found to be significantly skewed across conditions according to results from a Shapiro-Wilks test. Therefore, LMERs were used to reduce the potential biasing of statistical models by data skewness.

LMERs were used to establish the effect of fixed factors STIMULATION CONDITION (rTMS/sham), STIMULUS TYPE (symbolic/spatial), and RESPONSE TYPE (symmetric/asymmetric) on mean RT and the SD of RT (Hypothesis 2). Participant ID was included as a random effect.

4.2.6.2 Supplementary analyses

Simple reaction time task

For the simple RT task, mean RT was found to be significantly skewed across multiple conditions of interest according to results from a Shapiro-Wilks test. Furthermore, 6 participants failed to follow task instructions correctly on some task conditions, resulting in 8 missing observations. Therefore, to reduce the potential biasing of statistical models by data skewness while also avoiding dropping 6 full datasets from the analysis, LMERs were used to model mean RT across conditions of interest.
LMERs were used to establish the effect of fixed factors STIMULATION CONDITION (rTMS/sham), STIMULUS TYPE (symbolic/spatial), RESPONSE TYPE (left-unimanual/right-unimanual/bimanual) and TIME (pre/post) on mean RT. Participant ID was included as a random effect.

*Corticospinal excitability*

For corticospinal excitability, mean MEP amplitude was found to be skewed across all conditions of interest. To reduce the potential biasing of statistical models by data skewness, LMERs were used to model corticospinal excitability.

LMERs were used to establish the effect of fixed factors STIMULATION CONDITION (rTMS/sham), and TIME (pre/post) on corticospinal excitability. Participant ID was included as a random effect.

4.2.6.3 Exploratory analyses

*Assessing the impact of the number of movement frequency levels on stimulus type effects*

Based on results from our primary analyses, I performed an additional *a posteriori* exploratory analysis. The purpose of these exploratory analyses was to better understand why a significant main effect of STIMULUS TYPE or an interaction effect of MOVEMENT FREQUENCY x STIMULUS TYPE was not observed in the 4-finger bimanual rhythmic tapping task in current study, in contrast to results from Chapter 2.

The lack of an effect of STIMULUS TYPE did not appear to be purely due to sample size, since although the findings experiment 1 of Chapter 2 had a large sample size of 192 participants, the effects were largely replicated in experiment 2, which had a sample size of 20 participants (comparable to the current study). The difference in observed effects may have instead be caused
by differences in the number of levels of MOVEMENT FREQUENCY assessed. To satisfy the practical need to reduce total experiment time to stay within the time period of the predicted effects of 1 Hz rTMS on cortical activity, the version of the task used in the current study only assessed task performance at 3 levels of MOVEMENT FREQUENCY (1.4, 2.2, 3 Hz), while the task used in Chapter 2 derived these same measurements across 12 levels of MOVEMENT FREQUENCY (1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8, 3, 3.2 Hz). This raises the possibility that either: (1) the current study simply did not collect data at enough levels of MOVEMENT FREQUENCY to overcome random noise within the effect of STIMULUS TYPE, or (2) the more gradual increase in MOVEMENT FREQUENCY used in Chapter 2 instituted a learning effect that increased the benefit of spatial cues versus symbolic cues compared to the relatively rapid increases in MOVEMENT FREQUENCY in the current chapter.

To address this question, I re-analyzed mean absolute error of relative phase data collected in Experiment 2 of Chapter 2 but constrained the analysis to the 3 levels of MOVEMENT FREQUENCY used in the current study, instead of the 12 levels used in the original Chapter 2 analysis. If the lack of an effect of STIMULUS TYPE is due to an inability of 3 levels of MOVEMENT FREQUENCY to overcome random noise within the effect of STIMULUS TYPE (scenario 1), then I would expect to observe a similar lack of effect of STIMULUS TYPE in the re-analysis of Chapter 2 data. If the lack of an effect of STIMULUS TYPE is due to differences in the rate of increase of MOVEMENT FREQUENCY (scenario 2), then I would instead expect to observe a significant effect of STIMULUS TYPE in the re-analysis of Chapter 2 data. I predicted that results would follow scenario 1, and that the MOVEMENT FREQUENCY x STIMULUS TYPE would not be replicated with 3 levels of MOVEMENT FREQUENCY.
The exploratory analysis approach mirrored that used in the primary analyses of the current study. Extreme outlier observations were removed from the dataset and two distinct LMERs with fixed effects of STIMULUS TYPE (symbolic/spatial), RESPONSE PATTERN (symmetric/asymmetric), and MOVEMENT FREQUENCY (1.4, 2.2, 3 Hz) were run on mean absolute error data. Participant ID was included as a random effect.

4.3 Results

4.3.1 4-finger bimanual rhythmic tapping task

Mean absolute error – Results from the 4-finger bimanual rhythmic tapping task are shown in Figure 4.5. Mean absolute error increased with movement frequency across task conditions, as was evidenced by a main effect of MOVEMENT FREQUENCY between 1.4 and 3 Hz conditions ($\beta = 8.48$, 95% confidence interval [CI; 4.27 12.69], $p <0.001$). Estimated marginal means indicated that mean absolute error was significantly increased between 1.4 Hz and 2.2 Hz ($\mu = 4.21 \pm 1.35$, $p = 0.01$), 1.4 Hz and 3 Hz ($\mu = 12.04 \pm 1.36$, $p < 0.0001$), and 2.2 Hz and 3 Hz ($\mu = 7.84 \pm 1.35$, $p < 0.0001$) conditions. The main effect of MOVEMENT FREQUENCY should be considered in the context of by a significant MOVEMENT FREQUENCY x RESPONSE PATTERN interaction effect ($\beta = 8.69$, 95% CI [3.66 13.72], $p <0.001$), which indicated that the detrimental effects of asymmetric patterns compared to symmetric patterns on mean absolute error significantly increased at 3 Hz compared to 1.4 Hz. Estimated marginal means were calculated to better understand the simple main effects of RESPONSE PATTERN across all levels of MOVEMENT FREQUENCY. Asymmetric response patterns were found to significantly increase mean absolute error compared to symmetric patterns at 3 Hz ($\mu = 8.32 \pm 1.28$, $p < 0.0001$), but not at 1.4 Hz ($\mu = 0.31 \pm 1.29$, $p = 0.99$) or 2.2 Hz ($\mu = 1.91 \pm 1.28$, $p = 0.67$). Unexpectedly, there was no significant MOVEMENT FREQUENCY x STIMULUS TYPE interaction effect between
1.4 and 3 Hz conditions (p = 0.84). There was also no significant main effect of STIMULATION CONDITION (p = 0.7) or any significant interaction effects involving STIMULATION CONDITION (Figure 4.5A).

