Mirror Activity in the Macaque Motor System

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Doctor of Philosophy

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DEDICATION

This thesis is dedicated to the memory of Hagop Vartzbedian.
Declaration

I, Steven Jack Jerjian, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. The work was completed without assistance except:

1. Critical stages of surgical procedures were performed by Dr Alexander Kraskov.

2. The experimental work was performed as part of an ongoing research program in Dr Kraskov’s laboratory. All stages involved discussion and collaboration with members of the research group.

3. Experiments in chapter 6 were performed in collaboration with Dr Marco Davare.

4. MRI scans were acquired with assistance from Dr David Thomas.

5. Spike discrimination software, MRI maps, and some assistance with programming, were provided by Dr Alexander Kraskov.

Signature: ....................................
Abstract

Mirror neurons (MirNs) within ventral premotor cortex (PMv) and primary motor cortex (M1), including pyramidal tract neurons (PTNs) projecting to the spinal cord, modulate their activity during both the execution and observation of motor acts. However, movement is not produced in the latter condition, and mirror responses cannot be explained by low-level muscle activity. Relatively reduced activity in M1 during observation may help to suppress movement.

Here, we examined the extent to which activity at different stages of action observation reflects grasp representation and suppression of movement across multiple levels of the mirror system in monkeys and humans. We recorded MirNs in M1 and F5 (rostral PMv), including identified PTNs, in two macaque monkeys as they performed, observed, and withheld reach-to-grasp actions. Time-varying population activity was more distinct between execution and observation in M1 than in F5, and M1 activity in the lead-up to the observation of movement onset shared parallels with movement withholding activity. In separate experiments, modulation of short-latency responses evoked in hand muscles by pyramidal tract stimulation revealed modest grasp-specific facilitation at the spinal level during grasp observation. This contrasted with a relative suppression of excitability prior to observed movement onset or when monkeys simply withheld movement. Additional cortical recording experiments examined how contextual factors, such as observing to imitate, observing while engaged in action, or observation with reduced visual information, modulated mirror activity in M1 and F5. Finally, single-pulse transcranial magnetic stimulation (TMS) in healthy human volunteers was used to examine changes in corticospinal excitability (CSE) during action observation and withholding.

Overall, the results reveal distinctions in the profile of mirror activity across premotor and motor areas. While F5 maintains a more abstract representation of grasp independent of the acting agent, a balance of excitation and inhibition in motor cortex and spinal circuitry during action observation may support a flexible dissociation between initiation of grasping actions and representation of observed grasp.
Impact statement

"Every representation of a movement awakens in some degree the actual movement which is its object."

Principles of psychology (James, 1890)

Mirror neurons in the primate brain modulate their activity during the execution of grasping actions, and also during the passive observation of actions performed by another agent. This intriguing property provides a direct instantiation of a link between perception and action, espoused by psychology at least since the days of William James.

Although action observation does not produce any movement in the observer, mirror activity extends to pyramidal tract neurons, which project directly to the spinal cord and are intimately associated with spinal circuits for movement control. This challenges our understanding of how the brain distinguishes signals for self-movement generation from those related to other’s movements.

The work presented in this thesis addresses this issue by examining the profile of mirror activity at multiple levels of the macaque motor system. We found evidence that the time-course of mirror neuron activity flexibly supports both the suppression of movement and the shared representation of executed and observed grasping actions. Building on previous work, we identified distinctions between ventral premotor and primary motor cortex mirror neurons in this regard, and went on to show that activity patterns at the cortical level are recapitulated in sub-threshold modulations in the spinal pools innervating hand muscles during action observation. This dissociation between signals related to movement initiation and grasp representation may be important in the context of distinguishing self and other actions. In one additional study, we found that observation activity during ongoing movement is superimposed on activity during execution in premotor, but not motor cortex mirror neurons. Overall, the data presented advance our knowledge of the contributions of different areas within motor circuitry during action observation and withholding.
Study of the mirror neuron system and its role in movement suppression during action observation may have important clinical applications. Many devastating neurological disorders are characterised by excessive or impaired movement due to damage or abnormal activity within motor circuitry. For example, cortical signals related to movement generation, action observation, and motor imagery are often intact within tetraplegic patients, and therefore provide potentially useful control signals for brain-machine interface technologies. The results in this thesis also provide a framework for studying mirror activity in sub-cortical areas with connections to motor cortex, such as the subthalamic nucleus. A greater understanding of the functional role of this area in movement generation and suppression could provide new insights into mechanisms underlying the pathological slowing of movements in Parkinson’s disease and the therapeutic effects of deep brain stimulation.
Acknowledgements

Good science is a collaborative endeavour, and it is no understatement to say that the work presented in this thesis would have suffered tremendously without the support of many colleagues, friends and family. First and foremost, I am sincerely grateful to my primary advisor, Alexander (Sasha) Kraskov, who diligently taught, guided, and trusted me over the course of a challenging project. You welcomed my questions and ideas, pushed me to think critically and explain myself clearly, and fostered my independence in many aspects of research. Maneesh Sahani provided useful feedback on the more mathematical aspects of the thesis, anchored me to the bigger picture which is all too easy to lose sight of, and gave me the opportunity to present at lab meetings. It was a privilege and inspiration to share in the knowledge, stories and humour of Roger Lemon, whose wisdom arrived at critical junctures on several occasions. Marco Davare, Stephan Waldert and Ganesh Vigneswaran were all generous with their advice and support. Tabatha Lawton patiently taught me how to train the monkeys, and how to handle (and enjoy!) their unique personalities. Dominika Klisko, Lianne McCombe, Adam Keeler, Alexandra Jagla and Yeung-Yeung Leung all also helped greatly with animal care and management over the years. I owe special thanks to Jonathon Henton and Spencer Neal for their engineering skills, Stuart Baker for his help with EMG surgeries, David Thomas for help with MRIs, Martin Lawton for his veterinary expertise, and Deborah Hadley, Ligia Diana-Craciun, Imran Sayed, Chris Seers, and Julie Savidan for administrative and other assistance. The PhD itself was made possible through funding from Brain Research UK.

On a more personal level, I have been blessed with wonderful and supportive friendships within and beyond Queen Square, and I hope these will continue to grow in the years to come. Football, climbing, the fifth floor cake club, Friday pub trips, and the occasional karaoke sessions were great fun, and provided welcome breaks during difficult periods. Finally, I owe a debt to my family and loved ones, especially Mum, Dad, my brother and sisters, and Ellie. You always believed in me, more so when I myself did not. You encouraged my curiosity, celebrated my successes as your own and reminded me that, with hard work, all hurdles could be surmounted. I could not have done it without you.
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<td>1DI</td>
<td>first dorsal interosseous</td>
</tr>
<tr>
<td>5-FU</td>
<td>5-fluorouracil</td>
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<tr>
<td>AbDM</td>
<td>abductor digiti minimi</td>
</tr>
<tr>
<td>AbPB</td>
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<td>abductor pollicis longus</td>
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<td>ADL</td>
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<td>BA</td>
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<td>Blood Oxygen Level-Dependent</td>
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<td>DO</td>
<td>displacement onset</td>
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<td>HO</td>
<td>hold onset</td>
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<td>ICMS</td>
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<td>IFC</td>
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<td>IP</td>
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<td>IPL</td>
<td>inferior parietal lobule</td>
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<tr>
<td>ITI</td>
<td>inter-trial interval</td>
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<td>LFP</td>
<td>local field potential</td>
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<td>medial longitudinal fasciculus</td>
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<td>MNS</td>
<td>mirror neuron system</td>
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<td>MRI</td>
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<td>MTL</td>
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**List of Abbreviations**

- PMv: ventral premotor cortex
- PMRF: ponto-medullary reticular formation
- PPC: posterior parietal cortex
- PSpF: post-spike facilitation
- PSTF: post-stimulus facilitation
- PSTH: peri-stimulus time histogram
- PSTS: post-stimulus suppression
- PT: pyramidal tract
  - PTN: pyramidal tract neuron
- RST: reticulospinal tract
- SICI: short intra-cortical inhibition
- sIN: segmental interneuron
- SMA: supplementary motor area
- SPL: superior parietal lobule
- SpTA: spike-triggered average
- STN: subthalamic nucleus
- STS: superior temporal sulcus
- StTA: stimulus-triggered average
- SVD: singular value decomposition
- TMS: transcranial magnetic stimulation
  - pp-TMS: paired pulse TMS
  - rTMS: repetitive transcranial magnetic stimulation
- UID: unidentified neuron
- WHG: whole-hand grasp
1. Introduction

1.1. The primate hand

1.1.1. The importance of the hand

Skilled use of the hand is a hallmark of the primate and human motor repertoire, providing unique capacities for interaction with the external environment. In his 1871 treatise on 'The Descent of Man', Charles Darwin proposed that the freedom and dexterity of the hands, along with bipedality and larger brains, was a critical factor in the evolution of early humans (Darwin, 1871). The eminent neurologist Hughlings Jackson considered hand motor function to be among the most complex evolved aspects of the nervous system (Jackson, 1884). The breadth and depth of possible hand movements requires a well-developed control system (Schieber & Santello, 2004) and demands large resources, which is borne out by the disproportionate representation of the hand within the motor cortex (Penfield & Boldrey, 1937). The system underlying hand control is particularly vulnerable to injury and disease (Jackson, 1884; Eisen et al., 2014), and the importance of the hand is exemplified by the fact that quadriplegic patients overwhelmingly give highest priority to regain of hand and arm function (Anderson, 2004).

1.1.2. The organisation of the motor system controlling the hand

Early insights into the neural organisation of hand control were derived from studies of cytoarchitecture and anatomically identified pathways, as well as stimulation and lesion studies. Brodmann, (1909) identified two main anatomical areas (Brodmann Area (BA) 4 and 6) anterior to the central sulcus, which lacked a granular layer IV. Surface stimulation of these electrically excitable areas in monkeys and humans led to an approximate definition of the more caudal area 4 as primary motor cortex (M1). The more rostral BA6 was divided into the premotor cortex on the lateral surface, and the supplementary motor area (SMA) on the medial wall. Both premotor and primary motor cortex were found to contain broad
1.2. The cortical control of grasp

somatotopic representations of the face, and proximal and distal parts of the body (Penfield & Rasmussen, 1950; Woolsey et al., 1952), although further anatomical and physiological work has led to a finer parcellation of these areas (Matelli et al., 1985; Rizzolatti et al., 1998, see Figure 1.1, and following sections). Many pyramidal cells within layer V of these areas, particularly from primary motor cortex (M1), send projections to the spinal cord (Dum & Strick, 1991), and show a distributed termination within the spinal grey matter, primarily on the contralateral side (Kuypers, 1981; Porter & Lemon, 1993; Morecraft et al., 2013). Despite being relatively small in number, these neurons have long been regarded as overwhelmingly important for skilled control of the hand (Porter & Lemon, 1993). In pioneering lesion studies, Lawrence & Kuypers demonstrated that monkeys with bilateral lesions to the medullary pyramid can largely recover independent gross limb movements, but suffer irreversible damage to fine, fractionated movements of the fingers (Lawrence & Kuypers, 1968a). In the years since, the advent of methods for stable recordings of single neurons in awake, behaving animals, and the capability for more precise mapping of outputs and functions using stimulation and non-invasive imaging techniques, has heralded substantial work. This has led to refinement and re-evaluation of our understanding of the cortical and spinal contributions to the complex and varied repertoire of skilled hand movements in primates and humans (Schieber, 2011).

1.2. The cortical control of grasp

In order to successfully interact with objects in our environment through grasping actions, the brain must first extract, via visual input, relevant properties such as size, shape, texture, and orientation, and spatial location. Progressive visuomotor transformations must then take place in order to produce appropriate muscle commands for transportation of the limb through space, shaping of the hand, and accurate prehension of fingers around the object (Jeannerod et al., 1995; Fagg & Arbib, 1998). This process is typically performed effortlessly and automatically in healthy adults, yet the complex transformations described are mediated on a rapid timescale by an extensive and interconnected circuitry spanning cerebral neocortex, sub-cortical motor centres within the cerebellum and basal ganglia, and a sophisticated spinal machinery.
1.2. The cortical control of grasp

Within the circuitry underlying this transition from perception to action, it has been evident for some time that object-related visual information diverges early on via two main cortico-cortical pathways. A ventral stream, linking primary and secondary visual areas to inferotemporal cortex via area V4, was initially hypothesised to support object recognition, while a more dorsal stream, projecting to inferior parietal areas, was proposed to underpin perception of object location (Ungerleider & Mishkin, 1982). The distinct deficits present in human patients and lesioned monkeys with damage within each pathway led to a reformulation of the “what” and “where” description, with greater emphasis placed on the output functions of these pathways, rather than their perceptual aspects. Within this influential framework, the ventral stream represents semantic features of the target object, while the dorsal stream encodes pragmatic information regarding how to effectively grasp the object (Goodale & Milner, 1992; Jeannerod, 1994). In simple terms, the ventral stream supports perception, and the dorsal stream provides information for action, although interaction between these pathways is undoubtedly fundamental in order to generate unified percepts and facilitate online control of complex object-oriented actions (van Polanen & Davare, 2015). The dorsal stream has been further subdivided along the medial-lateral axis (Figure 1.1), with a dorsomedial circuit projecting from medial intraparietal area (MIP) and V6A in the superior parietal lobule to dorsal premotor cortex (PMd) more involved in reach planning, and the lateral part (anterior intraparietal area (AIP)-F5) more associated with grasp (Tanne-Gariépy et al., 2002; Rizzolatti & Matelli, 2003; Borra et al., 2008; Grafton, 2010). However, the real-time execution of reach and grasp evolves as an interactive and co-ordinated process, and the neuro-anatomical and physiological segregation of these two pathways is far from complete (Rozzi et al., 2006; Borra et al., 2008; Fattori et al., 2010; Davare et al., 2011). In the following sections, I appraise the anatomical and functional properties of key nodes within the primate cortical grasping network, and discuss the flow of information between these nodes.
1.2. The cortical control of grasp

1.2.1. Areas of the cortical grasping network

Parietal Cortex

Anterior Intraparietal Area

AIP is located rostrally within the anterolateral bank of the intraparietal sulcus (IPS), and is strongly interconnected with premotor area F5 (Luppino et al., 1999; Borra et al., 2008; Gerbella et al., 2011). It also has local connections with other areas within the inferior parietal lobule (IPL) and the lateral intraparietal area (LIP), as well as prefrontal areas 46 and 12 (Borra et al., 2008). Early electrophysiology studies indicated that neurons within the posterior parietal cortex become active during hand manipulation (Hyvärinen & Poranen, 1974; Mountcastle et al., 1975), and lesions in the inferior parietal area impair reaching and grasping actions (Haaxma & Kuypers, 1975). Building on this work, the response properties of single neurons in area AIP within the IPS have been extensively studied while monkeys viewed and grasped three-dimensional objects of different shapes and sizes in light and dark conditions (Taira et al., 1990; Sakata et al., 1995; Murata et al., 2000). These studies revealed the presence of distinct subtypes of neurons, whose responses were primarily motor, visual, or both visual and motor. Motor neurons fire similarly during object manipulation in light and dark settings and visual neurons do not fire during object manipulation in the dark. Visuomotor neurons lie in between, firing in both light and dark settings, but to a lesser degree in the dark (Sakata et al., 1995; Murata et al., 2000). AIP responses during hand manipulation are often selective for specific 3-D objects, grasps, and orientations (Murata et al., 2000; Raos et al., 2006; Baumann et al., 2009), and a recent multi-dimensional approach involving large simultaneous recordings and a wide range of objects concluded that AIP neurons primarily encode the visual properties, such as shape, of graspable objects (Schaffelhofer & Scherberger, 2016). Reversible inactivation of AIP using muscimol, an agonist of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), results in pronounced deficits in appropriate initial shaping of the contralateral hand for grasp (Gallese et al., 1994).
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Ventral Intraparietal Area

The ventral intraparietal area (VIP) receives input from areas in the dorsal visual stream, such as the middle temporal area (MT/V5) (Maunsell & Van Essen, 1983), and from somatosensory areas within the IPL (Seltzer & Pandya, 1986). Neurons in VIP are sensitive to the direction and speed of moving stimuli (Colby et al., 1993), and have unimodal tactile, visual, or bimodal receptive fields. Visual receptive fields are head-centred, and tactile and visual receptive fields within bimodal cells are often closely aligned (Duhamel et al., 1998). This area has therefore often been associated with the representation of peri-personal space within a head-centred reference frame (Colby et al., 1993; Duhamel et al., 1998).