Standard deviation of absolute error – SD of absolute error increased with movement frequency across task conditions, as was evidenced by a main effect of MOVEMENT FREQUENCY between 1.4 and 3 Hz conditions (β = 10.29, 95% CI [0.18 20.41], p = 0.046). Estimated marginal means indicated that mean absolute error was significantly increased between 1.4 Hz and 3 Hz (µ = 17.19 ± 2.35, p < 0.0001), 2.2 Hz and 3 Hz (µ = 12.14 ± 2.34, p < 0.0001) but not between 1.4 Hz and 2.2 Hz (µ = 5.05 ± 2.36, p = 0.1) conditions. The main effect of MOVEMENT FREQUENCY should be considered in the context of a significant MOVEMENT FREQUENCY x RESPONSE PATTERN interaction effect (β = 14.47, 95% CI [0.95 27.98], p = 0.036), which indicated that the detrimental effects of asymmetric patterns compared to symmetric patterns on mean absolute error significantly increased at 3 Hz compared to 1.4 Hz. Estimated marginal means were calculated to better understand the simple main effects of RESPONSE PATTERN across all levels of MOVEMENT FREQUENCY. Asymmetric response patterns were found to significantly increase mean absolute error compared to symmetric patterns at 3 Hz (µ = 16.45 ± 3.07, p < 0.0001), but not at 1.4 Hz (µ = 0.46 ± 3.14, p = 0.99) or 2.2 Hz (µ = 4.13 ± 3.08, p = 0.76). Unexpectedly, there was no significant MOVEMENT FREQUENCY x STIMULUS TYPE interaction effect between 1.4 and 3 Hz conditions (p = 0.97). There was also no significant main effect of STIMULATION CONDITION (p = 0.55) or any significant interaction effects involving STIMULATION CONDITION (Figure 4.5B).

Transition occurrence – Logistic regression analysis revealed that the probability of a transition occurrence did not significantly increase across levels of STIMULATION CONDITION (p =
0.99), STIMULUS TYPE ($p = 0.13$), RESPONSE PATTERN ($p = 0.13$), or MOVEMENT FREQUENCY (1.4-3 Hz: $p = 0.64$, 1.4-2 Hz: $p = 0.38$; **Figure 4.5C**).
Figure 4.5. Bimanual rhythmic tapping task results.

Predicted task performance values in the rhythmic bimanual tapping task derived from linear mixed effect and logistic regressions for sham and real 1 hertz rTMS conditions. (A) Mean absolute error of relative phase. (B) Standard deviation of absolute error. (C) Probability of transition out of required tapping pattern. Points represent predicted values and error bars represent 95% confidence intervals. There was no significant effect of rTMS on outcome measures when compared to sham stimulation.
4.3.2 4-finger bimanual choice reaction time task

Mean reaction time – Mean RT was slower when responses were cued with symbolic stimuli compared to spatial stimuli as was evidenced by a significant main effect of STIMULUS TYPE (β = 136, 95% CI [110 162], p < 0.001). Similarly, mean RT was slower when asymmetric responses were required compared to symmetric responses, as was evidenced by a significant main effect of RESPONSE TYPE (β = 35, 95% CI [3 68], p = 0.033, d = 2.19). These main effects should be considered in the context of a significant STIMULUS TYPE x RESPONSE TYPE interaction effect (β = 39, 95% CI [14 64], p = 0.003, d = 0.63). Estimated marginal means were calculated to further interrogate the interaction effect. Asymmetric responses were found to be significantly slower than symmetric responses when trials were cued with symbolic stimuli (µ = 73 ± 15, p = 0.0002, d = 0.77) but not when cued with spatial stimuli (µ = 36 ± 15, p = 0.094, d = 0.5). There was no significant main effect of STIMULATION CONDITION (p = 0.44) or any significant interaction effects involving STIMULATION CONDITION (Figure 4.6A).

Standard deviation of reaction time – SD of RT was greater when responses were cued with symbolic stimuli compared to spatial stimuli as was evidenced by a significant main effect of STIMULUS TYPE (β = 32, 95% CI [15 48], p < 0.001, d = 1.06). This main effect should be considered in the context of a significant STIMULUS TYPE x RESPONSE TYPE interaction effect (β = 30, 95% CI [12 48], p = 0.001). Estimated marginal means were calculated to further interrogate the interaction effect. Asymmetric responses were found to be significantly slower than symmetric responses when trials were cued with symbolic stimuli (µ = 33 ± 10, p = 0.01, d = 0.47) but not when cued with spatial stimuli (µ = 6 ± 10, p = 0.92, d = 0.15). There was no significant main effect of STIMULATION CONDITION (p = 0.83) or any significant interaction effects involving STIMULATION CONDITION (Figure 4.6B).
Figure 4.6. Bimanual 4-choice reaction time task results.

Mean (A) and standard deviation (B) of reaction time (RT) values for symmetric and asymmetric response types collected in the spatially cued and symbolically cued bimanual 4-choice RT tasks. There was no difference in outcome measures for sham (left) versus real rTMS (right). RT was significantly faster when movements were cued with spatially congruent cues. Furthermore, the cost of asymmetric movements to the magnitude and variability of RT was significant only when movement was cued with abstract symbolic cues.
4.3.3 Simple reaction time task

Mean RT did not significantly differ across levels of STIMULATION CONDITION ($p = 0.083$), TIME ($p = 0.13$), RESPONSE TYPE (unimanual-right/unimanual-left: $p = 0.5$; unimanual-right/bimanual: $p = 0.14$), or STIMULUS TYPE ($p = 0.11$). Furthermore, there were no significant interaction effects (Figure 4.7).

4.3.4 Corticospinal excitability

Corticospinal excitability did not differ significantly across levels of TIME ($p = 0.86$) or STIMULATION CONDITION ($p = 0.265$), and there was no significant TIME x STIMULATION CONDITION interaction effect ($p = 0.71$) (Figure 4.8).

Figure 4.7. Simple reaction time results.