![Figure 1.1. Lateral view of the macaque brain](image)

**Figure 1.1. Lateral view of the macaque brain.** Frontal areas F1-F7 are classified according to Matelli et al., (1985). The intraparietal sulcus (IPS) is shown unfolded, with the dotted line denoting the fundus of the sulcus. The traditional, reciprocally connected, dorsolateral grasping (AIP/PFG, F5 and M1 (F1)), and dorsomedial reaching (MIP/V6A, F2 (PMd), and F1) circuits are shown in red and blue, respectively. ASs, superior arcuate sulcus; ASi, inferior arcuate sulcus; CS, central sulcus; IPS, intraparietal sulcus; STS, superior temporal sulcus; AIP, anterior intraparietal area; MIP, medial intraparietal area; VIP, ventral intraparietal area. Areas PF, PFG & PG constitute the convexity of the inferior parietal lobule. a-p anterior-posterior, d-v dorsal-ventral
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**Inferior Parietal Lobule**

The IPL convexity can be divided somatotopically along its rostro-caudal axis, into three areas (PF, PFG, and PG), with overlapping representations of mouth, hand and arm movements, respectively (Rozzi et al., 2008). Neurons within area PFG in the IPL modulate their discharge during hand grasping actions, and appear to do so depending on the higher-order goal, or context, of the motor act in which the grasp is embedded (Fogassi et al., 2005; Rozzi et al., 2008; Bonini et al., 2010, 2011, 2012). In these studies, neurons in PFG have typically been recorded during grasping actions that form part of a sequence which ends with different goals of placing or eating, and many of these neurons show selectivity for one or other of the action goals. In PFG goal-selective neurons, later peaks in activity are associated with higher preferences (Bonini et al., 2010). Important control tests in which the placing container is located near the mouth indicate that this action goal selectivity is not trivially due to low-level differences in action parameters, such as kinematics.

**Superior Parietal Lobule**

Areas MIP and V6A in the superior parietal lobule (SPL) constitute part of the dorso-medial pathway within posterior parietal cortex, and through their projections to PMd, are thought to be involved in reaching control (Eskandar & Assad, 1999; Rizzolatti & Matelli, 2003; Galletti et al., 2003). V6A neurons modulate their activity during reaching in light and dark conditions (Fattori et al., 2001; Galletti et al., 2003), but have also been shown to be selective for different grasp types and presented objects (Galletti et al., 2003; Fattori et al., 2010, 2012; Breveglieri et al., 2015). V6A and the dorsomedial visual pathway are therefore likely to be involved in aspects of reach-to-grasp movements spanning both transport and shaping of the hand.
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Ventral Premotor Cortex

F5

Area F5 is located in the rostral part of the ventral premotor cortex (PMv), within the posterior bank of the inferior limb of the arcuate sulcus. It shares strong reciprocal connectivity with areas of the posterior parietal cortex (Luppino et al., 1999; Rizzolatti & Luppino, 2001; Borra et al., 2008; Gerbella et al., 2011) and M1 (Muakkassa & Strick, 1979; Godschalk et al., 1984; Matelli et al., 1986; Dum & Strick, 2005), as well as direct and indirect connections with other premotor and prefrontal areas (Rizzolatti & Luppino, 2001; Gerbella et al., 2011; Kurata, 2018).

F5 activity has long been associated with visual guidance of the hand (Godschalk et al., 1981), and many F5 motor neurons respond to hand and mouth actions which achieve a particular goal, such as grasping, tearing and holding. Within a category such as grasping, neurons can often be subdivided further, for example into those selective for precision grip or whole-hand grasp (Rizzolatti et al., 1987, 1988). On this basis, it has been proposed that F5 contains a vocabulary of motor actions, or “motor prototypes”, which encode particular grasping actions or parts of actions (Rizzolatti et al., 1988; Rizzolatti & Luppino, 2001). Evidence for goal encoding in F5 neurons was supported by a clever study which established that, following a period of training, F5 neurons respond similarly during the use of normal pliers and “reverse” pliers to perform a grasping action with the same goal, even though the intrinsic motor commands to achieve the goal are necessarily different in the two conditions (Umiltá et al., 2008). In parallel with their recordings in IPL, Bonini and colleagues have reported that F5 neurons are often selective for the final goal of grasping actions (Bonini et al., 2010, 2011, 2012).

Inactivation of the bank region of F5 via muscimol injection produces deficits in hand preshaping for grasp, which are only corrected after tactile contact with the object, causally linking F5 to the visuomotor transformations necessary for grasping (Fogassi et al., 2001). Detailed assessments of the motor and visual responses in F5 neurons during grasping of 3-D objects during the same paradigm used to study AIP neurons has identified motor and visual-motor neurons in F5, which display preferences for specific grasps, or

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1.2. The cortical control of grasp

defined sets of grasps (Murata et al., 1997; Raos et al., 2006). The visual-motor neurons are commonly known as “canonical” neurons, and frequently show matched selectivity in the visual and motor conditions, thus providing a critical link between the visual properties of graspable objects, and the motor action effective for actually grasping them. A further group of F5 motor neurons also show visual responses to the observation of the grasping actions of others (Gallese et al., 1996; Rizzolatti et al., 1996a). These “mirror neurons” are discussed in detail in section 1.4. The relatively higher proportion of motor neurons in F5 relative to AIP, and the lack of purely visual responses in F5, have underpinned a framework in which AIP provides F5 with affordances based on the visual properties of objects, from which F5 then selects the appropriate motor prototype for grasping (Fagg & Arbib, 1998; Rizzolatti & Luppino, 2001; Schaffelhofer & Scherberger, 2016). The critical role of F5 in these visuomotor transformations has been confirmed and extended by numerous studies. Umiltá and colleagues found that F5 neurons exhibit strong object-grasp specificity, which begins prior to movement (during object presentation), and persists into the grasping stages of the task (Umiltá et al., 2007). The multi-dimensional approach adopted by Schaffelhofer & Scherberger, (2016) found that F5 neurons showed a greater selectivity for different grip types, rather than different objects. Using targeted dimensionality reduction, a recent study with a wide range of delay periods showed that initial F5 population activity across all delays reflected grip-specific movement preparation. For longer delays, F5 activity evolved dynamically through different states, which related to the reaction time or withholding of upcoming reach-to-grasp movements (Michaels et al., 2018). Similarly to AIP, information on grasp type in F5 is context-specific (Fluet et al., 2010), and can be decoded with high accuracy in F5 neurons, either alone, or in combination with activity in AIP (Townsend et al., 2011).

Using a wide set of grip types and hand configurations, Schaffelhofer et al., (2015) demonstrated that hand configuration could be reliably decoded during both planning and movement stages in F5 (and AIP), and that decoding errors were often assigned to a neighbouring class. Within the local field potential (LFP), the low frequency signal thought to reflect the summed input to many neurons in the vicinity of the electrode (Logothetis, 2003), accurate decoding of grasp type during action execution is also possible (Spinks et al., 2008; Waldert et al., 2015). Although LFP signals are typically less informative than single-neuron spikes for decoding purposes, their greater long-term
stability renders them particularly useful for brain-machine interface (BMI) control (Waldert et al., 2009; Bansal et al., 2012).

Based on architectonic evidence, F5 has been further subdivided into three areas, namely; anterior (F5a) and posterior (F5p), lying within the post-arcuate bank, and a section on the convexity (F5c) (Belmalih et al., 2009). These anatomically distinct areas are interconnected, but by virtue of their different connectivity patterns with other areas, likely subserve different functional roles (Gerbella et al., 2011). F5p contains sparse and large layer V pyramidal cells, and comprises the majority of F5’s corticospinal (CS) projections, and cortico-cortical connections to M1 (Kurata, 2018). It is also the predominant location where canonical neurons have been found. F5a, on the other hand, contains more densely packed medium-sized pyramidal cells (Belmalih et al., 2009), and is more strongly connected with prefrontal areas 46 and 12, and posterior parietal areas, but not with M1 (Matelli et al., 1986; Gerbella et al., 2011; Kurata, 2018). This suggests that F5a may be more removed from the motor output and preferentially integrate prefrontal and parietal inputs before projecting to the other F5 sectors (Gerbella et al., 2011; Kurata, 2018), and physiological evidence demonstrating that F5a neurons can show shape-selectivity without motor responses supports this (Theys et al., 2012a).

**F4**

Area F4 lies immediately adjacent to F5, forming the caudal part of inferior area 6, and contains representations of the face, neck, and arm. Single neurons in this area typically fire during proximal arm movements (Godschalk et al., 1981; Gentilucci et al., 1988), and exhibit tactile, or congruent tactile and visual, receptive fields (Fogassi et al., 1996). Anatomical tracing indicates that F4 primarily receives input from VIP in the IPS, and together these two areas have been associated with the encoding of peripersonal space for movement towards object locations (Fogassi et al., 1996; Luppino et al., 1999).

**Dorsal Premotor Cortex**

Traditionally, caudal PMd has been associated with proximal arm movements and the control of reaching. Neurons in this area show set-related activity during movement
1.2. The cortical control of grasp

preparation in instructed delay tasks (Weinrich & Wise, 1982; Riehle & Requin, 1989; Boussaoud et al., 1995; Crammond & Kalaska, 1996, 2000), related to the direction of the movement the animal subsequently makes (although see Churchland & Shenoy, 2007b; Scott, 2008), consistent with the idea that PMd plays an important role in the preparation of arm movements under visual guidance (Godschalk et al., 1981). More distal movements are also represented in PMd (Godschalk et al., 1995), with distal forelimb movements concerning the wrist and digits tending to be located in the more lateral parts of PMd (area F2) (Raos et al., 2003). Areas of PMd containing digit representations project to M1, and are also strongly interconnected with the digit representations in ventral premotor cortex (PMv) (Dum & Strick, 2005). A study by Raos et al. found that neurons in the distal forelimb representation of PMd exhibit visuomotor responses during grasping (Raos et al., 2004). Further systematic investigations of reaching and grasping activity for different reaching directions and grasp types have confirmed that there is a great deal of anatomical overlap between single units encoding reach and grasp in premotor areas, such that both ventral and dorsal aspects of premotor cortex (PMC) contain anatomical and functional representations of proximal and distal limb movements (Stark et al., 2007a; Lehmann & Scherberger, 2013; Takahashi et al., 2017). In humans, virtual lesioning of PMd via transcranial magnetic stimulation (TMS) disrupts coupling between grasp and lift phases during a precision grip task (Davare et al., 2006).

Primary Motor Cortex

The primary motor cortex (M1) lies in the precentral gyrus (BA4), anterior to the central sulcus or Rolandoic fissure, and is characterised by its agranular cytoarchitecture and the presence of large pyramidal (Betz) cells in a thick layer V (Brodmann, 1909). It predominantly receives inputs from motor thalamus, ventral and dorsal aspects of premotor cortex (PMv and PMd, respectively) and the supplementary motor area (SMA) (Dum & Strick, 2005), with some evidence for smaller connections from somatosensory and parietal areas (Gharbawie et al., 2011). M1 provides the dominant drive to the spinal cord for control of the hand via the descending corticospinal tract (CST), with up to 50% of the total frontal lobe CS projections arising from M1 (Dum & Strick, 1991; Porter & Lemon, 1993). Classical investigation into the function of M1 via lesioning of M1 or the CST output unequivocally demonstrated the key role of M1 in hand and limb control
1.2. The cortical control of grasp

(Tower, 1940; Lawrence & Kuypers, 1968a; Woolsey et al., 1972; Porter & Lemon, 1993), as relatively independent finger movements are profoundly disrupted. Stimulation studies, meanwhile, outlined a somatotopically organised map of outputs within M1 (Penfield & Rasmussen, 1950; Woolsey et al., 1952), and the gross outline of this map, with medial and lateral M1 containing representations of leg and face respectively, is well accepted. However, there is evidence against finer detailed somatotopy of individual fingers within the M1 hand area, with both anatomical and functional studies reporting highly overlapping representations of individual fingers (Schieber & Hibbard, 1993; Schieber & Poliakov, 1998; Rathelot & Strick, 2006).

It has been known for some time that M1 contains neurons whose discharge is correlated with the movement of individual fingers (Schieber & Hibbard, 1993), or tuned to different movement parameters, such as force, joint position, direction, or velocity (Evarts, 1968; Cheney & Fetz, 1980; Georgopoulous et al., 1986; Kakei et al., 1999; Moran & Schwartz, 1999; Stark et al., 2007b). Within the hand area of M1, many neurons show tuned activity for particular types of grasp (Muir & Lemon, 1983; Umiltá et al., 2007; Schaffelhofer et al., 2015; Schaffelhofer & Scherberger, 2016). In contrast to the selectivity previously discussed in AIP and F5, grasp selectivity is poor during object presentation and movement preparation, but is relatively strong during grasping execution (Umiltá et al., 2007; Schaffelhofer & Scherberger, 2016), consistent with M1’s powerful direct influence on the distal musculature for the control of the fingers (Muir & Lemon, 1983; Porter & Lemon, 1993; Rathelot & Strick, 2009). Muscimol inactivation of M1 induces severe paralysis and hypotonia in contralateral fingers (Fogassi et al., 2001), and human patients with lesions in the motor cortex or descending CST (see section 1.3, p.37), show decreased individuation in finger movements (Lang & Schieber, 2003, 2004).

1.2.2. Cortico-cortical transfer of information

The key nodes in the lateral grasping circuit are strongly anatomically connected, and the functional properties of neurons in these areas show a graded evolution from primarily visual responses to object features in AIP, to the motor commands necessary for the successful generation and performance of the grasping action in M1. The transfer of this information between the nodes is essential for the precise control of grasp in both monkeys.
1.2. The cortical control of grasp

and humans (Jeannerod et al., 1995; Davare et al., 2011). With respect to F5 and M1, a series of studies have used stimulation methods in macaques to unequivocally demonstrate a physiological and functionally relevant link between these two areas. F5 neurons are antidromically activated by stimulation of the M1 hand area (Godschalk et al., 1984), and high-intensity single-pulse stimulation in PMv was shown to produce largely inhibitory responses in M1 (Tokuno & Nambu, 2000). Single-pulse stimulation in F5 does not evoke responses in spinal motoneurons or muscle activity in sedated monkeys by itself. However, sub-threshold conditioning shocks preceding single shocks in M1 at short latencies can substantially facilitate the amplitude of late, indirect CS volleys in lightly sedated and anaesthetised monkeys (Shimazu et al., 2004; Schmidlin et al., 2008, see Figure 1.2). This produces a corresponding facilitation in post-synaptic responses in motoneurons (Shimazu et al., 2004), and in short-latency evoked electromyography (EMG) responses in peripheral muscles (Cerri et al., 2003). Inactivation of M1 via muscimol injection abolishes these effects, and motor effects evoked by repetitive intra-cortical microstimulation (rICMS) in F5 are also blocked following M1 inactivation (Schmidlin et al., 2008). Prabhu et al., (2009) used intracortical stimulation through implanted microwires to demonstrate that facilitation of EMG responses occurs in a grasp-specific manner in the awake, behaving monkey, and a further study showed that single neurons in M1 show short-latency excitatory and inhibitory responses to low-intensity stimulation in F5, and vice versa (Kraskov et al., 2011). Furthermore, axonal recordings in CS neurons confirm that intracortical stimulation in F5 produces synaptic responses via M1 (Maier et al., 2013). Altogether, these results strongly suggest that F5 exerts its primary effect on spinal circuitry for the purposes of grasping actions via its connections with M1.

In healthy human subjects, virtual lesioning of AIP (Davare et al., 2010) and PMv (Davare et al., 2006) via TMS disrupts appropriate hand shaping and force scaling during grasping actions. Paired pulse TMS (pp-TMS) paradigms in which a sub-threshold conditioning TMS pulse is applied over PMv before a supra-threshold test pulse over M1 indicate that PMv produces grasp-specific facilitation of motor-evoked potentials (MEPs) (Davare et al., 2008, 2009). pp-TMS over M1 prior to grasping movement onset also results in grasp-specific facilitation (Cattaneo et al., 2005; Prabhu et al., 2007b), but only when visual information is available online (Prabhu et al., 2007a), suggesting this facilitation is transient and occurs only shortly before the grasping movement is initiated.
1.3. Descending control of the hand

The visuomotor transformations which take place in cortical areas can only be brought to bear on performed actions through the descending motor pathways in the spinal cord, which drive the spinal motoneurons within Sherrington’s “final common path”, and produce the complex patterns of muscle activity seen during skilled movement (Schieber, 1995; Brochier et al., 2004). In primates and humans, a number of different descending pathways exist, which are phylogenetically preserved to varying degrees, and characterised by distinct origins, paths and termination patterns (Lemon, 2008). The corticospinal tract (CST) has traditionally been considered as the most important pathway in primates for
skilled movements such as grasping (Lawrence & Kuypers, 1968a; Porter & Lemon, 1993), and has therefore also been the only descending pathway seriously examined in the context of action observation.

1.3. Descending control of the hand

1.3.1. The corticospinal tract

The CST originates from a number of cortical areas in macaques, including M1, primary somatosensory cortex, and premotor areas, PMd, PMv, and the SMA, and CS projections also arise from cingulate motor areas, somatosensory cortex, and even the posterior parietal cortex (PPC) (Dum & Strick, 1991). CST fibres pass through the internal capsule and cerebral peduncles before arriving at the medullary pyramid, after which the vast majority of fibres cross the midline (Kuypers, 1981; Porter & Lemon, 1993; Morecraft et al., 2013). CST terminations are widespread within the dorsal horn, ventromedial and dorsolateral intermediate zones of the spinal cord grey matter, and in higher primates, there are substantial direct projections to the motoneuron pools in lamina IX within the ventral horn (Kuypers, 1982; Lemon, 2008). The diverse origins and terminations of the CST imply that it subserves a number of different functions (Lemon, 2008).

A particularly relevant feature of the CST subserving fine finger control is the cortico-motoneuronal (CM) system, which is formed by direct, monosynaptic connections from descending fibres onto spinal alpha motoneurons in the ventral horn (Bernhard & Bohm, 1954). These connections are commonly identified physiologically via averaging of muscle activity with respect to the spiking activity of individual CS neurons. The presence of post-spike facilitation (PSpF) in the spike-triggered averages (SpTAs) of one or more muscles consistent with a monosynaptic latency point towards CM identity (Fetz & Cheney, 1980; Muir & Lemon, 1983), although PSpFs can also arise through synchrony effects (Baker & Lemon, 1998; Schieber & Rivlis, 2005). The CM system is highly developed in humans (Kuypers, 1981; Palmer & Ashby, 1992; Maertens de Noordhout et al., 1999), and is also present in apes (Kuypers, 1981) and old world monkeys (Lemon & Griffiths, 2005). It is found in some New World monkeys but is virtually absent in squirrel monkeys (Bortoff & Strick, 1993; Nakajima et al., 2000), and rodents (Alstermark et al., 2004; Lemon, 2008). Overall, the extent of the CM system is well correlated with functional dexterity when compared across species (Heffner & Masterton, 1975, 1983;
1.3. Descending control of the hand

Lemon, 2008), and CM connections are particularly strong to intrinsic hand muscles, with weaker ones to proximal limb muscles (Palmer & Ashby, 1992; Maier et al., 1993; Porter & Lemon, 1993; McKiernan et al., 1998). These findings support the observations of Lawrence & Kuypers, (1968a) and emphasise the role of the CM system in controlling fractionated movements of individual fingers (Kuypers, 1981; Muir & Lemon, 1983; Buys et al., 1986; Bortoff & Strick, 1993).

Detailed anatomical work using retrograde transneuronal tracers has demonstrated an overlapping distribution of CM projections to different target muscles in the hand and arm, and a range of fibre sizes within the CM system (Rathelot & Strick, 2006). In light of the conventional bias in electrophysiology experiments towards recording from cells with faster (larger) axons, the relative abundance of slow CM fibres suggests that there are substantial gaps in our knowledge of the physiological response properties of CM cells (Firmin et al., 2014; Kraskov et al., 2019). Although a finely segregated somatotopic map within the CM population is not apparent, CM projections predominantly arise from caudal (“new”) M1, which lies within the rostral bank of the central sulcus. By contrast, the more rostral parts of M1, and the premotor and secondary motor areas giving rise to CS projections, do not appear to have direct CM projections (Rathelot & Strick, 2009). Correspondingly, intra-cortical microstimulation (ICMS) in new M1 evokes excitatory post-synaptic potentials (EPSPs) in spinal motoneurons whose latency is consistent with monosynaptic activation (Witham et al., 2016), although this study also found that slowly conducting fibres in old M1 can generate weak, late monosynaptic effects.

CM cells are separated by just one synapse from their target muscles, and the relationship between their activity in relation to parameters of movement and muscle activity has therefore been of great interest. CM cell activity occurs over a range of levels of force and muscle activity (Cheney & Fetz, 1980), and frequently covaries with activity in target muscles (Muir & Lemon, 1983; Buys et al., 1986; Lemon et al., 1986; Bennett & Lemon, 1996; McKiernan et al., 2000; Griffin et al., 2008). On the other hand, the activity of many CM cells can also be dissociated from target muscle activity (Muir & Lemon, 1983; Maier et al., 1993; Bennett & Lemon, 1996; Schieber & Rivlis, 2007; Griffin et al., 2008; Griffin et al., 2015). This is not altogether surprising however, as CM cells project to multiple muscles (Shinoda et al., 1981), often show post-spike effects in more than one muscle.
1.3. Descending control of the hand

(Cheney & Fetz, 1980; Buys et al., 1986), and one CM cell also provides only a fraction of the cortical drive to a given target muscle. For this reason, several thousand spikes are typically required for the SpTA procedure.

The majority of descending inputs within the CST reach spinal motoneurons disynaptically, via segmental interneurons (sINs), which lie within the same lower cervical segments as forelimb motoneurons (Morecraft et al., 2013), or the propriospinal pathway, which is predominantly located within upper cervical segments (C3-C4) (Alstermark et al., 1999; Isa et al., 2006). Recordings from sINs indicate that these neurons show preparatory activity during instructed delay periods prior to wrist movements (Prut & Fetz, 1999). Cervical sINs produce divergent post-spike effects in intrinsic hand muscles (Takei & Seki, 2010), and during grip performance, may subserve muscle synergies, upon which more fractionated patterns can then be imposed by the CM system (Takei et al., 2017). C3-C4 propriospinal transmission to forelimb motoneurons is strong in cats (Illert et al., 1977), but is limited in both sedated (Maier et al., 1998) and awake (Olivier et al., 2001) primates. These weak effects appear to be due to feed-forward inhibition by the CM system, as systemic application of strychnine, a glycinergic antagonist, disinhibits the circuit and reveals C3-C4 mediated responses to stimulation of the pyramidal tract (Alstermark et al., 1999; Isa et al., 2006). Nonetheless, there is evidence that this system exists even in humans (Pierrot-Deseilligny, 2002), and also makes some contribution to reach and grasp behaviour, since monkeys can recover precise grasping after a lesion at C5 (Sasaki et al., 2004), but not after one at C2 (Alstermark et al., 2011).

1.3.2. The reticulospinal tract

Lesions of the CST result in permanent loss of skilled hand function (Lawrence & Kuypers, 1968a), and subsequent lesions of medial brainstem pathways including the reticulospinal tract (RST) in recovered animals impair gross movements (Lawrence & Kuypers, 1968b). These findings, in conjunction with anatomical evidence for terminations in the ventral horn and intermediate regions, led the RST to be ascribed a role in postural control and gross bilateral co-ordination, and have framed much of our understanding on the respective roles of the CST and other descending pathways in motor control (Kuypers, 1982; Porter & Lemon, 1993). More recently, Davidson & Buford
applied electrical stimulation to the ponto-medullary reticular formation while recording EMG responses in ipsilateral and contralateral upper arm and shoulder muscles during primate reaching. Stimulus-triggered averages (StTAs) revealed post-stimulus facilitation (PStF) effects predominantly in flexor muscles, such as the biceps, while post-stimulus suppression (PStS) effects were more frequent in extensor muscles e.g. triceps (Davidson & Buford, 2004, 2006). PStF responses were typically earlier in onset, shorter and larger, and bilateral responses were common (Davidson & Buford, 2006). SpTAs of EMG activity compiled for cells in the ponto-medullary reticular formation (PMRF) also reveal post-spike effects in flexors and extensors of both arms (Davidson et al., 2007). Overall, these results indicate a role for the RST in flexion of the arm during reaching and bilateral coordination. Some of the responses in these studies were present in flexors and extensors of the wrist, and a growing body of evidence suggest a further role for the RST in the control of hand and finger movements, and for recovery of function in patients with CST damage (Baker, 2011). Using intracellular recording in spinal motoneurons, and stimulation in nerve cuffs and the medial longitudinal fasciculus (MLF), Riddle et al., (2009) identified direct and indirect connections of the RST to cervical spinal motoneurons innervating intrinsic muscles of the hand, evincing a role for the RST in fine finger control. A follow-up study which recorded from spinal interneurons found a high proportion of convergent inputs (71/163 cells) from CST and RST pathways, including many of those associated with control of the hand (Riddle & Baker, 2010). Single neurons in the PMRF can also modulate their activity during index finger flexion and extension (Soteropoulos et al., 2012).

### 1.4. Mirror neurons

At the time when F5’s critical role in the visuomotor transformations necessary for grasp was becoming increasingly apparent (Rizzolatti et al., 1988; Jeannerod et al., 1995), another class of visuomotor neuron was discovered in this area, which specifically responded to the observation of goal-directed motor acts (di Pellegrino et al., 1992). These mirror neurons (MIRNs), named for the frequent similarity in their discharge during the executed and observed actions, provoked considerable interest because of this intriguing property, which further questioned the purely executive role of the motor system, and
blurred the boundary between perceptual and motor processes.

F5 MirNs have been studied extensively (Kilner & Lemon, 2013; Rizzolatti & Fogassi, 2014), and cells with mirror-like properties have since been identified across a number of anatomically interconnected areas within the cortical circuits for reaching and grasping. The evidence for MirNs within the key nodes of this network is discussed below, and synthesised in the context of an extended mirror neuron system (MNS) within the primate brain. The widespread activity during passive action observation within a system integral to the execution of visually-guided movement suggests mechanisms to decouple motor cortical activity from muscles must be in place (Schieber, 2011), and further challenges our understanding of the appropriate generation and suppression of movement.

1.4.1. Locations and properties of mirror neurons

F5

Following the discovery of MirNs over 25 years ago (di Pellegrino et al., 1992), a more extensive characterisation was published a few years later (Gallese et al., 1996). In this study, 92/532 (17.3%) of all recorded cells in F5 modulated their activity during hand actions performed by both the monkey and an experimenter, and were therefore classed as MirNs. Critically, the observed action effective in activating these neurons was frequently congruent with the execution action which the neuron also responded to. Congruence was often relatively broad, such that the observation responses appeared to generalise across several actions. Some MirNs were defined as strictly congruent, where the effective action in the execution and observation conditions was identical (Gallese et al., 1996; Rizzolatti et al., 1996a).

The representation of observed actions directly within an area already thought to encode the goals of motor actions performed by the monkey (Rizzolatti et al., 1988), led to the suggestion that this goal representation is extended to the actions of others. An early study supporting this demonstrated that occlusion of the critical stage of the grasping action (prehension around the object) did not abolish the activity of many F5 MirNs (of 37 MirNs tested in all conditions, 19 (51%) continued to respond in the “hidden” condition), thus presumably reflecting a continued prediction of the state of the action (Umiltá et al., 2001).
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Some MirNs also respond to the sounds associated with the actions which they code for motorically, even in the complete absence of visual information (Kohler et al., 2002; Keysers et al., 2003). Furthermore, although the early studies reported that MirNs did not generalise to actions performed with tools (Gallese et al., 1996; Rizzolatti et al., 1996a), prolonged training reveals tool-responding MirNs in F5 (Ferrari et al., 2005). These responses often remain weaker than the comparable action performed directly with the hand, which more closely resembles the monkey’s own motor template for the action (Rochat et al., 2010), but support the notion the MirNs can encode the goal of motor action, and also highlight the influence of sensorimotor experience on mirror activity (Catmur et al., 2007; Heyes, 2010).

A number of studies have provided additional insights into the functional properties of F5 MirNs. Caggiano et al., (2009) demonstrated that observation responses can be selective for the spatial location of the observed action. They initially recorded from 105 MirNs in F5 while monkeys observed actions performed either in the monkey’s peri-personal space, or extra-personal space. The activity of 50 neurons (47%) was not different between the two spatial locations, while 27 neurons (26%) preferred actions performed in peri-personal space, and 28 neurons (27%) were selective for extra-personal space. Observation of actions at multiple distances confirmed that space-selective neurons modulated their activity as a monotonic function of distance. In a follow-up experiment, in which a panel was introduced in front of the monkey on some peri-personal space trials such that they could no longer reach the object, 9 of 21 neurons changed their spatial preference, indicating that they encoded space in an operational, rather than metric manner (Caggiano et al., 2009). An important conclusion derived from this study was that MirN activity does not solely reflect a high-level “recognition” of the motor action, but may also relate to possible actions which could involve interacting with the observed object or action. In a similar vein, MirNs can also show differential activity depending on the viewpoint of the observed action. 75% of tested F5 MirNs exhibited a preference for at least one of subjective, lateral, and frontal (0°, 90°, 180°, respectively) views. The testing of action viewpoints necessitated the use of video stimuli, and an important first test established that these activated MirNs in a similar manner to naturalistic stimuli, albeit with a higher proportion of neurons responding to naturalistic stimuli (Caggiano et al., 2011). This same view-dependent encoding is detectable in the LFP, with the subjective viewpoint
producing higher power in the same low-frequency band (2-10Hz) often associated with the execution of movement (Caggiano et al., 2015). In natural behaviour, the spatial location and viewpoint of observed actions are linked, and a recent study using live actions showed that these two features of MirN activity can be integrated. F5 MirNs which were selective for peripersonal space preferred a subjective, rather than lateral, viewpoint, while neurons which were unselective for space were also more likely to be view-invariant (Maranesi et al., 2017). These results hint at a unique importance for actions from a subjective perspective, which may arise because of its inextricable association with self-action. Indeed, a comparison of responses during grasping in light and dark conditions indicates that F5 MirNs appear to be more responsive to visual feedback from the hand during the grasping stage of self-action, than simultaneously recorded non-MirNs (Maranesi et al., 2015).

In addition to spatial features, the perceived value of an action may determine its relevance to the observer. In accordance with this, MirNs in F5 have been reported to modulate depending on the subjective value associated with a grasped object. This value-dependence was strongest and most skewed when comparing food vs non-food objects, but was also apparent when comparisons were made between two non-food objects which were explicitly associated with different levels of reward, or when the object and reward level were independent (Caggiano et al., 2012). Critically, the reward level of a given trial was always made clear to the monkey beforehand, which may indicate that the MirNs modulating their activity for different reward levels were encoding a prediction of the expected outcome of the trial.

In line with the idea that MirNs encode observed action goals, 65.7% of F5 MirNs recorded in the same grasp-to-eat v.s. grasp-to-place paradigm previously discussed (Fogassi et al., 2005, see p.31) showed selectivity for one or other of the overall action goals, with the vast majority preferring grasp-to-eat (Bonini et al., 2010). An extension of this task to examine social context (by placing in the mouth, container or experimenter’s hand) found that 10% of goal-selective neurons had a preference for the social interaction of placing in the hand (Coudé et al., 2018). Context-dependence in MirN activity is also supported by a recent study which used a Hidden Markov model approach, and demonstrated that MirNs in PMv monitor the sequence of behavioural events before and during movement, and do so in a
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similar way across executed and observed actions (Mazurek et al., 2018).

As well as representing observed actions, there is some evidence that grasping MirNs in F5 represent actions which are withheld. Bonini et al. trained monkeys to perform a Go/NoGo task in separate execution and observation blocks, and found MirNs which responded to the NoGo condition, usually in either the execution or observation block, but not both. 72% of cells responding to the execution NoGo condition did not show mirror activity, whereas all cells responding to observation NoGo were MirNs (Bonini et al., 2014b). Within this task, the cues at the start of each trial within a block were perfectly predictive of the upcoming action. This was reflected in the activity of NoGo-responsive MirNs - 59.6% of these MirNs begin to modulate their discharge ahead of the onset of the observed action, in contrast to only 17.4% of MirNs which did not respond in the NoGo condition (Maranesi et al., 2014). Recently, it was reported that MirNs also tend to become active earlier than non-MirNs during action execution (Mazurek & Schieber, 2019). This predictive discharge is in line with the various studies already described that show MirNs activation is not dependent on visual input (Umiltá et al., 2001; Kohler et al., 2002) but can be context-dependent (Bonini et al., 2010; Caggiano et al., 2012; Coudé et al., 2018; Mazurek et al., 2018). Furthermore, proactive eye movements, which occur during action observation (Flanagan & Johansson, 2003), may be an important driver of MirN activity (Maranesi et al., 2013). Together, there is considerable evidence that mirror activity in F5 exhibits a strong predictive component, according to a generative model of expected action consequences (Kilner et al., 2007).

As well as grasping actions, neurons which motorically encode mouth actions have also been found to respond to the observation of communicative and ingestive actions performed in front of the monkey (Ferrari et al., 2003). Interestingly, the response of mouth MirNs is largely intransitive (not object-directed), contrasting with the conclusions from initial evidence that an object interaction is required for macaque grasp-related MirNs to fire (Gallese et al., 1996; Rizzolatti et al., 1996a; Umiltá et al., 2001; Rizzolatti & Craighero, 2004). More recently, a representational similarity analysis, in which the kinematics of the observed actions were compared to the activity of MirNs, found that intransitive actions do elicit mirror activity, and this activity reflects the kinematics of the observed action (Papadourakis & Raos, 2017). The authors noted that previously, kinematic effects on MirN activity during goal-directed grasping were only examined
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during action execution conditions (Bonini et al., 2010), although even during execution, there is evidence for encoding of kinematic parameters (Kakei et al., 2001). These results are also more consistent with evidence that both the goals and kinematics of observed actions appear to be encoded within the human MNS (Gangitano et al., 2001, 2004; Cattaneo et al., 2009; Sartori et al., 2012; McCabe et al., 2015, see subsection 1.4.2, p.51). The overlapping representations of grasping goals and action kinematics could partly reflect the interplay between the dorsomedial reaching and dorsolateral grasping networks during action observation, and different task requirements may account for some of the differences in results across studies.