Mean reaction time was unchanged from pre to post sham (left) and real rTMS (right), regardless as to which stimuli type was used to cue responses and whether left hand, right hand, or bimanual responses were required. Points represent group mean values and error bars represent standard error of the mean.
4.3.5 Assessing the impact of movement frequency levels on stimulus type effects

A re-analysis of mean absolute error data from Chapter 3 (experiment 2) with analysis constrained to 1.4 Hz, 2.2 Hz and 3 Hz levels of MOVEMENT FREQUENCY largely mirrored results from the primary analysis of the current study. Mean absolute error increased with movement frequency across task conditions, as was evidenced by a main effect of MOVEMENT FREQUENCY between 1.4 and 3 Hz conditions ($\beta = 7.11$, 95% CI [0.26 13.96], $p = 0.042$). Estimated marginal means indicated that mean absolute error was significantly increased between 1.4 Hz and 2.2 Hz ($\mu = 7.55 \pm 2.23$, $p = 0.0047$), 1.4 Hz and 3 Hz ($\mu = 16.27 \pm 2.21$, $p < 0.0001$), and 2.2 Hz and 3 Hz ($\mu = 8.72 \pm 2.21$, $p = 0.001$) conditions. Again, the main effect of MOVEMENT FREQUENCY should be considered in the context of by a significant MOVEMENT FREQUENCY x RESPONSE PATTERN interaction effect ($\beta = 13.22$, 95% CI [4.59 21.84], $p = 0.003$), which indicated that the detrimental effects of asymmetric patterns compared to symmetric patterns on

Figure 4.8. Corticospinal excitability across sham and real rTMS.

Corticospinal excitability was unchanged from pre to post sham (A) and real rTMS (B). Horizontal black bars represent group mean. Circles represent individual participants. For change scores (C), lines link individual participants across stimulation conditions.
mean absolute error significantly increased at 3 Hz compared to 1.4 Hz. Estimated marginal means were calculated to better understand the simple main effects of RESPONSE PATTERN across all levels of MOVEMENT FREQUENCY. Asymmetric response patterns were found to significantly increase mean absolute error compared to symmetric patterns at 3 Hz ($\mu = 12.63 \pm 2.63$, $p = 0.0002$), but not at 1.4 Hz ($\mu = 2.63 \pm 2.68$, $p = 0.93$) or 2.2 Hz ($\mu = 6.38 \pm 2.73$, $p = 0.2$). There was no significant MOVEMENT FREQUENCY x STIMULUS TYPE interaction effect between 1.4 and 3 Hz conditions, although the interaction effect did approach significance ($\beta = 8.31$, 95% CI [-0.35 16.98], $p = 0.06$) (Figure 4.9).

**Figure 4.9.** Re-analysis of mean absolute error values from Chapter 2 experiment 2.

(A) Task design from chapter 2 (experiment 2) assessed bimanual rhythmic tapping at 12 levels of movement frequency. In the original analysis, 12 levels of movement frequency were included as a continuous variable in the linear mixed effect regression, and response type (symmetric/asymmetric) and stimulus type (spatial/ symbolic) were found to significantly effect task performance at higher frequencies. (B) A re-analysis of the data constrained statistical modeling to the 3 levels of movement frequency used in the current study (1.4, 2.2, 3 Hz). This re-analysis replicated a significant effect of response type (symmetric/asymmetric) at 3 Hz compared to 1.4 Hz but failed to replicate a significant effect of stimulus type (spatial/symbolic) at 3 Hz compared to 1.4 Hz. This re-analysis indicates that the task design used in the current study may have been underpowered to overcome noise within the effect of stimulus type.
4.4 Discussion

In the current study, I found no effect of 1 Hz rTMS over right PMd on the stability of bimanual rhythmic finger tapping relative to a sham rTMS control condition. As expected, asymmetric movements were less stable than symmetric movements at high movement frequencies. However, when compared to sham stimulation, rTMS was not associated with a significant decrease in asymmetric movement stability relative to symmetric movement stability. Similarly, task conditions in which movement frequency was cued with abstract symbolic stimuli were not significantly less stable after rTMS compared to conditions in which movement frequency was cued with spatially congruent stimuli (Figure 4.5). Bimanual choice RT (Figure 4.6), bimanual simple RT, and left and right hand unimanual simple RT (Figure 4.7) were also unchanged after rTMS relative to sham stimulation, regardless of the stimulus type used to cue movement or whether asymmetric or symmetric responses were required. Corticospinal excitability was not affected by rTMS or sham stimulation (Figure 4.8), indicating that the inhibitory effects of 1 Hz rTMS did not spread to right M1. Taken together, these findings speak against the notion that PMd is specifically tasked with programming discrete or rhythmic asymmetric movements or responsible for managing cognitive load during movement. Instead, they support the hypothesis that right PMd is one node in a broader cortical network, which is capable of maintaining behavioural output in response to exogeneous challenges posed by rTMS.

4.4.1 The brain can compensate for neuronal challenge to right PMd and maintain rhythmic bimanual behaviours

Previous neurophysiological evidence has demonstrated a link between PMd activity and asymmetric bimanual rhythmic control\(^4\,6\,197\), while behavioural evidence has associated asymmetric bimanual control with an increased load on cognitive resources\(^10,14\,16\). Therefore, I
predicted that detriments to the stability of temporal coupling of rhythmic bimanual movements after rTMS over right PMd would scale with the degree of cognitive load engendered by task conditions. Symmetric and asymmetric rhythmic tapping were assessed with flickering spatially congruent visual stimuli demonstrating the target movement frequency and with flickering abstract symbolic visual stimuli demonstrating the target movement frequency. In chapter 3 I demonstrated that cueing movement frequency with spatially congruent stimuli significantly increases the stability of bimanual rhythmic tapping behaviours when compared to abstract symbolic stimuli, probably by reducing the strain on cognitive resources. Therefore, these task manipulations provided a method to effectively test whether right PMd was responsible for programming asymmetric rhythmic movements, or mode broadly responsible for managing increased cognitive load during rhythmic bimanual behaviours.

Rather than observing detriments to task performance that scaled with the degree of cognitive load, or detriments to performance specifically in asymmetric tapping conditions, I found no change in task performance after rTMS over right PMd compared to sham stimulation. This result speaks against the notion that right PMd plays a special role in programming asymmetric rhythmic movements or managing increased cognitive load in rhythmic bimanual movement contexts. A potential alternative explanation is that right PMd is one node in a broader cortical network responsible for controlling rhythmic bimanual behaviours, with the cortical network capable of compensating for neuronal challenge to right PMd to maintain behavioural output. Evidence for the capacity for the brain to compensate for neuronal challenge to PMd comes from a study which applied 1200 pulses of 1 Hz rTMS to left PMd prior to the performance of unimanual decision-making task with the right hand. This work found that choice RT was slowed immediately after rTMS over left PMd but returned to baseline levels ~4 minutes after stimulation. Additionally, this
rebound in choice RT was associated with an increase in blood-oxygen dependant signal in a network of premotor regions beyond left PMd, including right PMd, right M1, left SMA, and bilateral CMA. This increase in activation suggests that a broader network of cortical regions was able to compensate for inhibition of left PMd to stabilize decision making processes. In a similar vein, the application of short trains of rTMS over left PMd “online” during preparation of unimanual responses in a simple RT paradigm is shown to have no effect on RT\textsuperscript{118}. However, application of rTMS over PMd was shown to concurrently disrupt PMd-M1 interhemispheric circuits. Given that PMd-M1 interhemispheric circuits are thought to play a role in sculpting corticospinal output during unimanual preparation\textsuperscript{72,122,198}, this further suggests that a broader network of regions can account for challenge to PMd to maintain behavioural output.

Similar compensatory processes may have occurred in the current study. Studies using fMRI have demonstrated that a network of frontal and parietal regions including right PMd but also that the superior parietal cortex and the intraparietal cortex are activated during difficult bimanual rhythmic tasks that require continuous movement of two hands in asymmetric directions\textsuperscript{197}. Similarly, asymmetric rhythmic bimanual finger tapping is associated with increases in activation in right PMd, but also in the left supramarginal gyrus, bilateral SMA, and bilateral CMA\textsuperscript{4,5}. Therefore, given that a network of frontal-parietal cortical regions beyond right PMd is recruited during bimanual rhythmic behaviours, perhaps the application of 1 Hz rTMS over right PMd in the current study led to an increase in activation in this network of cortical regions, mitigating any potential disruption to behaviour. The involvement of a larger network may have been masked in previous studies which found increased switching from asymmetric to symmetric bimanual tapping behaviours after application of single pulses of TMS over PMd\textsuperscript{4,6}, since a period of ~4 minutes is needed for compensation processes to take effect. This may have given the false impression that
PMD is critical for asymmetric bimanual control, when in reality a larger cortical network with built-in redundancies is recruited during asymmetric bimanual behaviours.

**4.4.2 The stabilizing effects of cueing movement frequency with spatially congruent stimuli are nosier than the stabilizing effect of movement symmetry**

Behavioural results differed somewhat from what was observed in chapter 3 using the same task manipulations. While symmetric responses were significantly more stable than asymmetric responses at a frequency of 3 Hz as expected, there was no significant benefit to stability when movement frequency was cued with spatially congruent stimuli compared to abstract symbolic stimuli. This was unexpected given that in chapter 3 I showed that switching the stimulus type from spatially congruent to abstract symbolic decreased stability at high movement frequencies. A critical difference between the current study and this previous investigation was the previous investigation assessed bimanual rhythmic tapping at 12 movement frequencies, beginning at 1 Hz and increasing by 0.2 Hz after each block, while the current study assessed bimanual rhythmic tapping at only 3 movement frequencies, beginning at 1.4 Hz and increasing by 0.8 Hz after each block. This difference in task design raises the possibility that either: (1) the effect of stimulus did not emerge because there is some beneficial learning effect inherent to spatially congruent stimuli which only emerges when movement frequency is increased at a slow rate, or (2) not enough data was collected at higher frequencies to overcome noise within the effect of stimulus type. To investigate these possibilities, I performed a re-analysis on data from chapter 3, but restricted the analysis to the 3 movement frequencies used in the current study. Similar to the current study, I found no significant effect of stimulus type when only 3 levels of movement frequency were included in the model but did observe a significant accuracy benefit for symmetric compared to asymmetric responses. This favours the view that the effects of stimulus type on
temporal coupling of rhythmic biannual movements may be noisier than that of response type, with greater variance from block to block. Given that I assessed performance at only a single movement frequency (3 Hz) where the effect of stimulus type is potent, I may have not been able to overcome the noise inherent to this factor to yield a significant effect of stimulus type.

The failure to replicate the effects of stimulus type from our previous investigation raises the possibility that our adapted task design with 3 just levels of movement frequency may not have been capable of effectively capturing hypothesized changes in performance across stimulation conditions. However, I note that the competing hypotheses regarding the role of PMd in bimanual rhythmic behaviour as a programmer of asymmetric movements or a manager of cognitive load both predicted that there would be a greater change in performance in asymmetric compared to symmetric conditions (Figure 4.1). Such an effect of rTMS was not observed even though the effect of response type did emerge as expected. Overall, the conclusion that there was no effect on behaviour across sham and real rTMS seems reasonable. However, future investigations using tasks of this nature should assess performance at a greater number of movement frequencies to reliably capture the differential effects of spatially congruent stimuli and abstract symbolic stimuli on task performance.