Notwithstanding the electrophysiological bias towards recordings from large pyramidal cells with fast-conducting axons (Kraskov et al., 2019), the anatomical identity of MirNs has largely remained a mystery. Using antidromic stimulation and collision testing methods, Kraskov et al., (2009) found that physiologically identified PTNs in F5 can exhibit mirror activity. This study also identified a new class of MirNs, which suppressed their activity during observation, while still increasing their firing rate during action execution, suggesting a potential mechanism for the suppression of self-movement during action observation, since these cells unequivocally differentiate between self and others’ actions. Importantly, this study also provided a clear demonstration that mirror activity cannot be trivially ascribed to low-level muscle contractions by the observing monkey (Kraskov et al., 2009).

Previously, MirNs had been considered to reside within area F5c (Belmalih et al., 2009; Maranesi et al., 2012). Unlike F5p, where F5 PTNs and canonical neurons are predominantly found, inactivation of F5c does not produce deficits in hand shaping (Fogassi et al., 2001). However, functional magnetic resonance imaging (fMRI) activation during the observation of grasp is found in all three sectors of F5 (Nelissen et al., 2005), and a recent systematic assessment of neuronal responses to both object presentation and grasping observation using linear probes indicated that canonical and mirror neuron populations overlap substantially, and single neurons can fall into both categories (Bonini et al., 2014a). This study also found that canonical responses were typically restricted to peripersonal space, whereas action observation responses showed a more graded preference for spatial location, consistent with the findings of Caggiano et al., (2009).
Dorsal Premotor Cortex

It has been known for some time that PMd also contains visually-responsive neurons (Fogassi et al., 1999), and several studies have shown that it also contains cells with mirror-like response properties. In a task which examined directional tuning in PMd neurons during both the execution and observation of cursor motion on a screen, 76% of cells showed directional tuning during the instructed-delay periods of action observation, and population responses during execution and observation were similar (Cisek & Kalaska, 2004). The authors considered the frequent onset of responses before any cursor movement to reflect a correlate of mental rehearsal of motor actions learned through stimulus-response association, which is distinct, although possibly functionally related, to the mirror activity induced by visual observation of natural grasping actions (Cisek & Kalaska, 2004). A subsequent study similarly found cells in PMd with congruent directional tuning both when a monkey executed a cursor movement, and when it later observed its own movements (Tkach et al., 2007). For both executed and observed natural cursor movements, mutual information estimates indicated that neural activity carried most information about cursor movement occurring in the future, consistent with the capacity of MirNs to fire in a predictive manner (Bonini et al., 2010). This was not the case for artificial movements, where the observed cursor movement occurred at a simple constant velocity (Tkach et al., 2007).

A key difference between these studies and those in classic MirN experiments, was the use of cursor movements abstractly related to the monkey’s own movements, rather than more naturalistic reach and grasp actions. This discrepancy was addressed in a recent study, which identified neurons in PMd which responded to the execution and observation of reach-to-grasp actions, and showed similar grasp selectivity and execution-observation matching to simultaneously recorded MirNs in F5 (Papadourakis & Raos, 2019). In contrast to the finding of Cisek & Kalaska, (2004), the majority of grasping observation responses in PMd began after the onset of experimenter movement. Some PMd (and F5) neurons showed suppression responses during action observation, although the proportions (8% and 6%, respectively) were lower than the findings of Kraskov et al., (2009) in F5.
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Inferior Parietal Lobule

Following the discovery of MirNs in F5, it was a natural next step to examine upstream areas within the grasping circuitry for evidence of mirror activity which may be inherited and transformed by F5 during action observation. Within the rostral sector of the IPL (area PFG) (Pandya & Seltzer, 1982; Rozzi et al., 2006), many neurons with motor properties also respond to the observation of goal-directed grasping actions, and have therefore also been classed as MirNs (Gallese et al., 2002; Fogassi et al., 2005; Rozzi et al., 2008; Bonini et al., 2010). Using the same grasping-to-eat vs. grasping-to-place task which had been used to study motor properties of IPL neurons, Fogassi et al., (2005) found that the activity of around 75% (31/41) of IPL MirNs were also tuned to the goal of the grasping action in the observation condition, with the majority (~74%, 23/31) preferring the grasp-to-eat condition. This finding was later replicated in a comparison of IPL and F5 MirNs (Bonini et al., 2010), with functionally similar responses found within the MirNs of both areas.

In contrast to the execution condition, during observation, the two areas contained similar proportions of neurons with preferences for grasping-to-eat (50-60%), grasping-to-place (~35%), or no preference, and both areas exhibited similar, positive correlations between action goal preferences in the motor and visual conditions (Bonini et al., 2010), suggesting strongly overlapping representations of observed action goals within these two anatomically interconnected areas.

The discovery of MirNs in the IPL also provided an important clue regarding the possible origin of mirror activity, which was under debate since F5 itself does not directly receive visual input. The rostral part of superior temporal sulcus (STS), contains neurons which discharge during the observation of biological actions, including goal-directed hand movements (Perrett et al., 1989). These neurons are not defined as MirNs as they rarely discharge during the monkey’s own actions, but STS projects strongly to the rostral IPL (Seltzer & Pandya, 1994), which in turn projects to F5 (Godschalk et al., 1984; Matelli et al., 1986; Luppino et al., 1999; Rozzi et al., 2006; Borra et al., 2008; Gerbella et al., 2011).

Given the strong connections between AIP and IPL, and AIP’s responses to visual presentation of objects (Sakata et al., 1995; Murata et al., 2000; Baumann et al., 2009;
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Theys et al., 2012b) and key role in visuomotor grasp (Gallese et al., 1994; Murata et al., 2000; Schaffelhofer & Scherberger, 2016), it should be expected that AIP also contains MirNs. Accordingly, responses to action observation have indeed been reported in AIP neurons (Pani et al., 2014). In an object grasping task performed under visual or memory-guidance, 59% of AIP neurons were active during both executed and observed grasps. Interestingly, the neurons active during action observation also showed significantly higher activity during object presentation, resembling the overlap of canonical and mirror activity found in F5 (Bonini et al., 2014a). Maeda et al., (2015) found that 54 of 235 neurons recorded across macaque AIP and PFG responded to visual feedback of own-hand action during a grasping task, with 33 of these classed as MirNs by virtue of their response to executed and observed actions.

A combined anatomical tracer and fMRI study in monkeys corroborated the functional linkage between STS and F5 via parietal cortex. Injections of retrograde tracers into AIP and PFG indicated that the two areas receive primary input from two different areas of STS, which show responses to action observation. During the grasping observation task, the AIP route showed preferential activation when only the hand-object interaction was visible, whereas the PFG route became more active when the whole actor was visible. Although this implies the existence of parallel pathways, the strong interconnection between AIP and PFG indicates that information arising from these two pathways is shared (Nelissen et al., 2011). Recently, a systematic study reported selective encoding of different observed actions within posterior AIP in particular (Lanzilotto et al., 2019). As previous studies had focused on grasping actions only, and found little evidence for responses to observed actions not occurring from a first-person perspective (Maeda et al., 2015), this study emphasises the role of AIP as an additional key parietal hub of the action observation network.

M1

The initial report by Gallese et al., (1996) determined that mirror activity was absent in macaque M1, and considered this a natural solution for preventing the overflow of mirror activity into unintended self-movement. However, contradictions arising within the non-invasive literature regarding the presence or absence of mirror activity in M1 (Fadiga
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et al., 1995; Rizzolatti et al., 1996b; Hari et al., 1998, see subsection 1.4.2, p.51) prompted a closer re-examination. The aforementioned study by Tkach et al. found modulation at the level of both single-neurons and the LFP in M1 during the observation of cursor movements towards targets, which was congruent with activity during the monkey’s own movements of the cursor in terms of directional tuning (Tkach et al., 2007). In another study, approximately half of M1 neurons modulated their activity during the observation of a step tracking task, with the majority (62%) shifting their preferred direction tuning during observation relative to execution (Dushanova & Donoghue, 2010). In monkeys performing a naturalistic precision grip grasping task, Vigneswaran et al., (2013) identified M1-PTNs which showed mirror activity. In a similar manner to previous work (Kraskov et al., 2009), the study reported both facilitation and suppression responses during action observation, with facilitation responses during observation markedly reduced compared to execution. Given the proximity of M1 to the spinal output and its innervation of the lower cervical cord (Dum & Strick, 1991; He et al., 1993), this “disfacilitation” provided strong additional evidence that action observation activity is insufficient to drive spinal machinery in the same manner as during action execution, and thus is prevented from causing overt movement in the observer (Vigneswaran et al., 2013; Kraskov et al., 2014). Although the identification of PTNs is an important step in the right direction in measuring mirror activity in known cell populations, the output targets and effects of these cells at the spinal level is not known, except in the case of the direct excitatory connections onto motoneurons provided by CM cells (see subsection 1.3.1, p.38). Preliminary evidence suggests that CM cells can show mirror activity (Vigneswaran et al., 2013), but more systematic investigation awaits. Downstream of M1, 14C-deoxyglucose imaging has revealed metabolic suppression bilaterally within the cervical enlargement in monkeys which had observed grasping actions, whereas executed actions induced an ipsilateral increase in metabolic activity (Stamos et al., 2010). The nature of the study however, in which monkeys were sacrificed immediately after task performance, meant that execution and observation changes could not be studied within the same subjects. Despite some work in humans, the net effects of action observation at the spinal level are not well understood.
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An extended mirror neuron system

The previous sections have discussed the properties of MirNs identified in a range of anatomically and functionally interconnected cortical areas. Beyond the traditional areas of the reaching and grasping network, mirror-like activity has also been identified in SPL (Breveglieri et al., 2019), areas of the medial frontal cortex (MFC), such as SMA and pre-SMA (areas F3 and F6) (Mukamel et al., 2010; Yoshida et al., 2011; Livi et al., 2019), prefrontal cortex (Rozzi & Fogassi, 2017), and even substructures beyond the primate neocortex, including the medial temporal lobe (MTL) (Mukamel et al., 2010), subthalamic nucleus (STN) (Alegre et al., 2010, Kraskov et al., unpublished observations), and cerebellum (Calvo-Merino et al., 2006). The existence of an extended MNS is therefore generally well accepted (Bonini, 2016; Bruni et al., 2018), and substantial corroboration for this has been derived from studies in humans.

1.4.2. Mirror neurons in humans

Single-unit recordings in humans are rarely possible for ethical reasons, and so most evidence for the existence of MirNs in humans has arrived indirectly, via fMRI, electro-encephalography (EEG) / magneto-encephalography (MEG), and TMS. In one study however, where it was possible to record extracellularly from single neurons in 21 patients implanted with electrodes for seizure monitoring, many neurons in MFC and MTL regions responded to the execution and observation of grasping actions and facial expressions (Mukamel et al., 2010). The recording locations were determined by clinical need, and included the SMA, pre-SMA, and anterior cingulate cortex in the MFC, and amygdala, hippocampus, parahippocampal gyrus, and entorhinal cortex in the MTL. This contrasts with the majority of MirN studies, which have focused on lateral areas of cortex (Figure 1.1, but see Yoshida et al., 2011; Livi et al., 2019). These findings suggest that mirroring is a widespread property in the primate brain, but may subserve different functions in different areas (Mukamel et al., 2010). This study also found neurons which suppressed their activity during observation, consistent with the suppression MirNs found in monkey F5 and M1 (Kraskov et al., 2009; Vigneswaran et al., 2013).
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Motor resonance within corticospinal pathways - evidence from neurophysiology

Action observation and execution both induce a desynchronisation in 15-25Hz (beta) oscillatory activity over M1, revealed by the inhibition of the rebound following stimulation of the median nerve (Hari et al., 1998). The temporal dynamics of beta oscillations are similar for executed and observed movements (Nishitani & Hari, 2000), and modulation in the beta range during action observation is influenced by the spatial location (side of the screen) in which the observed action takes place (Kilner et al., 2009a). EEG recordings have shown that there is an increase in the readiness potential, a correlate of movement preparation, in advance of the onset of predictable observed movements (Kilner et al., 2004).

TMS permits non-invasive examination of corticospinal excitability (CSE) in awake subjects via quantification of changes in the size of short-latency MEPs which are produced in muscles, typically in the contralateral arm and hand, following stimulation over M1 (see Bestmann & Duque, 2016). An early study by Fadiga et al. found that action observation resulted in increased MEP amplitude relative to non-action conditions. Critically, this increase showed some muscle specificity, with proximal muscles but not the opponens pollicis (OP), a thumb flexor, showed increased MEPs during arm elevation (Fadiga et al., 1995). This provided important evidence for a mirror mechanism similar to that reported in monkeys (Gallese et al., 1996), and the excitability changes were postulated to be mediated by the cortico-cortical connections of premotor area F5 to M1 and the spinal cord. The modulation of MEPs during observation of intransitive arm movements was thought to reflect a possible difference between monkey and human MirNs, although responses to observed intransitive movements in monkey MirNs have been described recently (Kraskov et al., 2009; Papadourakis & Raos, 2017).

Despite only providing a coarse window into net modulation during action observation, TMS offers high temporal precision, and has therefore also been used to examine the timing of modulation in the putative human MNS (Fadiga et al., 2005; Naish et al., 2014). If changes in CSE excitability during action observation reflect the same mirror mechanisms studied in the monkey literature, then these changes should vary over the course of an observed action, and modulate with the nature of the action and muscles.
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involved. Numerous studies, using the delivery of TMS at different times on different trials, and under different conditions, have shed substantial light on our understanding of the human MNS, and its correspondence with the one in monkeys. CSE excitability is indeed modulated with the phase of the ongoing action, and covaries with observed finger aperture (Gangitano et al., 2001). A follow-up study by the same group found that this modulation disappears when the kinematics of the action diverge from what is naturally expected (Gangitano et al., 2004), and was hypothesised to result from a mismatch between the predicted goal of the action and the subsequently observed motor action. A study by Cattaneo et al., (2009) demonstrated that, during the observation of a grasping task using normal or reverse pliers, MEPs in the OP muscle are modulated in a manner reflecting the predicted goal of the action. Some studies have also provided evidence for a fast and non-specific component of CSE modulation early during action observation (Lepage et al., 2010; Lago & Fernandez-del-Olmo, 2011).

On the other hand, a number of studies have indicated that CSE modulation falls in line with actual observed action kinematics. Cavallo et al., (2012) found that MEP modulation followed the observed hand movement during a pliers grasping task. MEPs are also modulated in line with the force requirements for lifting objects of different weights (Alaerts et al., 2010a), and kinematic cues are particularly relevant for this relationship (Alaerts et al., 2010b; Senot et al., 2011). Differences in kinematics are often not consciously detected by participants, but appear to be sufficient to influence the level of MEP modulation during action observation (Sartori et al., 2012). A study comparable in design to that of Gangitano et al., (2004) in the use of natural and unnatural actions, found that CSE modulation did not decrease during the observation of unnatural actions, but simply followed the functional role of the muscle involved (the first dorsal interosseous (1DI)) (Gueugneau et al., 2015). This suggests that the representation of the observed action is updated in real-time, and the distinction with previous results may arise from the use of real-time, rather than artificially slowed, videos of the observed actions. Kinematic modulation of MEP amplitude during action observation is also independent of the availability of information about the action goal (McCabe et al., 2015).

Contextual information can also directly affect CSE. The presentation of “incorrect” cues (e.g. cues for precision grip prior to whole-hand grasp observation or vice versa), or
ambiguous ones, initially induces CSE modulation compatible with the cued or expected action in relevant muscles, which is later altered to match the actual viewed action (Cavallo et al., 2013; Janssen et al., 2015). Congruent contextual information also enhances the facilitation of CSE during action observation (Riach et al., 2018), and incongruent contextual information may result in a suppression of CSE (Senot et al., 2011). Changes in CSE induced by contextual information occur in a muscle- and grip-specific manner, and are strengthened by the addition of kinematic cues (de Beukelaar et al., 2016; Cretu et al., 2019). Paired-pulse stimulation between PMv and M1 suggests that this grasp-specific facilitation is mediated by interactions between these areas (de Beukelaar et al., 2016), in a comparable manner to their interactions during grasping execution (Davare et al., 2008, 2009). Providing congruent, but not incongruent, auditory contextual cues also enhances CSE during action observation (Alaerts et al., 2009b), consistent with results in monkeys, which suggest a multi-modal representation within the MNS (Kohler et al., 2002; Keysers et al., 2003). Overall, CSE during action observation is likely to reflect an interplay between top-down contextual information, which provides a prediction of the upcoming movement, and bottom-up visual inputs, provided by kinematic cues. Contextual priors can be updated based on incoming visual information regarding the observed action, which fits within a Bayesian predictive coding framework (Kilner et al., 2007; Cretu et al., 2019).