4.4.3 Limitations

A major limitation of the current study was that no neural measure capable of indexing a change in right PMd activity was collected alongside behavioural measures. Therefore, I cannot rule out the possibility that there was simply no effect of rTMS on brain activity or that effects were highly variable between participants. While 1 Hz rTMS is typically understood to inhibit cortical activity in healthy participants, the effects can be variable between subjects199–203. Furthermore, the time course of rTMS induced effects on cortical inhibition differs between individuals204. Such mixed
effects of rTMS over PMd may have produced a confounding factor that added noise to behavioural measures. Because I did not collect concurrent measures of cortical activity our statistical models were not able to account for this potential confounding factor. Furthermore, while I have discussed the likelihood that compensatory processes facilitated the maintenance of behavioural output despite inhibition of right PMd based on previous research, the lack of a concurrent neural measure means I have no evidence that such neural compensation actually occurred in the current study, or what the spatial extent of compensation was. To effectively index cortical inhibition and account for potential compensatory processes, it is critical that future investigations concurrently collect neural measures before and after rTMS. Functional MRI is well suited in this regard since it has excellent spatial resolution and has previously been shown to able to capture neural compensation after rTMS over PMd. Advanced diagnostic TMS methods may also be useful for indexing the inhibitory and compensatory effects of rTMS. While I collected single pulses of TMS before after rTMS in the current study, the purpose of this measure was to verify that rTMS over right PMd did not directly inhibit right M1. Measures of corticospinal excitability alone are less well equipped to capture changes in PMd activity. The application of dual coil TMS to measure interhemispheric circuits between PMd and M1 may be better placed to directly capture the degree of inhibition of PMd after rTMS. Similarly, dual coil TMS could be used to measure changes in activity in other cortical regions hypothesized to produce compensatory activity after inhibition of PMd with rTMS, such as SMA, M1, and the posterior parietal cortex. While I demonstrated that PMd-M1 interhemispheric circuits may be less active during bimanual movements in experiment 1, they may become active when compensation is triggered by neuronal challenge to contralateral PMd.
As discussed, another limitation is that bimanual rhythmic tapping data was not collected at sufficient levels of movement frequency to accurately capture the effects of spatially congruent stimuli versus abstract symbolic stimuli on behaviour. My previous investigations in chapter 3 indicate that the effect is well captured with 12 levels of movement frequency. While it was necessary to cut down the levels of movement frequency to keep the length of behavioural assessments within the 30-40 minute time period of change induced by rTMS, reducing to 6 levels rather than 3 would have been more appropriate. Furthermore, our previous work indicates that the effects of stimulus type and response symmetry on tapping stability only emerges at higher frequencies, where smooth continuous movement strategies tend to be preferred (2.2-3.2 Hz). Therefore, future investigations may be better placed to focus on higher movement frequencies when investigating the neural control of rhythmic bimanual movements.

A related limitation is that the sample size of the current study may have been statistically underpowered to adequately assess the effects of 1 Hz rTMS over right PMd on behaviour. The decision to collected 28 participants was based on the finding in Chapter 3 (Experiment 2) that the effects of stimulus type and response type on bimanual rhythmic tapping were robust in a sample of 20 participants. However, the subsequent finding that these behavioural effects was limited to higher movement frequencies indicates we may have needed to collect a greater number of participants to effectively assess any potential effect of rTMS on behaviour using a design with a more limited set of movement frequencies. Therefore, as well as collecting bimanual rhythmic tapping data at an appropriate level of movement frequencies, future studies should also conduct preliminary pilot testing to acquire estimates of effect size so that statistical power can be more adequately calculated a priori, and ensure that a large enough sample size is recruited.
4.4.4 Conclusion

Results from the current study indicate that inhibiting activity in right PMd with 1 Hz rTMS has no effect on the stability of bimanual rhythmic tapping, on bimanual choice RT, or on simple RT, regardless of the perceptual quality of the stimulus used to cue movement timing or whether asymmetric or symmetric responses are required. This speaks against the notion that right PMd alone plays a special role in programming asymmetric movements or in managing cognitive load. The null result in the context of previous research instead suggests that compensatory activity in a network of cortical regions may have been engaged to rescue any potential detrimental effects to behaviour that would otherwise be caused by inhibition of right PMd.
5 Conclusion

The goal of this dissertation was to specify the functional role of PMd in the control of rhythmic asymmetric finger movements. The experiments described in this thesis investigated: how activity in interhemispheric circuits connecting PMd with M1 differs for preparation of unimanual and bimanual movements (Chapter 2), whether manipulation of the perceptual quality of visual stimuli used to cue movement timing affects the stability of symmetric and asymmetric rhythmic bimanual finger movements (Chapter 3), if movement quality relates to the stability of rhythmic bimanual finger movements (Chapter 3), and finally whether right PMd is responsible for programming movement or managing cognitive load during rhythmic bimanual behaviours (Chapter 4). Below, I summarize the major findings of each research chapter. The limitations of this body of work are considered, followed by a discussion of potential future directions for the field.

5.1 Summary of chapter aims and main findings

Chapter 2: The primary aim of chapter 2 was to determine whether preparation related changes in PMd-M1 interhemispheric inhibition (IHI) are different for unimanual and bimanual movements. A secondary aim was to determine whether preparation related changes in PMd-M1 IHI differs between hemispheres. I investigated these questions by indexing PMd-M1 IHI between both hemispheres at rest and during the preparation of unimanual and bimanual movements in a simple reaction time paradigm. I was specifically interested in assessing changes late (50 ms) and early (100 ms) in the “pre-movement period” – after the presentation of the imperative “GO” stimulus but before reaction time – since the degree of release of PMd-M1 IHI release early in unimanual preparation has been shown to correlate with the capacity to maintain asymmetric rhythmic bimanual movements at high frequencies. I found that changes in PMd-M1 IHI were similar for both right and left PMd. I also discovered that PMd-M1 IHI was released early but not late in
preparation for unimanual movements; this finding agrees with previous investigations\textsuperscript{8,77,120,121,123}. The same pattern of change was not found during preparation of bimanual movements, where PMd-M1 IHI did not change significantly from resting baseline levels. An exploratory analysis further revealed that the degree of release of PMd-M1 IHI early in unimanual preparation depended on participant mean reaction time.

\textit{Chapter 3}: There were 2 primary aims for chapter 3. The first aim was to determine whether spatially congruent cues increase the speed of planning of discrete symmetric and asymmetric bimanual finger movements compared to abstract symbolic cues. The second aim was to determine whether spatially congruent movement frequency cues increase the stability of rhythmic symmetric and asymmetric bimanual finger movements compared to abstract symbolic movement frequency cues. In experiment 1, I found that bimanual choice reaction time was significantly faster when movement was cued with spatially congruent stimuli which mapped to the position of task-relevant fingers compared to abstract symbolic stimuli which denoted the required response with words. Reaction time was generally slower for asymmetric movements, but the difference between symmetric and asymmetric reaction times was magnified when movement was cued with abstract symbolic stimuli. These findings emulate results from bimanual reaching paradigms\textsuperscript{18–21}, and provide further evidence that response selection processes responsible for translating abstract stimuli into action impose a bottleneck on asymmetric response planning. The stability of bimanual coupling in a rhythmic bimanual tapping paradigm generally decreased with increasing movement frequency. Stability was also shown to depend on whether the required response patterns were symmetric or asymmetric, and whether movement frequency was cued by flickering spatially congruent or abstract symbolic stimuli. Both of these factors contributed equally to coupling stability. This suggests that altering the perceptual features of task elements can significantly affect
rhythmic bimanual finger coordination, in a manner that is similar to what is found in the planning of discrete bimanual actions.

A secondary aim of chapter 3 was to determine if movement strategies change with increasing movement frequency in symmetric and asymmetric 4-finger rhythmic tapping tasks. This was addressed in experiment 2, where I found that increasing movement frequency of rhythmic tapping was associated with a switch from repeated discrete movements to smooth continuous movements. This change in movement quality is important since these two types of movement are likely controlled by distinct neuro-cognitive systems. The change in movement quality could explain why there was a non-linear fit between movement frequency and coupling stability, and why the effects of symmetry and spatial congruence only emerged at higher movement frequencies. Overall, results from chapter 3 demonstrate that there is a cognitive-perceptual component to rhythmic bimanual tapping which has not been fully appreciated in attempts to link asymmetric rhythmic control with neural activity. They also demonstrate the importance of considering the movement strategy used to achieve task goals when investigating principles of bimanual coordination.

Chapter 4: The aim of chapter 4 was to determine whether right PMd is specifically involved in programming rhythmic asymmetric bimanual movements, or generally involved in managing cognitive load. Findings from chapter 2 provided a platform to specifically test these competing hypotheses. The excitability of right PMd was transiently reduced using 1 Hertz repetitive TMS and participants performed a truncated version of the rhythmic bimanual finger tapping task developed in chapter 2. Task performance was compared against a second sham stimulation session. Neither of the competing hypotheses were supported. Instead, 1 Hz rTMS was found to have no significant effect on the stability of bimanual rhythmic finger movements. In the context
of previous research which examined the effect of rTMS over PMd in a decision-making paradigm\textsuperscript{166}, this result suggests that the brain is able to compensate for challenge to right PMd to maintain rhythmic bimanual behaviours.

5.2 Synthesis of main findings

When considered together, these findings make several novel contributions to our understanding of the functional role of PMd in asymmetric rhythmic bimanual control. Behavioural results from chapters 2 and 3 clearly demonstrate that the manipulation of cognitive-perceptual task elements can have powerful effects on rhythmic bimanual coordination principles. This emphasizes the importance of considering the degree to which central cognitive resources are generally taxed when investigating how symmetric and asymmetric bimanual movements are controlled by the brain. The finding that movement quality may have underpinned a change in coordination principles also underscores the importance of explicitly considering movement strategy when designing investigations aimed at elucidating the neural control of rhythmic movements.

Formal consideration of cognitive-perceptual factors facilitated an experimental design which was capable of separating the change in kinematics between symmetric and asymmetric movements from the general increase in cognitive load between the two movement patterns. This allowed a direct test of competing hypotheses regarding the specific role of right PMd in asymmetric rhythmic bimanual control. Specifically, it facilitated a test of whether right PMd is responsible for programming of asymmetric actions or whether it is responsible for managing cognitive load during asymmetric rhythmic bimanual movement. Rather than finding support for either hypothesis, disruption of right PMd with rTMS had no effect on rhythmic bimanual control. This can be seen as a rejection of the premise that right PMd alone is of critical importance for asymmetric rhythmic bimanual control. Instead, it suggests that the control of rhythmic bimanual
movements should be conceptualized as carried out by a distributed network of brain regions. While PMd may be a critical node within this network, my results suggest that it cannot be easily separated from the action of the network as a whole.

PMD-M1 interhemispheric circuits have been proposed as mechanism through which PMd organizes bilateral descending corticospinal output during asymmetric rhythmic bimanual behaviours. However, this proposal is based on a comparison between unimanual preparatory related changes in PMd-M1 interhemispheric inhibition and asymmetric rhythmic bimanual performance. The finding in chapter 2 that PMd-M1 interhemispheric circuits are not active during preparation of simple bimanual movements casts doubt on the notion that this circuit is directly involved in shaping descending output during asymmetric rhythmic bimanual movements. Instead, the degree of release of PMd-M1 IHI in response to movement cues may represent a marker of general cognitive efficiency, i.e., the capacity to process task-relevant information in a fast and efficient manner. Increased cognitive efficiency as indexed during simple unimanual RT tasks could have benefits during cognitively demanding and unfamiliar motor tasks, such as asymmetric rhythmic bimanual tasks. Results from chapter 4 further support this viewpoint. Presumably right PMd-left M1 interhemispheric circuits were disrupted by the application of rTMS over right PMd, yet no change in behaviour was observed.

While the degree to which PMd-M1 interhemispheric circuits are actually recruited during rhythmic bimanual movements remains an open question, results from chapter 2 did demonstrate that PMd is capable of influencing contralateral corticospinal excitability. PMd-M1 IHI was shown to be transiently released 50 ms after the presentation of an imperative “GO” stimulus during unimanual preparation, which replicated results from several previous investigations. Importantly, this capacity was found in both left and right PMd. These findings are particularly interesting in
the context of results from chapter 4, which suggest that compensation in other brain regions beyond right PMd can sustain behavioural output after rTMS-induced inhibition of right PMd. Left PMd may have been one such node in the distributed compensatory network. Therefore, given that left PMd was shown to be capable of influencing right hemisphere corticospinal excitability in chapter 2, compensatory processes may have been facilitated by left PMd-right M1 interhemispheric circuits.

5.3 Limitations

There are several overarching limitations of this dissertation work to consider, in addition to the study-specific limitations that have been discussed in each chapter. The primary limitation of this thesis is that there was no concurrent measure of neural activity taken after application of rTMS in the experiment described in chapter 4. Therefore, while we have discussed the possibility that compensation in cortical areas beyond right PMd may have sustained behavioural output after neural challenge to right PMd at length, this idea is based solely off results from other investigations. There are plausible alternative explanations for why there was a null effect of rTMS on behaviour. For example, rTMS may simply have had no effect on activity in right PMd, or rTMS may have had mixed effects between participants which clouded statistical outcomes. One Hz rTMS has been shown to have variable effects on brain activity between subjects when delivered over M1, although it is not well established if this is also the case with PMd. The collection of concurrent measures of neural activity in PMd and beyond would have allowed us to establish if neural compensation actually occurred, and parse out potential individual differences in response to rTMS over right PMd. This would have provided a deeper understanding of the relationship between right PMd activity and the bimanual behaviours of interest. Functional
MRI and the dual coil TMS assessments used in chapter 2 are both well placed to address this limitation in future work.