Gallese et al., (1996) emphasised that many MirNs showed a clear matching between their preferred executed and observed actions (although this congruence was often broad). In line with this matching, CSE modulation during action observation in humans is muscle-specific, typically in the direction expected or actually observed when the muscle is used during performance of the action (Fadiga et al., 1995; Maeda et al., 2002; Montagna et al., 2005; Urgesi et al., 2006; Alaerts et al., 2010a; Sartori et al., 2012; McCabe et al., 2015; Bunday et al., 2016). For example, Maeda et al., (2002) found larger MEP changes in the 1DI muscle during observation of index finger movements, and larger changes in abductor pollicis brevis (AbPB) (a key abductor of the thumb), during thumb movements. Numerous studies have also reported facilitation of MEPs in the 1DI muscle, but not abductor digiti minimi (AbDM), a little finger abductor, during observation of precision grip (Catmur et al., 2007; Sartori et al., 2012; McCabe et al., 2015; Bunday et al., 2016). A similar muscle-specificity has been shown for wrist extensor and flexor muscles during wrist extension-flexion tasks (Borroni et al., 2005; Alaerts et al., 2009c). Catmur et al., (2007) found
that prior sensorimotor training in which subjects perform index finger movements after observing little finger movements, and vice versa, reverses the mirror effect, such that MEPs become relatively greater in the IDI for observed little finger movements, and greater in the AbDM for index finger movements. This counter-mirroring has led to suggestions that sensorimotor learning configures the human MNS (Catmur et al., 2007; Heyes, 2010, see subsection 1.4.3, p.59).

CSE modulation can also depend on the orientation of the observed action (Maeda et al., 2002), and the laterality of the observed body part (Aziz-Zadeh et al., 2002). First-person perspective viewing appears to enhance MEP facilitation (Maeda et al., 2002), and from this viewpoint, MEPs elicited in the right hand are larger when observing right-hand actions (Aziz-Zadeh et al., 2002). There are also interactions between laterality and orientation of the hand (Alaerts et al., 2009a), which may be attributable to the presence of both extrinsic and intrinsic reference frames in motor and premotor cortex (Kakei et al., 1999, 2001).

An important, but understated, aspect of the early study by Fadiga et al., (1995) was the exclusion of a “rest” condition, due to the large variability in responses during this condition. The reported facilitation during observation of grasp was therefore relative to the simple observation of objects, and a dimming detection task (Fadiga et al., 1995). Several studies have noted that MEPs during the observation of grasp are strongly facilitated relative to an extra-task baseline. On the other hand, MEPs can remain unmodulated, or even become suppressed relative to an intra-task baseline or other conditions, suggesting that the former facilitation may be largely due to attentional effects (Lago & Fernandez-del-Olmo, 2011; Bunday et al., 2016; Hannah et al., 2018b). The finding of muscle-specific facilitation is therefore often based on an interaction across muscles or conditions (Bunday et al., 2016). An intra-task baseline likely provides a better comparison with extracellulary recorded MirN activity, which is typically assessed by comparing activity during stages of observed movement to a within-trial baseline. Different definitions of the baseline condition in TMS studies may explain some of the conflicting evidence on relative facilitation or suppression effects (Naish et al., 2014).

Single pulses of TMS can produce direct excitation of corticospinal neurons, and indirect activation of the CST via oligosynaptic pathways, resulting in direct (D), and multiple indirect (I-) waves in recorded volleys (Edgley et al., 1997; Ziemann & Rothwell, 2000; Di
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Lazzaro et al., 2008, see Figure 1.2). MEPs recorded in muscles reflect the net summation of these different elements, as well as excitability of the downstream spinal circuits themselves. Paired pulse TMS (pp-TMS), in which a sub-threshold pulse is delivered just prior to a supra-threshold test pulse in order to condition the subsequent MEP, can be used to delineate the cortical contribution to CSE changes during action observation. Different intervals between the paired stimuli produce different effects - short intervals (<5ms) result in inhibition (short intra-cortical inhibition (SICI)) of the test stimulus response, whereas slightly longer intervals can produce facilitation (Di Lazzaro et al., 2004). Strafella & Paus, (2000) performed single and paired-pulse stimulation over M1 during observation of hand and arm movements at different intervals while recording from the 1DI and biceps muscles. Unconditioned MEPs were larger in 1DI for observed hand actions and in biceps for arm actions. SICI produced by pp-TMS with a 3ms interval, and facilitation at a 12ms interval, were both reduced in 1DI during observation of hand-writing, and in biceps during observation of arm movements, demonstrating a modulation of cortical excitability during action observation (Strafella & Paus, 2000).

The H-reflex response, which occurs in muscles following electrical stimulation of muscle spindle afferents within peripheral nerves, can provide a measure of spinal excitability during action observation. Using this approach, Baldissera et al., (2001) found that the H-reflex response in the flexor digitorum superficialis (FDS) muscle increased during hand opening and decreased during finger closure. This modulation at the spinal level was the opposite of the previously reported pattern seen via TMS (Fadiga et al., 1995), suggesting a dissociation between cortical and spinal changes during action observation. However, a follow-up study showed that this modulation was enhanced by conditioning the H-reflex response with a TMS pulse, and the pattern of modulation closely matched the actual pattern of EMG in the FDS muscle when subjects performed the same action themselves, supporting the notion that actions are represented within the cortical and spinal motor systems of the observer (Montagna et al., 2005). This finding also underlines an important consideration in study design when probing the MNS in humans, which is not always fulfilled. Since MirNs by definition must be active during both action execution and observation, ascribing fluctuations in CSE to the MNS must consider the patterns of muscle activity during execution of the same actions.
In a cyclic wrist extension-flexion task, Borroni and colleagues showed that H-reflex modulation closely matched the sine wave oscillations of the observed movement cycle (Borroni et al., 2005). An important caveat for interpretation of H-reflex responses is that the pre-synaptic terminals of the Ia afferents within the spinal cord can be subject to homosynaptic inhibition, meaning that the same stimulus intensity can produce different responses independent of any task-related changes (Knikou, 2008).

The extent of the human MNS - evidence from neuroimaging

Non-invasive imaging, initially using positron emission tomography (PET) but increasingly fMRI, has become popular for examining the effects of action observation across a range of brain regions, and for probing the functional homology between monkey and human brains (Hardwick et al., 2018). Early studies using PET and fMRI reported activation during the observation of hand and arm actions within PMv and pars opercularis of the posterior inferior frontal gyrus (IFG) (Grafton et al., 1996; Rizzolatti et al., 1996b; Decety et al., 1997; Iacoboni et al., 1999). Observed hand, mouth, and foot actions are somatotopically segregated within premotor cortex (BA6 and BA44) (Buccino et al., 2001), and the Blood Oxygen Level-Dependent (BOLD) signal, the primary variable of interest in fMRI, is also increased in parietal areas during action observation (Iacoboni et al., 1999; Buccino et al., 2001).

BA44 forms part of Broca’s area, a key cortical motor speech centre, which has fuelled the hypothesis that MirNs held an instrumental role in human language evolution (Rizzolatti & Arbib, 1998, see subsection 1.4.3, p.59). However, the conclusion that this area forms part of the functional homologue of monkey F5 (Rizzolatti et al., 2001), and is activated by action observation, has been challenged by meta-analysis results (Grèzes & Decety, 2001; Molenberghs et al., 2012).

Contextual information regarding the observed action goal has also been shown to modulate the BOLD response. In a task in which subjects observed videos containing different contexts, actions, or intentions (context and action combined), activation in posterior IFG and PMv was greater during the intention conditions than in the context or action conditions (Iacoboni et al., 2005). This suggests that the MNS supports encoding of context or intention, which fits with results in the monkey literature (Bonini et al., 2010).
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A study on different groups of expert dancers reported that BOLD activation in premotor cortex, STS, and parietal areas was greater if subjects were viewing dance moves they were familiar with performing themselves, supporting the suggestion that observed actions are mapping onto the motor repertoire of the observer (Calvo-Merino et al., 2005). Similarly, a comparison of male and female ballet dancers, who are visually familiar with dance moves of both genders, but have gender-specific motor experience, revealed significant BOLD activation of left premotor cortex, bilateral IPS, and bilateral cerebellum in a manner which depended on the level of previous motor experience (Calvo-Merino et al., 2006).

The MNS, by virtue of the direct matching of observed and executed actions, has been proposed to subserve imitation behaviours. Iacoboni et al., (1999) found greater BOLD modulation in MNS areas such as the IFG pars opercularis, during observation for imitation and imitation execution compared to symbolically cued execution. In a more complex imitation task involving the reproduction of complex guitar chords, the IFG pars opercularis and rostral parietal cortex showed increased BOLD activation during observation for imitation and the subsequent delay period than during non-imitation conditions. Activation in the frontal cortex also extended dorsally, into an area corresponding to PMd, during imitative observation, possibly reflecting elements of motor preparation for the subsequent imitation of action (Buccino et al., 2004). The MNS has been linked with automatic and stimulus-response forms of imitation, as well as “true” imitation, which involves learning and performance of new actions. Specifically, a more direct mirror pathway has been proposed to underlie automatic imitation such as seen in neonates. Although evidence suggests that macaque monkeys do not engage in true imitation, a more indirect mirror pathway may then emerge during development which enables the suppression of automatic imitation and controlled performance of more complex, true, imitative actions (Ferrari et al., 2009).

In recordings from macaque monkeys, statistics are performed at the level of individual neurons, whereas fMRI studies typically perform group analyses on smoothed voxel data (Kilner & Lemon, 2013). At the individual subject level, overlapping BOLD activity during action execution and observation could arise from non-overlapping voxels. However, unsmoothed data from single subjects has revealed shared voxel activation within BA6/44, IPL and other areas, including PMd, SMA and SPL, during the execution
and observation of hand movements (Gazzola & Keysers, 2009). This study also found reduced activation in M1 during observation, consistent with findings of suppression during observation at the single-neuron level (Vigneswaran et al., 2013).

Furthermore, conventional fMRI analysis cannot unequivocally determine whether activation within the same voxels across two different behaviours is driven by overlapping, or separate populations. Modulation within single neurons during both execution and observation forms a cornerstone of the hypothesis that observed actions are directly matched with the motor representation of the same action. To address this, Kilner et al., (2009b) leveraged the phenomenon of repetition suppression, whereby neurons reduce, or “adapt” their firing in response to successive repeated presentations of activity-evoking stimuli. They found a reduction in BOLD activity, within and across subjects, in left IFG when action execution was followed by the same observed action, or vice versa, strongly suggesting that the modulation in the human MNS during execution and observation was occurring within highly overlapping neural populations, as suggested by the monkey literature. By contrast, Lingnau et al., (2009) reported repetition suppression within modality (execution or observation), and if observation occurred first, but not for execution followed by observation. Evidence for repetition suppression in monkey MirNs is also mixed. Initial reports provided evidence against repetition suppression, but examined only two repetitions (Caggiano et al., 2013), and multiple repetitions did produce an effect (Kilner et al., 2014).

1.4.3. Mirror neuron functions and controversies

The discovery of MirNs generated a wave of interest among both scientific and lay communities, revolving around the implication for our understanding of the links between perception and action, and social behaviour among conspecifics. It has been argued that MirNs provide a neurobiological basis for a “motoric” understanding of the goals of the actions of other individuals, via the direct mapping of observed actions onto the motor system which the observer would use to perform the actions themselves (Gallese & Goldman, 1998; Rizzolatti & Craighero, 2004; Rizzolatti & Fogassi, 2014). This action understanding hypothesis has laid the burden for a wide range of social behaviours upon the MNS, from language understanding (Rizzolatti & Arbib, 1998) to empathy, while
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dysfunction of the MNS has been linked with autism spectrum disorder (Iacoboni & Dapretto, 2006). However, causal evidence for the influence of MirNs in these cognitive and social domains is thus far lacking, and this hypothesis has been strongly criticised for its inconsistency with a number of empirical observations (Hickok, 2009, 2013, 2014). In the context of object-oriented action, the notion of high-level understanding is more consistent with semantic functions of the visual ventral stream (Jeannerod et al., 1995), and is at odds with the finding that mirror activity has predominantly been found within dorsal stream areas, and occurs in a relatively fast and automatic manner. It also fails to account for the fact that inferring action goals from observed movements is a fundamentally ill-posed problem. The lack of a one-to-one relationship between observed movements and action goals means that the forward model which predicts the sensory consequences of motor commands given the action goals cannot be directly inverted during action observation. A solution for this has been proposed in the form of a predictive coding framework (Kilner et al., 2007; Kilner, 2011). According to this model, prior expectations and contextual cues provide an initial prediction of the action goal, and the observer can then compare the observed kinematics with the kinematics predicted by their own motor system, and update the likely goal based on a minimisation of the prediction error. This minimisation can be iteratively achieved at each stage of the anatomical hierarchy, and thus allow for inference of action causes (Kilner et al., 2007). Here, it is important to note that inferring the motoric cause of an action does not necessarily imply or serve an understanding of the action goal, or vice versa (Kilner, 2011). For example, in fMRI studies in dancers of different genres, genders, and experience levels, greater BOLD activation occurred within premotor and parietal areas when individuals observed actions which were within their personal motor repertoire (Calvo-Merino et al., 2005, 2006). On the other hand, humans can, with experience, recognise actions and infer intentions of their pet dogs, despite being physiologically incapable of producing many of the same actions themselves. The lack of empirical evidence for the action understanding hypothesis and the reverse inference problem thus expose a logical fallacy, namely that the observation of actions which we can understand and perform activates motor areas, therefore activation of these motor areas during action observation provides understanding of the observed action.

An influential alternative, albeit not mutually exclusive, hypothesis posits that MirNs are a natural outcome of associative learning (Heyes, 2010). As discussed in subsection 1.4.2
1.4. Mirror neurons

(p.51), muscle-specific facilitation measured via responses to TMS can be modified based on experienced associations of executed and observed actions (Catmur et al., 2007). The key implication of this hypothesis is that sensorimotor experience of our own hand actions during development leads to the formation of an association between the motor commands for action and the sensory consequences, and this gradually and inevitably extends to the actions of others (Kilner et al., 2007). The finding that tool-responding MirNs only appear after macaques undergo prolonged training to use the tool (Ferrari et al., 2005) is consistent with shaping of the MNS by experience (Catmur et al., 2007; Heyes, 2010).

The MNS has also been proposed to mediate motor imitation, the matching of self-movement to another’s (Jeannerod, 1994; Rizzolatti et al., 2001; Rizzolatti & Craighero, 2004; Buccino et al., 2004; Iacoboni & Dapretto, 2006). Imitation is fundamental to the development of motor and social skills, and this theory provides a plausible phenomenological explanation for why observation of action can often expedite skill learning. Imitation produces activity within the human MNS (Nishitani & Hari, 2000), and sensorimotor training with compatible movements increases the speed of automatic imitation of observed hand actions (Press et al., 2007), whereas incompatible training produces interference effects (Capa et al., 2011). Ferrari et al. have speculated that largely automatic imitative behaviour in early life may take place via a “direct” mirror pathway. Later on, the refinement of motor control may then permit the mirror system to be harnessed by more indirect pathways for more complex and genuine imitation (Ferrari et al., 2009). The presence of PTNs which suppress their discharge during action observation may provide a mechanism for the suppression of automatic imitation while observing others’ actions (Kraskov et al., 2009, 2014; Vigneswaran et al., 2013).

1.4.4. Decoupling motor cortex activity from muscles

The link between perception and action, which is apparent in MirNs, can be traced as far back as the psychologist William James (James, 1890), and action observation is by no means unique in this regard. Observation, imagination, and preparation of action all occur without overt muscle or movement activity, but activate some of the same circuitry and motor programs responsible for action execution (Jeannerod, 1994). Motor imagery and mental rehearsal both evoke activity in motor areas, which overlap with those of the MNS.
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As discussed previously, the mere visual presentation of graspable objects activates many neurons with motor properties in both AIP (Sakata et al., 1995; Murata et al., 2000; Baumann et al., 2009) and F5 (Murata et al., 1997; Raos et al., 2006; Umiltá et al., 2007; Fluet et al., 2010). Some M1, and many PMd neurons modulate their activity during the preparatory period of instructed-delay tasks, and this activity can reflect a number of different movement parameters, including the force, direction and speed of upcoming movements (Evarts, 1968; Tanji & Evarts, 1976; Weinrich & Wise, 1982; Riehle & Requin, 1989; Riehle & Requin, 1993; Alexander & Crutcher, 1990; Moran & Schwartz, 1999; Churchland et al., 2006a). Preparatory activity changes are also present to some degree at the level of the spinal cord (Prut & Fetz, 1999; Fetz et al., 2002).

This decoupling of cortical and muscle activity is particularly relevant in the context of M1 neurons, which are linked both anatomically and functionally to the motoneurons innervating forelimb muscles (Porter & Lemon, 1993; Rathelot & Strick, 2006, see subsection 1.3.1, p.38). At the single-neuron level, there is substantial evidence that the discharge of M1 cells can be flexibly and rapidly dissociated from movement, even within PTNs and CM cells (Fetz & Finocchio, 1971; Schieber, 2011; Vigneswaran et al., 2013).

The puzzling heterogeneity of single-neuron response profiles and resulting lack of consensus over what parameters of movement M1 represents, and to what extent (Fetz, 1992; Churchland & Shenoy, 2007b; Scott, 2008) have prompted a shift in perspective from the “representational” framework, towards one which emphasises motor cortex as a dynamical control system where future activity is a function of current activity, input, and noise (Todorov & Jordan, 2002; Churchland & Shenoy, 2007b; Shenoy et al., 2013; Cunningham & Yu, 2014). The corollary of this perspective is that, while single-neuron readouts may resemble certain parameters of movement, such correlations are not integral to the generation of useful muscle activity, and could be largely coincidental or epiphenomenal (Fetz, 1992; Scott, 2008; Shenoy et al., 2013). Instead, this framework assumes that the correlated activity of large neural populations, such as those sampled by large-scale recordings, evolves within a manifold, which is a constrained subspace within the complete high-dimensional space of firing rates. The dominant patterns within this manifold capture a large degree of the covariance in neural activity, and can explain why
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neural and muscle activity evolve in certain ways (Shenoy et al., 2013). For example, this framework has provided valuable insights into the seeding of appropriate movement dynamics by condition-dependent preparatory activity (Churchland & Shenoy, 2007a; Churchland et al., 2010, 2012). It has also indicated that this preparatory activity avoids causing premature movement by residing in a muscle-null subspace (Kaufman et al., 2014), orthogonal to the subspace of movement activity (Elsayed et al., 2016).

The generality and mathematical intuitions of the dynamical systems approach, where each neuron is considered as a “cog within a dynamical machine” make it an attractive tool for making sense of often confusing single-neuron responses within and across different behaviours (Shenoy et al., 2013; Gallego et al., 2018). However, this approach also overlooks the known existence of distinct anatomical populations, such as M1-PTNs and CM cells, despite incontrovertible evidence that these cells make unique and important contributions to movement control (Soteropoulos, 2018; Lemon & Kraskov, 2019).

Although the activity of putative pyramidal and interneurons in motor areas provides some evidence against an explicit gating mechanism to suppress movement during preparation (Kaufman et al., 2010, 2013), the overlapping distributions of the spike waveforms of these two cell types in macaques (Vigneswaran et al., 2011) emphasises the importance of robust physiological identification in order to parse the contributions of different cell types to movement generation and suppression.

In humans, TMS and H-reflex responses have frequently been used to study motor system processes during action preparation, and a number of studies have reported time-varying suppression of CSE during pre-movement delay periods (Hasbroucq et al., 1999; Duque & Ivry, 2009; Duque et al., 2010; Lebon et al., 2016; Hannah et al., 2018a; Ibáñez et al., 2018). Different cortical and sub-cortical components of this suppression have been hypothesised to reflect “competition resolution” between different possible responses, and general “impulse control”, which globally prevents premature responses, respectively (Duque et al., 2010, 2012). Preparatory delay period changes may also play an important role in the movement initiation process itself, by setting an appropriate state from which a specific, desired movement can be generated (Churchland et al., 2012; Greenhouse et al., 2015; Elsayed et al., 2016; Hannah et al., 2018a).
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1.4.5. Inhibition of movement

The study of volitional inhibition has a long history in psychology and cognitive neuroscience, and has generally come under the domain of executive function and impulse control. Much attention has been devoted to areas of inferior frontal cortex (IFC), which become active during stopping in Go-NoGo, and stop-signal paradigms (Aron, 2009; Aron et al., 2014). Patients with right IFC lesions show disrupted inhibition in a stop-signal reaction time task, and the level of behavioural impairment and volume of affected cortex are significantly correlated (Aron et al., 2003). IFC may exert its inhibitory effect via the STN, within the basal ganglia (Aron et al., 2014).

Beyond the higher-level cognitive processes involved in impulse control, premotor and motor cortex appear to play a role in withholding, braking, pausing, or stopping responses. During center-out reaching, M1 and PMd population activity is implicitly gated from producing muscle activity during movement preparation (Kaufman et al., 2014). Achieving particular states during movement preparation may support appropriately timed movement (Churchland & Shenoy, 2007a), although is not necessarily required during non-delayed reaches or switched reaches (Ames et al., 2014). In the study of reaching and grasping, the traditional effect of PMv on M1 has been presumed to be excitatory, with this interaction critically involved in the shaping of the hand for accurate object grasping (Umiltá et al., 2007; Davare et al., 2008, 2009; Prabhu et al., 2009). However, inhibitory interactions between the two areas can be revealed in awake monkeys, both via single-pulse stimulation (Kraskov et al., 2011), and paired-pulse stimulation at longer conditioning-test pulse intervals (Prabhu et al., 2009). PMv exerts an inhibitory effect over M1 at rest (Davare et al., 2008, 2009), and the output from M1 is suppressed prior to grasp (Prabhu et al., 2007b). This inhibition disappears or becomes grasp-specific facilitation during object-driven grasp (Prabhu et al., 2007b; Davare et al., 2008, 2009), emphasising that the interaction between PMv and M1 is dynamic. Additionally, PMv may play an important role in the reprogramming of movements when the required action is altered, since the traditional facilitation seen during pp-TMS over PMv and M1 during movement initiation becomes inhibitory if action reprogramming is required (Buch et al., 2010). In a Go/No-Go task combined with single-pulse TMS over M1, Hoshiyama et al., (1996) found MEP facilitation of agonist (radial extensor), and suppression of antagonist (ulnar flexor),
muscles in the Go condition of a reaction-time hand movement task, but suppression of both muscles during the NoGo condition, suggestive of generalised inhibition in the pyramidal tract (PT). Sohn et al., (2002) used single-pulse TMS and pp-TMS to test volitional inhibition in M1. SICI was increased on NoGo trials when the subjects had to refrain from movement, around the subject’s reaction time on Go trials. This inhibition was apparent in both an agonist for the index finger extension movement required, and an unused, control muscle, supporting the suggestion that volitional inhibition in M1 is non-selective.

Descending activity within the CST can have both excitatory and inhibitory effects at the level of the spinal cord (Porter & Lemon, 1993). PTNs and even CM cells can become active hundreds of milliseconds prior to movement, suggesting mechanisms for suppression of movement, at the level of the cortex, spinal cord, or both, must exist. During action observation, suppression-MirNs, which unequivocally disambiguate between action execution and observation conditions, may be particularly important for flexible and rapid dissociation of motor cortex from muscle activity (Vigneswaran et al., 2013; Kraskov et al., 2014). Together with the weaker discharge in facilitation MirNs during action observation relative to execution (Vigneswaran et al., 2013), this activity clearly results in an overall disfacilitation of the spinal cord during observation. There is some evidence for suppression at the level of the spinal cord during action observation in both humans and monkeys (Baldissera et al., 2001; Montagna et al., 2005; Stamos et al., 2010). This suppression could reflect active inhibitory mechanisms at the spinal level, or simply a relative disfacilitation driven by the reduction in corticospinal outflow (Vigneswaran et al., 2013; Kraskov et al., 2014).

1.5. Thesis outline

This thesis involved multi-electrode recordings of cortical activity, and EMG recordings during stimulation in awake, behaving macaques, as well as TMS studies in healthy human volunteers.

In chapter 2, I describe general methods regarding the non-human primates used in multiple experiments within this thesis. In chapter 3, I present data from single neurons,
1.5. Thesis outline

including identified PTNs, recorded in areas M1 and F5, while trained macaque monkeys executed, observed, or withheld reach-to-grasp actions. The properties of MirNs in F5 have been studied extensively, but little is known about the relationship of this activity to the mirror activity recently identified in M1. This chapter therefore quantitatively compares the profiles of neuronal discharge across action execution and action observation in premotor and motor cortical MirNs. Additionally, the neural correlates of action suppression during action observation are assessed by comparing activity during action observation and the cued withholding of action.

The net effect of grasping observation activity in descending pathways has been little studied, and evidence for spinal modulation during action observation is inconclusive. In chapter 4, I took advantage of the MEPs evoked by direct stimulation of the PT to test the effects of action observation and action withholding on spinal motoneuron pools. These results provide important additional insights into our understanding of the net effects of mirror activity within the CST, and its effects on downstream targets.

Chapter 5 examines factors which may influence the level of activity during action observation in three brief experiments. For example, while passive action observation provides visual information, the somatosensory feedback which is obtained during self-action is notably absent. Accurate grasping execution requires extraction and use of proprioceptive and tactile information regarding object features, whereas these inputs are absent during passive action observation. We investigated MirN activity as monkeys observed actions while simultaneously engaged in another action, and also when monkeys had to extract information from the observed grasp and replicate it in order to successfully complete the trial. Previously, single-neuron recordings showed that mirror activity does not necessarily depend on online, trial-by-trial visual information, however this property has not been studied in M1 MirNs, which may show a greater dependence on the specific kinematics of observed actions. We therefore also recorded MirNs while monkeys observed actions for which the level of visual information regarding the grasp was systematically altered. In these three experiments, neurons were also recorded during the basic, passive action observation condition, which allowed a comparison of their activity across the different observation conditions.

A large body of evidence suggests that humans also have MirNs with similar and possible
1.5. Thesis outline

additional functional properties to those found in macaques. In chapter 6, I used single-pulse TMS over M1 to investigate modulation of CSE in healthy human volunteers performing a similar behavioural task involving action observation and withholding. I also assessed modulation with the motor system during active observation, when subjects executed a grasp which was congruent or incongruent with one which they were simultaneously observing.

Finally, chapter 7 draws together the findings from the preceding chapters, and highlights unanswered questions which future studies may tackle.
2. Non-human primate methods

This chapter describes general methods regarding non-human primate subjects, and the design of the basic mirror task. Specific details of the variants of the mirror task used in individual experiments, and data analysis methods, are outlined in the relevant ensuing chapters.

2.1. Behavioural task

2.1.1. Monkeys

Experiments involved two adult male purpose-bred rhesus macaque monkeys (*Macaca mulatta*), M48 and M49, weighing 12.0kg and 10.5kg, respectively. All procedures were approved by the Animal Welfare and Ethical Review Body at the UCL Queen Square Institute of Neurology, and carried out in accordance with the UK Animals (Scientific Procedures) Act, under appropriate personal and project licences issued by the UK Home Office. The monkeys were single-housed based on veterinary advice, in a unit with other rhesus monkeys, with natural light and access to an exercise pen and forage area. At the time of writing, both monkeys are still alive.

2.1.2. Training

After acclimatising to the housing area, monkeys were trained to enter a transport box through a door in the home cage, which could then be used to move the monkey into the laboratory. Here, monkeys were trained to interact with objects for small food rewards, in preparation for training on the task. Monkeys were then trained to accept neck restraint via a smooth metal collar, which enabled us to transfer the monkey from the transport box to a primate chair within the experimental recording rig. A loose-fitting plate was placed above the hips. After implantation of a headpiece for head fixation (see subsection 2.2.2, p.73), monkeys were trained to accept head restraint, using a metal disc and horseshoe plate to secure the head to the experimental rig for stable single-unit recording. Training on the
2.1. Behavioural task

Basic version of the mirror task was well underway before head fixation. Positive reinforcement and standard operant conditioning techniques were used at all stages of training, although negative reinforcement was occasionally used where deemed necessary and effective (Mason et al., 2019).

2.1.3. Experimental task

In each session, the monkey sat opposite a human experimenter, with a custom-built experimental box apparatus between them (Figure 2.1A). The monkey was presented with two target objects in peri-personal space, a trapezoid affording precision grip (PG), and a sphere affording whole-hand grasp (WHG) (Figure 2.1A, inset). Each trial began after a short inter-trial interval (ITI) (1-2s), with the monkey depressing two homepads with both hands and the experimenter depressing a homepad on their side. A controllable LCD screen (14cm x 10cm) became transparent (LCDon, Figure 2.1B,C), and the object area was illuminated with white light. After a delay (0.25s in M48, variable 0.25-0.45s in M49), two amber LEDs illuminated on one side or the other to indicate the target object for the current trial. After a further delay (0.8s in M48, variable 0.8-1.2s in M49), a single green or red LED indicated the trial type. When a green LED was presented on the monkey side (Go), the monkey released the active (right) homepad (homepad release (HPR)), and made a reach-to-grasp movement towards the target object using their right hand. The monkey then grasped the object using a trained grasp (displacement onset (DO)), rotated the object into a window (> 30° rotation) and held for 1 second (hold onset (HO) to hold off). A constant frequency tone indicated that the monkey was in the hold window, and a second, higher frequency tone after 1s indicated successful completion of the hold. The monkey then released the object and returned to the homepad, and another high frequency tone indicated correct completion of the trial. Observation trials followed the same sequence, except that the experimenter performed the same reach-to-grasp and hold movement in front of the monkey, who remained still, with both hands on the homepads. On NoGo trials, a red LED required the monkey to simply remain on the homepads for the duration of the trial. After a delay (0.7s in M48, 1.0s in M49), a single tone indicated the end of the trial. The monkey received a small fruit reward directly to the mouth for each successfully completed execution, observation or NoGo trial. All trial types were presented in pseudo-randomised order, with relative proportions of 8:3:2 for
2.1. Behavioural task

each object. The larger proportion of execution trials were used to ensure the monkeys remained attentive and were regularly expected to move. Error trials, where there was a failure to respond appropriately within the constraints of the task (e.g. releasing the homepad before the Go cue), triggered a low frequency error tone and were immediately aborted by the experimental software. The monkey was not rewarded and these trials were excluded from further analysis.
2.1. Behavioural task

Figure 2.1. Basic mirror task design (A). Schematic of the custom-built experimental box, showing target objects, their corresponding LEDs, LCD screen, and homepads. Inset shows the trapezoid and sphere objects, and the respective precision and whole-hand grasps performed by the monkeys on execution trials. (B). Pseudo-random trial presentation sequence, shown as 2-D representation of monkey’s view of object area. All trials began in the same way, with the object area illuminated (LCDon), and upcoming object/grasp cued (e.g. trapezoid, PG). Each trial was then indicated as Execution (green LED on monkey side), Observation (green LED on experimenter side), or NoGo (red LED on monkey side). (C). Homepad and object displacement signals on Go trials, and digital task events. LCDon LCD screen becomes transparent, ObjCue, object cue (amber LED); Go/NoGo, green/red LED; HPR, homepad release; DO, displacement onset; HO, hold onset; HOFF, hold offset; HPN, homepad return.
2.2. Procedures

2.2.1. Structural MRI scans

A 3T Siemens Trio Scanner was used to acquire T1- and T2-weighted magnetic resonance imaging (MRI) scans with 0.5mm³ voxel resolution. The calvarium surface was extracted in Brainsight software, and used to guide custom design of a headpiece for secure head fixation. The brain surface and locations of central and arcuate sulci were used to guide the optimal location for implantation of recording chambers and PT stimulation electrodes (see subsection 2.2.2). For scanning, monkeys were anaesthetised with a ketamine/medetomidine (Domitor) mix (i/m), which was topped up intravenously at 45 minute intervals. Monkeys were secured in a plastic stereotaxic holder with a surface coil for the procedure, which typically lasted 2.5 hours, with each scan lasting 45 minutes. Post-procedure recovery was expedited using antisedan (atipamezole, i/m) for reversal of anaesthesia, and monkeys were recovered in a padded cage overnight, before being returned to their home cage.

2.2.2. Surgical implants

Each monkey underwent four separate and well-spaced surgical procedures to prepare for experiments. First, monkeys were implanted with a ring-shaped bio- and MRI-compatible TekaPEEK (30% carbon) headpiece, firmly secured to the skull via four custom-designed bolt assemblies, with 9mm diameter discs placed epidurally, moved beneath the skull along burr holes (4mm length), and locked in place from above. We waited at least 8 weeks before attempting to train head fixation to allow for adequate osseointegration of the implanted headpiece.

In a second surgery, a custom-built TekaPEEK recording chamber was fixed using bone cement and dental acrylic to cover a craniotomy exposing the dura mater over M1 and F5. Fiducial markers on the chamber lid were measured in stereotactic co-ordinates during surgery, and used for subsequent triangulation of penetration locations (see section 2.3). In a third surgery, EMG electrodes were subcutaneously tunnelled from a connector on the headpiece through to the right arm and chronically implanted into 12 muscles in the right
arm and hand (muscle names are listed in subsection 2.3.1, p.77). Finally, two fine tungsten electrodes (240µm) were stereotaxically implanted in the left medullary pyramid in an anterior-posterior configuration. Each electrode was lowered within a guide tube until beyond the tentorium cerebelli, and then further lowered beyond the guide tube in 0.5mm steps, while single test stimuli of 300µA were delivered at regular intervals. The stimulus configuration was monopolar against an indifferent electrode in the scalp for the first electrode, or bipolar between the electrodes for the second. The final electrode position was determined on the basis of the threshold of the antidromic volley recorded via a ball electrode placed on the dura over M1. The guide tube was then carefully removed, and electrodes were fixed at a location below the intra-aural line which elicited the lowest threshold antidromic volleys (<50µA), with no additional signs of facial or hypoglossal activation (Kraskov et al., 2009). All surgical procedures were performed in aseptic conditions and under full general anaesthesia, induced with ketamine (10 mg/kg i/m) and maintained with 1.5-2.5% isoflurane in O₂, delivered via endotracheal intubation. Monkeys were intravenously infused with Ringer’s (Hartmann’s) solution at a rate of ~10ml/kg/h. Heart rate, respiration rate, SpO₂, end-tidal pCO₂, body temperature and blood pressure were monitored and recorded at regular intervals. Except for tunnelling stages of the EMG surgery, the monkeys were tightly secured in a metal stereotaxic frame, with atraumatic ear bars in the external auditory meatus. Eye bars on the infra-orbital margin and a palate bar securing the maxilla prevented rotation of the skull. Monkeys were recovered in a padded cage overnight, and received a full course of antibiotic, analgesic and anti-inflammatory medication as prescribed under veterinary advice.