Comparison of behavioural data collected in chapters 3 and 4 revealed another limitation regarding the design of the rhythmic bimanual tasks used in chapter 4. In chapter 4, there was a failure to replicate the beneficial effect of spatial congruence on rhythmic bimanual tapping stability observed in chapter 3. This seems to have been driven by collecting task data at too few levels of movement frequency. Due to a desire to constrain the total time limit of the experiment to fit within the predicted time frame of the transient effect of rTMS, the number of levels of movement frequency assessed for each version of the task was cut from 12 (in chapter 3 experiments) to 3 (for chapter 4 experiments). It appears that cutting from 12 to 6 would have been more appropriate, as this would have ensured the behavioural effect of interest was not lost to measurement noise.

In the context of the thesis as a whole, a limitation of chapter 2 is that changes in PMd-M1 interhemispheric inhibition during preparation were assessed during only 2 types of movement, i.e., discrete unimanual and symmetric bimanual movements. Given that we were primarily interested in the difference between symmetric and asymmetric bimanual control, an additional condition where participants performed asymmetric bimanual button presses would have added significant value. Furthermore, given that our findings from chapter 3 indicated that movement strategy evolves from repeated discrete movements to continuous movement with increasing movement frequency, knowledge on whether PMd-M1 interhemispheric interactions differ during preparation of these distinct classes of movement would have also helped to better establish a link between these circuits and rhythmic bimanual control. However, it should be noted additional task conditions in the described dual coil TMS paradigms used to measure PMd-M1 IHI incurs a
significant increase in experimental time, given that a large number of trials are needed to overcome measurement noise inherent to motor evoked potentials. Nevertheless, such task manipulations should be explored in future work to further build out our understanding of the function of PMd-M1 interhemispheric circuits in behaviour.

5.4 Future directions

A synthesis of the main findings and the consideration of thesis limitations demonstrates several avenues for future investigations that will further the understanding of the neural control of bimanual movement.

5.4.1 Indexing neural activity during rhythmic bimanual finger tapping after rTMS over PMd with fMRI

Given that it was demonstrated in chapter 4 that rhythmic bimanual finger coordination was not affected by rTMS over right PMd regardless of whether asymmetric responses were required or how action was cued by task stimuli, the natural next step to further probe this effect is to repeat the experiment with concurrent neuroimaging capable of indexing neural activity during behaviour. Functional MRI is well placed to achieve this goal, since it can map activity of the entire cortex with excellent spatial resolution, which may be necessary to account for the multiple regions that could be involved in compensating for neuronal challenge to rTMS. The finger tapping tasks use in the current thesis have already been shown to be amenable for translation to MRI scanner environments. Additionally, existing work has established a network of regions beyond right PMd that are active during rhythmic bimanual behaviours, which would allow hypotheses to be highly specific regarding where compensatory activity would occur in the brain. The suitability of fMRI in this regard has been demonstrated in previous work that successfully identified compensatory processes after application of rTMS over left PMd in a decision-making
paradigm\textsuperscript{166}. The addition of concurrent fMRI data would distinguish compensation across a network of brain regions from the possibility that rTMS simply had no effect. It would also facilitate a test of the presence of responders and non-responders to rTMS in the dataset, and control for the possibility that the effect of rTMS on cortical activity was mixed between participants.

5.4.2 Assessing the effect of rTMS induced inhibition of PMd on preparatory related activity in PMd-M1 interhemispheric circuits

Measuring the effects of rTMS over PMd on preparatory related changes in PMd-M1 interhemispheric circuits could help to more directly test if these circuits are involved in the control of rhythmic bimanual movements. It could also shed light on whether PMd-M1 interhemispheric circuits are important for enacting compensatory processes after disruption of right PMd. A previous finding that single pulses of TMS delivered over right PMd “online” slow choice reaction time only after 1 Hz rTMS has been delivered over left PMd suggests that contralateral PMd may a particularly critical region for facilitating compensation that sustains behavioural output\textsuperscript{166}. Therefore, it is possible that PMd may increase input to contralateral M1 after rTMS induced inhibition of contralateral PMd. Such increased input to contralateral M1 may be achieved via modulation of activity through the interhemispheric circuits probed in chapter 2. To address this question, the experiment outlined in chapter 4 could be repeated, but additional assessments used to measure preparatory related changes in PMd-M1 interhemispheric circuits used in chapter 2 could be added as post rTMS assessments. Measuring PMd-M1 IHI at rest could also serve as a potential index of individual sensitivity to rTMS. The bimanual 4-choice RT task could be removed to keep the experiment within the time period of transient inhibitory effects of 1 hertz rTMS. If the delivery of rTMS over right PMd was associated with an increase in the release of left PMd-right
M1 during motor preparation, this would be evidence that these circuits are important for rhythmic bimanual control and for enacting compensation. If the degree of change mapped to the degree of change in asymmetric rhythmic tapping performance versus sham stimulation, this would further strengthen the claim.

Beyond furthering our understanding of the relationship between PMd and asymmetric rhythmic bimanual control, this hypothetical study would help to address a broader question regarding the functional role of bilateral redundancy in the control of hand movements. Studies using electrocorticography\textsuperscript{149}, single neuron recordings\textsuperscript{150}, and fMRI\textsuperscript{151} all indicate that many unimanual finger, hand and arm movements are represented bilaterally in the brain. One hypothesis arising from this previous work is that bilateral redundancy allows for maintenance of motor control processes after unilateral injury to the brain, particularly early in recovery\textsuperscript{168,169}. Therefore, establishing whether PMd-M1 interhemispheric circuits up-regulate their activity in response to unilateral neuronal challenge by rTMS would provide further evidence for this hypothesis.