2.2.3. Chamber maintenance

The exposed dura within the recording chamber is susceptible to infection and was therefore cleaned regularly. 5-fluorouracil (5-FU) was applied to the dura surface for 5 minutes after recording sessions (or at 2-3 day intervals during non-recording periods), before being washed off thoroughly with sterile saline. 5-FU is a powerful anti-mitotic agent with bacteriocidal and bacteriostatic effects (Spinks et al., 2003), and thus prevents both the proliferation of fibroblast scar tissue, and infection. We also applied antibiotic gel (gentamycin) before resealing the chamber, to reduce the risk of infection. Occasionally, excessive tissue build-up rendered electrode penetrations extremely difficult and made it
2.3. Neuronal recordings

necessary to perform a dura strip in order to resume recordings. These were performed under full general anaesthesia (ketamine/medetomidine, i/m) and involved the use of fine scissors, forceps, a corneal hook, and suction to remove excess tissue (Spinks et al., 2003).

2.3. Neuronal recordings

![Array heads used for recording, with UK 10p piece (24.5mm diameter) shown for scale. Drive heads were attached to Eckhorn multielectrode manipulator microdrives. Insets show configuration and scale of guide tubes. Linear array guide tubes were separated by 500µm, square and circular array guide tubes were separated by 305µm. (E). A single loaded electrode protruding from linear array guide tube is indicated by red arrow. Black arrow indicates zeroing wire used for measurements. The gradient of the guide tubes was implemented to accommodate the slope of the dural surface.](image)

We used 16 and 7 channel Thomas Recording drives (Thomas Recording GmbH, Geissen, Germany, Eckhorn & Thomas, (1993)), each containing 1–5 quartz glass-insulated platinum-iridium electrodes (shank diameter 80µm, impedance 1–2MΩ) to record in the arm/hand regions of M1 and F5. On a given recording day, we either carried out dual recordings, recording in M1 using the 16-drive, and in F5 using the 7-drive, or recordings
in one area using a single drive. Linear array heads (spacing between adjacent guide tubes = 500$\mu m$) were used for initial mapping of M1 and F5, and subsequent recordings were conducted with square (16 drive) or circular (7 drive) heads to target more specific locations (305$\mu m$ spacing). The four array heads are shown in Figure 2.2A-D, and Figure 2.2E shows one electrode loaded into the linear array head for the 16 drive. Drives were secured to a metal plate on the rig above the monkey’s head, and angled for approximately perpendicular penetrations of the intact dura. Up to 5 fiducial points on the chamber lid were measured using a zeroing wire of known length placed within one of the guide tubes. Custom-written Matlab routines were used to transform the measured co-ordinates into an orthogonal system, which could then be mapped to a stereotaxic reference frame by minimising the rotational and translational error between these points and those measured stereotaxically during surgery. This procedure allowed us to note and compare penetration (and recording) locations across sessions (Figure 2.3 & 2.4). The drives were lowered until guide tubes containing the electrodes touched, and slightly dimpled, the dura surface. Electrodes were then slowly advanced one at a time using custom computer control software (Baker et al., 1999), while carefully listening for neural activity, until penetration was evident from the sound of neural activity in the recording or penetration of the dura, as viewed through a microscope (usually 1-2mm beyond the tip of the guide tube). After all electrodes had penetrated in turn, each one was slowly raised until no neuronal activity could be heard, or viewed on an oscilloscope. After penetration, we waited 10-15 minutes before any further electrode movement to allow recovery from any cortical depression and tissue destabilisation which may have been induced by penetration.

After a recording set was completed, repetitive intra-cortical microstimulation (rICMS) was delivered through each electrode in turn via an isolated stimulator to characterise output effects at recording locations. Sequences of 13 pulses at 333Hz (duty cycle 0.5Hz) were delivered every 1-1.5s at intensities up to 30$\mu A$ (M1), or 60$\mu A$ (F5), and the monkey’s body was carefully inspected for evoked motor responses.
2.3. Neuronal recordings

Figure 2.3. Structural MRI and example penetrations in M48. (A) shows a 3-D rendering of the brain surface. CS, central sulcus; AS, arcuate sulcus. (B) & (C) show coronal sections marking the same penetrations shown in (A) in M1 and F5, respectively. An additional transformation was required to translate between stereotaxic and MRI co-ordinates.

2.3.1. Recording parameters

Broadband signals from each drive were pre-amplified (x20, headstage amplifier), further amplified (x150), and bandpass-filtered (1.5Hz – 10kHz), and sampled at 25kHz via a PCI-6071E, National Instruments card. We simultaneously recorded EMG activity from 12 muscles in the contralateral arm and hand (first dorsal interosseous (1DI), thenar eminence, and abductor digiti minimi (AbDM), abductor pollicis longus (AbPL), extensor digitorum communis (EDC), extensor carpi ulnaris (ECU), flexor digitorum profundus (FDP), flexor carpi ulnaris (FCU), flexor carpi radialis (FCR), and brachioradialis (BRR), biceps extensor carpi radialis longus (ECR-L) (M48 only), and deltoid (M49 only). EMG was hardware high-pass filtered at 30Hz, amplified 2000x or 5000x, and sampled at 5 kHz. We also recorded analog signals of object displacement and homepad pressure (5kHz), as well as the timing of key task events at 25kHz resolution. All data was stored on laboratory computers for offline analysis.
2.3. Neuronal recordings

Figure 2.4. Map of recording chambers and electrode penetrations, shown in stereotaxic co-ordinates. M48 (left), and M49 (right). In both cases, the posterior edge of the recording chamber lay approximately above the central sulcus, and the anterior edge was close to the inferior limb of the arcuate sulcus. \textit{AP}, anterior-posterior; \textit{ML}, medial-lateral, \textit{HT}, height.

2.3.2. PTN identification

While searching for cells, PT stimulation was delivered between the two PT electrodes (Figure 2.5A). The search stimulus intensity was 250–350\(\mu\)A, and pulses were delivered every 0.6s (biphasic pulse, each phase 0.2ms). PTNs were identified as well-isolated cells which showed a robust and latency-invariant response (jitter \(\leq\) 0.1ms) to PT stimulation - larger jitters suggest synaptic, rather than direct antidromic, activation (Lemon, 1984).

In M49, the search stimuli consisted of 2 shocks 10ms apart (delivered every 0.6s), which provided additional confirmation of synaptic vs. antidromic activation. Antidromic responses invariably followed both shocks, but jitter in the antidromic response to the second shock was typically smaller since the influence of spontaneous activity was reduced (Swadlow et al., 1978). We recorded the antidromic latency (ADL) of each PTN, and determined threshold by slowly decreasing the stimulus intensity until the antidromic response disappeared. Changing the intensity also helped to disentangle cases where the antidromic responses of two PTNs with similar ADLs were superimposed. For absolute confirmation of PTNs identity, antidromic responses were subjected to a collision test. Spontaneous spikes were passed through a manually-defined window discriminator and used to trigger PT stimuli at a chosen latency. Altering this latency in 0.1ms steps enabled
us to determine the collision time, which was defined as the maximum time difference at which antidromic and orthodromic spike still collided, resulting in no spike at the recording electrode (Lemon, 1984, Figure 2.5B). PTN identification was always performed before task recordings, so this sample of cells was unbiased in terms of task-related activity, although was likely biased towards cells with fast-conducting, large axons (Vigneswaran et al., 2011; Firmin et al., 2014; Kraskov et al., 2019).

Figure 2.5. Identification of pyramidal tract neurons via antidromic stimulation and collision testing. (A). Bipolar stimulation (each phase 0.2ms, \(\sim 300\mu A\)) was delivered at the level of the medullary pyramid through chronically implanted tungsten electrodes. Antidromic responses can be identified at recording electrodes in the cortex. Stimulation also produces orthodromic responses via the lateral corticospinal tract (CST), which can elicit short-latency MEPs in hand and arm muscles. Other descending tracts, and indirect connections to motoneurons, are not shown for simplicity. (B). Top panel shows a single sweep with antidromic response (black) in M1-PTN at \(\sim 1.1\)ms. ADL, antidromic latency. If a spontaneous spike is used to trigger stimulation at a short latency, a collision will result within the axon and no antidromic response is seen at the recording electrode (red). Bottom panel shows all individual antidromic responses (black), and collisions (red). One antidromic response is delayed - this can result if the latency between the spontaneous spike and the stimulus is close to the collision time as the axon will be in a sub-normal conduction state when the stimulus is delivered.
2.3. Neuronal recordings

2.3.3. Spike discrimination

Offline spike sorting was performed using modified WaveClus software (Quiroga et al., 2004; Kraskov et al., 2009). Broadband data was first high-pass filtered (acausal 4th order elliptic 300Hz-3kHz, or subtraction of a median-filtered version of the signal). Threshold crossings were then sorted into clusters using an extended set of features, including wavelet coefficients, amplitude features, and the first 3 principal components. Manual curation of clusters outputted by the automatic procedure was possible by altering the temperature for the superparamagnetic clustering procedure, and by assigning unclustered spikes to curated templates. PTN spike shapes during task recordings were compared to the recorded waveforms of spontaneous spikes which resulted in successful collisions (Lemon, 1984; Kraskov et al., 2009). Single units were considered as those with a clean, consistent waveform and with inter-spike interval histograms uncontaminated below 1ms for bursting units.
3. Movement initiation and grasp representation in premotor and primary motor cortex mirror neurons

3.1. Abstract

PTNs within macaque rostral ventral premotor cortex (F5) and primary motor cortex (M1) provide direct input to spinal circuitry and are critical for skilled movement control, but can also be active during passive action observation. In M1, relatively reduced PTN activity during grasp observation may explain the lack of overt movement in this condition, and different functional contributions of F5 and M1 during the execution and observation of actions could support the flexible generation or suppression of grasping movements during these two conditions. Here, we recorded from single neurons, including identified PTNs in the hand and arm area of M1 (n=189), and in premotor area F5 (n=115) of two adult male macaques, while they executed, observed, or simply withheld (NoGo) reach-to-grasp and hold actions. Simultaneous electromyographic recordings from multiple arm and hand muscles confirmed that mirror activity during observation could not be attributed to small levels of muscle activity. Single neuron responses were heterogeneous, with classical and suppression MirNs in both cortical areas. At the population level, F5 mirror activity was more sustained and similar in profile to execution activity. In contrast, although some neurons mirrored during grasp and hold, M1 population activity during observation contained signatures of a withholding state in the lead-up to observed movement onset. Thus, while F5 maintains a more sustained representation of grasping actions, M1 and its output population may dissociate signals required for the initiation of movement from those associated with the representation of grasp in order to flexibly guide behaviour.
3.2. Introduction

The defining property of MirNs is that they modulate their firing both when a monkey performs an action, and when it observes a similar action performed by another individual (Gallese et al., 1996; Rizzolatti & Fogassi, 2014). Since their discovery in the macaque rostral ventral premotor cortex (F5), cells with mirror-like properties have been identified in parietal areas (Fogassi et al., 2005; Bonini et al., 2010; Lanzilotto et al., 2019), dorsal premotor cortex (PMd) (Cisek & Kalaska, 2004; Papadourakis & Raos, 2019), and even primary motor cortex (M1) (Tkach et al., 2007; Dus唤ova & Donoghue, 2010; Vigneswaran et al., 2013). MirNs thus appear to be embedded in an extended network (Bonini, 2016; Bruni et al., 2018), within a parieto-frontal circuit integral to the execution of visually-guided grasp (Jeannerod et al., 1995; Borra et al., 2017). The widespread activity within this circuitry during action observation takes place in the absence of detectable movement or muscle activity, despite the finding that even PTNs, which project directly to the spinal cord, can exhibit mirror properties (Kraskov et al., 2009; Vigneswaran et al., 2013).

While F5 MirNs often show similar levels of activity during execution and observation (Gallese et al., 1996; Kraskov et al., 2009), in M1-PTNs there is typically a reduced, or reversed, pattern of firing during observation relative to execution (Vigneswaran et al., 2013; Kraskov et al., 2014). By design, most action observation paradigms require movement suppression, and this disfacilitation of spinal outputs provides a rational, threshold-based explanation for why movement is not produced. However, there is substantial empirical evidence of both facilitation and suppression during movement execution in PTNs (Kraskov et al., 2009, 2014; Quallo et al., 2012; Vigneswaran et al., 2013; Soteropouloς, 2018) and putative pyramidal neurons (Kaufman et al., 2010, 2013), which suggests a more nuanced relationship between PTN activity and movement. At the spinal level, PTNs not only excite motoneurons via cortico-motoneuronal (CM) projections (Porter & Lemon, 1993; Rathelot & Strick, 2006), but also exert indirect effects via segmental interneuron (sIN) pathways, which in turn display their own complex activity before and during movement (Prut & Fetz, 1999; Takei & Seki, 2013). A dynamical systems approach (Shenoy et al., 2013) has recently suggested that movement-related activity unfolds in largely orthogonal dimensions to activity during
3.3. Methods

action preparation, such that movement is implicitly gated during movement preparation (Kaufman et al., 2014; Elsayed et al., 2016), and a similar mechanism has been hypothesised to operate during action observation (Mazurek et al., 2018). While the roles of F5 and M1 during the execution of visually-guided grasp have been studied extensively (Umiltá et al., 2007; Davare et al., 2008; Schaffelhofer & Scherberger, 2016), a more systematic understanding of the differences between action execution and observation activity in these two key nodes in the grasping circuitry could provide important insights into dissociations between representation of potential actions at the cortical level, and recruitment of descending pathways and muscles for actual action execution (Schieber, 2011). Along these lines, recent work comparing MirNs in premotor and motor cortex found premotor MirNs, but not those in M1, showed similar state transitions in execution and observation (Mazurek et al., 2018). State-space analyses have also previously found that F5 and the upstream anterior intraparietal area (AIP) exhibit different dynamics during immediate and delayed grasping actions (Michaels et al., 2018). Additionally, while previous work has examined grasp representation in F5 during inaction conditions (Bonini et al., 2014b), and reported little overlap between MirNs and neurons encoding self-action withholding, interleaved action and inaction within peri-personal space may provide a more ecologically valid framework for investigating movement suppression during action observation.

To explore the functional relationships between action execution, observation, and withholding, we compared the discharge of MirNs in M1 and F5 of two macaque monkeys, while they switched between executing, observing, and withholding reach-to-grasp and hold movements on a trial-by-trial basis. Electrical stimulation in the medullary pyramid was used to antidromically identify PTNs, and we leveraged the precise timing of task events within a naturalistic experimental paradigm to assess and compare the patterns of discharge of different populations of neurons across task conditions.

3.3. Methods

Details regarding animals, surgical preparations, basic experimental task design, and recording procedures, have been described in chapter 2.
3.3. Methods

3.3.1. EMG and behavioural analysis

EMG data for each channel was high-pass filtered (30Hz, 2nd order Butterworth), rectified, low-pass filtered (500Hz, 2nd order Butterworth), downsampled to 500Hz, and smoothed with a 100ms moving average. Signals were then aligned to the Go cue on individual trials, normalised to the 99th percentile amplitude across all trials and then averaged across trials within each condition. We recorded the timing of all relevant task events for subsequent alignment to analog signals. We defined reaction time on each execution and observation trial as the time between the GO cue and HPR, and movement time as the time between HPR and DO. For visualisation of displacement and homepad signals (Figure 3.1), individual trials were aligned to the Go cue. Signals were normalised to the 99th percentile amplitude across all trials and then averaged across trials within each condition.