### 5.4.3 Uncovering the limits of neural compensation

A central finding of the current thesis is that network-based approaches that consider single regions as nodes in a larger distributed system are needed to effectively answer questions regarding the neural implementation of bimanual coordination principles. The larger distributed system may be capable of compensating for challenge to any one node to sustain behavioural output. An important issue arising from this perspective is establishing the limits of compensation. Presumably if neuronal challenge is present in enough nodes of the distributed system eventually there would be a detrimental effect on behaviour. For example, if contralateral left PMd compensates for a rTMS induced challenge to right PMd, perhaps simultaneous disruption of left PMd would be associated with detriments to behavioural performance. Questions of this nature could be addressed by
applying rTMS over two cortical regions of interest simultaneously and comparing behavioural performance conditions in which rTMS is delivered over one region alone. By assessing effects of delivering rTMS over multiple pairs of regions in the hypothesized network, it may be possible to build a comprehensive picture of the limits of neural compensation in the context of complex behaviours such as asymmetric bimanual rhythmic finger movements. While such an approach may involve a high number of stimulation conditions, it may be necessary to gain meaningful insight into how these behaviours are controlled by the brain.

5.4.4 Exploring the relationship between movement strategy and bimanual coupling accuracy using explicit instructions

A key finding from chapter 3 was that increasing movement frequency in the bimanual rhythmic finger tapping task was associated with a shift in movement strategy from repeated discrete movements to continuous movement. In the context of previous research indicating these two types of movement are controlled by distinct neuro-cognitive systems\textsuperscript{42,43,46,48,49}, this change in strategy appears to have driven a change in the effect of response symmetry and spatial congruence of frequency cues on temporal coupling at higher frequencies. However, given that we did not systematically manipulate movement strategy, we cannot rule out that the change in effect of coupling was driven by movement frequency alone. To address this issue, participants could be asked to repeat the tasks as described, but with explicit instructions on what movement strategy to use throughout. If participants were required to try to adopt a repeated discrete strategy at all movement frequencies, instead of implicitly self-selecting strategies, perhaps the effects of spatial congruence and response symmetry would be diminished. Similarly, if participants were required to adopt a continuous strategy at all frequencies, perhaps these effects would emerge at lower
frequencies than we observed in the current thesis. These manipulations would help to establish whether the relationship between movement strategy and bimanual coupling are causally related.

5.4.5 Assessing how movement quality influences preparatory related changes in PMd-M1 interhemispheric circuits

The current thesis assessed preparatory related changes in PMd-M1 interhemispheric circuits during preparation of simple discrete finger movements, and how bimanual task requirements alter these changes. Another avenue for future investigations is to assess how movement quality affects preparatory related changes in inhibitory output. As discussed, previous research indicates discrete and continuous movements are controlled by distinct neural systems. Therefore, indexing whether activity in PMd-M1 interhemispheric circuits differs between preparation of discrete and continuous movements will help to increase understanding of the functional role of these circuits to behaviour. Given that breakdowns in the stability of asymmetric rhythmic bimanual finger movements tends to occur at frequencies where a continuous strategy is adopted, this may be a more appropriate type of movement preparation to relate to measurements of rhythmic bimanual control. The previous finding of a relationship between changes in PMd-M1 IHI during preparation of asymmetric bimanual continuous movements and subsequent movement accuracy strengthens this claim.

5.5 Conclusion

The coordination of asymmetric bimanual actions depends on interaction between many brain regions. Cognitive-perceptual factors can have strong effects on bimanual coordination principles. Changing how bimanual actions are cognitively represented by manipulating the perceptual quality of stimuli used to cue action is an effective way to parse out whether relationships between brain activity and asymmetric bimanual control are specific to asymmetric bimanual control or if they
are driven by the use of general cognitive functions leveraged across many types of motor control. Functional neuroimaging has demonstrated that a wide array of brain regions is active during asymmetric rhythmic bimanual behaviours, but subsequent brain stimulation studies indicated that right PMd may be of critical importance. While we set out to establish if right PMd was specifically critical as a: (1) programmer of asymmetric movement or a (2) manager of cognitive load, results from the current study speak against a single region view. Instead, they indicate that (3) PMd is one node in a larger distributed neural system with built in redundancies capable of compensating for neuronal challenge to right PMd. Ultimately, this emphasizes the need to move to network-based approaches in the ongoing mission to understand how bimanual coordination is realized by the brain.
Bibliography


70. Ugawa, Y., Hanajima, R. & Kanazawa, I. Interhemispheric facilitation of the hand area of 

71. Ni, Z. *et al.* Two phases of interhemispheric inhibition between motor related cortical areas 

72. Derosiere, G. & Duque, J. Tuning the Corticospinal System: How Distributed Brain 

73. Civardi, C., Cantello, R., Asselman, P. & Rothwell, J. C. Transcranial magnetic stimulation 
can be used to test connections to primary motor areas from frontal and medial cortex in 

74. Groppa, S. *et al.* The human dorsal premotor cortex facilitates the excitability of ipsilateral 
430 (2012).

75. Groppa, S. *et al.* A novel dual-site transcranial magnetic stimulation paradigm to probe fast 
facilitatory inputs from ipsilateral dorsal premotor cortex to primary motor cortex. 

76. Mochizuki, H., Huang, Y. & Rothwell, J. C. Interhemispheric interaction between human 

77. Koch, G. *et al.* Time course of functional connectivity between dorsal premotor and 

78. Bäumer, T. *et al.* Magnetic stimulation of human premotor or motor cortex produces 


154. Brinkman, C. Supplementary motor area of the monkey’s cerebral cortex: short- and long-
term deficits after unilateral ablation and the effects of subsequent callosal section. J. 

155. Kazennikov, O. et al. Effects of lesions in the mesial frontal cortex on bimanual co-

156. Wiesendanger, M., Wicki, U. & Rouiller, E. 9 - Are There Unifying Structures in the Brain 
Responsible for Interlimb Coordination? in Interlimb Coordination (eds. Swinnen, S. P., 
doi:10.1016/B978-0-12-679270-6.50014-0.

supplementary motor area and primary motor cortex: a paired-coil TMS study. Exp. Brain 

158. Rurak, B. K., Rodrigues, J. P., Power, B. D., Drummond, P. D. & Vallence, A. M. Test Re-
test Reliability of Dual-site TMS Measures of SMA-M1 Connectivity Differs Across Inter-

159. Rurak, B. K., Rodrigues, J. P., Power, B. D., Drummond, P. D. & Vallence, A.-M. Reduced 
SMA-M1 connectivity in older than younger adults measured using dual-site TMS. Eur. J. 

Associated with Motor Learning Are Somatotopic Specific. J. Neurosci. 37, 2377–2386 
(2017).