3.3.2. Single-neuron analyses

To assess task-dependent modulation during execution and observation, we initially defined seven task epochs of interest, as follows. (1) LCDon-CUEon (2) Object Cue: 500ms period before the Go/NoGo cue. (3) Early React: 0-150ms from the Go/NoGo cue (4) Late React: 150-300ms from the Go/NoGo Cue. (5&6). Early and Late Reach: the first and second halves of the HPR-DO interval, which varied in length on each trial. (7) Hold: 0-700ms from HO. Firing rates during execution and observation for each neuron and object were subjected to a one-way ANOVA with factor EPOCH (7 levels), followed by post-hoc comparisons to Baseline (Tukey-Kramer correction for multiple comparisons). Neurons showing a significant ANOVA result and at least 1 significant post-hoc result (p < 0.05) for a given condition, with a raw firing rate range of 5 spikes s\(^{-1}\) in at least one condition, were considered to be task-modulated. Neurons modulated during any of epochs 5-7 (Movement epochs) of execution and observation were classified as MirNs. All modulation statistics and analyses were performed within object - a neuron could therefore be classed differently for the two objects. For some analyses, we further categorised MirNs according to the sign of their maximum modulation during the Movement epochs of both execution and observation, for each object separately. Thus, MirNs could be subdivided into facilitation-facilitation (F-F), facilitation-suppression (F-S), suppression-suppression (S-S), or suppression-facilitation (S-F) types for each object, based on their responses to
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execution and observation, respectively.

For identification of putative CM cells, SpTAs of rectified EMG were compiled from -20 to +40ms around each spike, and non-stationary backgrounds were removed by subtracting a linear regression fit.

3.3.3. Population analyses

For all population analyses, spike times for each neuron were binned into firing rates, baseline-corrected and normalised. The exact details differed for different analyses, and these are described in turn below.

Heatmaps and population averages

To visualise neural population activity during the task, spike counts in 10ms bins were smoothed with a Gaussian kernel (unit area, standard deviation 50ms) and converted to spikes s\(^{-1}\). As the timing of events varied across trials, conditions and sessions, firing rates were aligned separately to Go, HPR, and DO events on each execution and observation trial as appropriate, so that the relative timing of these three events, covering the most dynamic period of the task, was matched across all conditions and units. For visualisation purposes, peri-stimulus time histograms (PSTHs) aligned to different task events were interpolated to produce one continuous firing rate for each condition. The Go/NoGo event was set as time 0, and HPR and DO were defined as the mean times across conditions, objects, and sessions. The average firing rate across conditions during the Baseline period (LCDon-ObjCue) was subtracted, and the resultant net firing rates were soft-normalised to the maximum absolute firing rate across all conditions + constant factor of 5. Each unit’s firing rate across all conditions was therefore limited to a maximum theoretical range of [-1,1], where negative normalised values correspond to suppression of the firing rate relative to the baseline (Kraskov et al., 2009; Vigneswaran et al., 2013). Comparisons of population activity were conducted on firing rates within the intervals defined for single-neuron analyses (also baseline-corrected and soft-normalised), using Wilcoxon sign-rank tests with Bonferroni correction for multiple comparisons.
3.3. Methods

Figure 3.1. EMG and behaviour during basic mirror task. (A). A single session in M48. Top panels show pre-processed, rectified, and normalised EMG activity for different muscles with clean recordings. Corresponding object displacement and homepad signals are shown in bottom panels. Execution EMG is presented for both objects separately, observation and NoGo EMG are pooled across objects, and shown with a 10x higher gain. Vertical markers at top of each trace indicate median time of key task events (LCDon, HPR, DO, HOFF) relative to Go/NoGo cue (vertical dashed lines). (B). Same as (A), but for M49. ECU, extensor carpi ulnaris; EDC, extensor digitorum communis; FCU, flexor carpi ulnaris; FDP, flexor digitorum profundus; FCR, flexor carpi radialis; IDI, first dorsal interosseous; AbDM, abductor digiti minimi; HH, human homepad; ML, monkey left homepad; MR, monkey right homepad; PG, precision grip; WHG, whole-hand grasp.
3.3. Methods

Correlation analyses

To make an initial analysis of the correspondence between execution and observation activity across the task, we averaged each neuron’s activity within each of the 7 task periods, and then across trials, for each condition. Activity was baseline-corrected by subtracting the average activity in the 250ms prior to LCDon, and then soft-normalised by the maximum absolute rate across conditions (within object), with a small constant (+5) added to the denominator to reduce the influence of low-firing neurons and improve interpretability of scatter plots. For each epoch, the net normalised execution and observation activity within a MirN population were extracted as N-dimensional vectors (N = number of MirNs), and the Pearson correlation coefficient between pairs of vectors was calculated. To compare observed correlation values to those expected by chance, we repeatedly shuffled (1000 iterations) the observation vector to destroy any within-unit relationships, and re-calculated the correlation coefficient, generating a null distribution of correlation values. Observed correlations were deemed significant if they fell beyond the range of 95% of the values in the null distribution. To examine the stability of cross-condition similarity in each population, we extended the cross-condition correlation procedure to correlate activity across timepoints, using time-resolved firing rates. To avoid trivial correlations induced by Gaussian smoothed firing rates, we calculated spike rates in 50ms non-overlapping bins, with the same multiple alignment as used for the population averages (Go, HPR, DO). We then correlated PSTH activity at execution condition timepoint \( t \) with activity at all timepoints \( t = 1...T \) in the observation condition, and vice versa, and then averaged across the diagonal. This produced a \( T \times T \) matrix containing the correlation values of each timepoint \( t \) with every other timepoint.

Decoding analyses

We used the Neural Decoding Toolbox (Meyers, 2013) to examine how well activity in each sub-population discriminated between conditions. We first ran the decoding across all three conditions (Execution, Observation, NoGo), and then repeated analysis using Observation and NoGo conditions only. Binned data (non-overlapping 50ms bins), singly aligned to the Go/NoGo cue for each trial, was used to form pseudo-populations of units for each population separately, using 10 trials from each condition (3x10 = 30 data points for each
3.3. Methods

condition in the 3-way decoding), and then randomly grouped into 10 cross-validation splits (3 data points per split). Firing rates were z-scored to reduce the bias of high-firing units in the classification. A maximum correlation coefficient classifier was trained on all but one of the splits, and then tested on the left-out split, and this procedure was repeated up to the number of splits, leaving out a different split each time. For increased robustness, the cross-validation splits were resampled 50 times, and decoding accuracy was averaged across these runs. To assess the significance of the observed decoding accuracy, we used a permutation test procedure. The classification was performed exactly as for the original data, except the relevant trial condition labels were shuffled beforehand. This was repeated 50 times to generate a null distribution of the decoding expected by chance, and the observed decoding accuracy was considered significant for a given bin if it exceeded all the values in the null distribution. To reduce the false positive rate, bins were considered truly significant only if they fell within a cluster of at least 5 consecutive significant bins.

Subspace analyses

To compare the trajectories of MirN activity in each sub-population, we applied principal component analysis (PCA). PCA identifies an orthogonal transformation for (correlated) data, where each successive dimension in the transformed space captures the maximum possible variance in the data, while remaining orthogonal to all other dimensions. Projection of data onto the leading principal axes can therefore be used to reduce dimensionality in a principled manner, and reveal low-dimensional structure which may otherwise be obscured. For application to neural data, we assume that neural activity lives within a high N-dimensional space at all times, but is constrained to a lower-dimensional manifold within this space, since neurons covary in their firing rates, and not all possible combinations of activity can be achieved. The goal of PCA is to identify this low-dimensional manifold, and this is made mathematically convenient via linear algebra procedures for matrix diagonalisation.
3.3. Methods

For any matrix $A$ of trial-averaged, mean-centred firing rates of dimensions $T \times N$, where $T$ is the number of observations (timepoints), and $N$ is the number of features (neurons), singular value decomposition (SVD) of $A$ yields:

$$A = USV^T$$  \hspace{1cm} (3.1)

where $U$ is a $T \times T$ unitary matrix, $V$ is an $N \times N$ unitary matrix, and $S$ is a $T \times N$ diagonal matrix. $U$ contains the left singular vectors (orthonormal eigenvectors of $AA^T$), $V$ the right singular vectors (orthonormal eigenvectors of $A^TA$) and $S$ contains the singular values along its diagonal and in descending order, which are shared between $U$ and $V$. Here, the columns of $V$ are the principal axes of the data, and the columns of $AV$ (or equivalently, $US$), are the principal components ("scores") i.e. the projections of the data onto the principal axes. Since the principal axes are ranked in order of the amount of variance they capture in the data (quantified in the singular values), dimensionality reduction is achieved simply by taking the first $k$ axes which explain a satisfactory level of variance, and discarding the rest, many of which will capture little variance in the data. Note that if the matrix $A$ is transposed (dimensions $N \times T$), then the interpretation of $U$ and $V$ is simply reversed. PCA can also be achieved via eigendecomposition of the covariance matrix of $A$.

$$C = \frac{A^TA}{T-1}$$  \hspace{1cm} (3.2)

$$C = VDV^T$$  \hspace{1cm} (3.3)

where $V$ and $D$ are matrices containing the eigenvectors and the eigenvalues, respectively, of $C$. The columns of $V$ are the principal axes, and the columns of $AV$ are the projections of the data onto these axes. Substituting Equation 3.1 into 3.2 demonstrates that the singular values derived from SVD are proportional to the square root of the eigenvalues of the covariance matrix:

$$C = \frac{VSU^TUSV^T}{T-1} = V S^2 \frac{1}{T-1} V^T$$  \hspace{1cm} (3.4)
3.3. Methods

Any data matrix containing the activity of the same \( N \) neurons e.g. during a different epoch or behaviour, can then be projected on to the same axes simply by multiplication with the columns of \( \mathbf{V} \), and the variance captured along each axes can be computed as \( \mathbf{D} = \mathbf{V}^T \mathbf{C} \mathbf{V} \), where \( \mathbf{C} \) is now the covariance matrix of the new data.

To apply this method to our data, PSTHs (firing rates in 10ms bins, convolved with a Gaussian kernel of unit area and 50ms standard deviation) were used to form pseudo-population firing rate matrices for each condition and neuronal sub-population. To prevent high-firing neurons from dominating the analysis, but preserve some relative range of firing rates, firing rates were soft-normalised by the total firing rate range across all times and conditions (+ a small constant of 5 spikes s\(^{-1}\)).

Trial-averaged execution data from 50ms before the HPR cue to 500ms after HO, separately for each object, was then used to form a peri-movement activity matrix \( \mathbf{M} \) (\( T \times N \), where \( T \) was the number of timepoints and \( N \) was the number of MirNs), which was then centred by subtracting the mean activity across time for each neuron (dimension). We projected trial-averaged execution and observation data spanning this time period onto the first \( k \) principal axes (\( k = 3; 3 \) dimensions typically captured >90% of the variance in \( \mathbf{M} \)), yielding \( k \) principal components for each condition, each with a fractional variance associated with it. We quantified the overlap, or “alignment” of observation activity within this space by normalising the total captured variance by the maximum observation variance which could be captured by \( k \) axes, according to the following equation (c.f. Elsayed et al., 2016).

\[
a = \frac{tr(\mathbf{V}_{\text{Exe}}^T \text{cov}(\mathbf{X}_{\text{Obs}}) \mathbf{V}_{\text{Exe}})}{tr(\mathbf{V}_{\text{Obs}}^T \text{cov}(\mathbf{X}_{\text{Obs}}) \mathbf{V}_{\text{Obs}})}
\]

\( \mathbf{V}_{\text{Exe}} \) and \( \mathbf{V}_{\text{Obs}} \) are the first \( k \) eigenvectors of \( \mathbf{X}_{\text{Exe}} \) and \( \mathbf{X}_{\text{Obs}} \), where \( \mathbf{X}_{\text{Exe}} \) and \( \mathbf{X}_{\text{Obs}} \) are the mean-centred execution and observation activity, respectively. \( tr \) denotes trace. The denominator is mathematically equivalent to the sum of the eigenvalues of the first \( k \) eigenvectors of \( \mathbf{X}_{\text{Obs}} \) and the alignment index is thus bounded between 0 (if \( \mathbf{X}_{\text{Exe}} \) and \( \mathbf{X}_{\text{Obs}} \) are fully orthogonal) and 1 (if \( \mathbf{X}_{\text{Exe}} \) and \( \mathbf{X}_{\text{Obs}} \) are perfectly overlapping). We compared true alignment values to a null distribution of alignment of 10,000 pairs of random, orthonormal subspaces, and a p-value was computed as the proportion of values in the null
distribution greater than the true alignment. \( P < 0.05 \) was considered significant (i.e. the true alignment value exceeded 95% of the values within the null distribution).

The above analysis was repeated for a subspace defined by observation activity in the same time period (50ms before HPR to 500ms after HO). To examine the state-space overlap between observation and NoGo, we used PCA to define a second set of 3 principal axes using trial-averaged observation data from across all neurons, 100-400ms after the Go cue. We then projected activity from all three conditions onto these axes, and quantified variance captured and alignment statistics in an analogous way to that for the movement period subspaces.

3.4. Results

We recorded single neurons in F5 and M1 of rhesus macaques performing and observing reach-to-grasp and hold actions, and assessed the profiles of activity across conditions and populations. We then compared modulation during the action observation condition, where monkeys were required to remain still, to neural activity when monkeys were explicitly cued to withhold movement.

3.4.1. EMG and behaviour

Monkeys were trained to a high level of performance before recording (>90% correct trials per session). For both monkeys, reaction and movement times were significantly faster than human experimenters (Table 3.1, Wilcoxon sign-rank test on session averages, all \( p < 1 \times 10^{-13} \)). As the trapezoid object was positioned contralateral to the reaching (right) arm, monkey movement times were 30-50ms longer than those for the sphere (Wilcoxon sign-rank test, both monkeys \( p < 1 \times 10^{-7} \)). To verify that neural activity during action observation and withholding was not confounded by muscle activity, we simultaneously recorded EMG from up to 12 hand and arm muscles. During action execution, we observed characteristic patterns of EMG for each grasp. In the action observation and NoGo conditions, on the other hand, EMG activity was negligible (Figure 3.1, observation and NoGo are plotted at x10 gain).
3.4. Results

<table>
<thead>
<tr>
<th></th>
<th>M48 Monkey</th>
<th>M49 Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PG</td>
<td>WHG</td>
</tr>
<tr>
<td>RT (ms)</td>
<td>310±25</td>
<td>267±22</td>
</tr>
<tr>
<td>MT (ms)</td>
<td>306±20</td>
<td>279±14</td>
</tr>
</tbody>
</table>

Table 3.1. Behaviour during recording sessions for basic mirror task. RT, reaction time; MT, movement time. Mean±SEM of median values from each session, rounded to nearest millisecond.

3.4.2. Effects of repetitive intracortical microstimulation

We delivered rICMS at 57 sites containing M1-PTNs, 124 sites with unidentified neurons (UIDs) in M1, and 111 sites in F5. Finger or thumb effects were elicited at 27/57 M1-PTN sites, 89/114 M1-UID sites, and 75/111 F5 sites. The majority of these sites in M1 had low thresholds (20/27 (74.1%) and 76/89 (85.4%) ≤20µA, PTNs and UIDs respectively), but not in F5 (27/75 (36.0%)).

3.4.3. Database

Single neurons were recorded across 25 sessions in M48, and 40 sessions in M49. Across the two monkeys, we recorded a total of 304 neurons for ≥10 trials per object for both execution and observation conditions (Table 3.2), and 302 of these were also recorded for ≥7 NoGo trials per object. 189 units were recorded in M1, and 115 in F5. 60 M1 neurons were identified as PTNs; the remaining 129 were UIDs. F5-PTNs were observed and recorded, however total numbers were low (15 in M48, 8 in M49), so all F5 neurons were considered as one population.

<table>
<thead>
<tr>
<th></th>
<th>M48</th>
<th>M49</th>
<th>Total</th>
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<tbody>
<tr>
<td>M1-PTN</td>
<td>36</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>M1-UID</td>
<td>78</td>
<td>51</td>
<td>129</td>
</tr>
<tr>
<td>F5</td>
<td>72</td>
<td>43</td>
<td>115</td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td>118</td>
<td>304</td>
</tr>
</tbody>
</table>

Table 3.2. Number of single-units recorded in each monkey and sub-population for at least 10 execution and 10 observation trials per grasp.
3.4. Results

3.4.4. Single-neuron responses

The complex naturalistic task set-up evoked a wide variety of responses in recorded neurons, particularly during action execution. A substantial proportion of neurons also showed responses to one or both of the action observation and NoGo conditions. Figure 3.2A shows a PTN recorded in M1, which maintained a steady baseline firing rate until the Go cue. During execution, HPR was accompanied by a suppression of firing for both grasps, followed by an increase leading up to DO, which was greater for WHG. This increased firing was maintained during the hold period, before falling below baseline as the monkey returned to the homepad. During observation, firing rates were considerably lower than during execution, and no suppression was apparent at movement onset. Firing rates increased to almost twice the baseline level during the hold period, before gradually decreasing, and were more similar across grasps than in execution. Another M1-PTN (Figure 3.2B) had a complex pattern of activity during execution, with a peak just before DO, particularly for PG, and a second peak prior to release of the object. During observation, the same unit showed a small, sustained increase in firing rate. The unit shown in Figure 3.2C was recorded in F5. In both action execution and observation conditions, the HPR to DO period showed a dramatic increase in firing for both grasps, peaking at >100 spikes s\(^{-1}\). In execution, hold period activity then stabilised at a lower rate, with activity for WHG sustained at a higher level. By contrast, observation activity decayed back to baseline relatively quickly at the beginning of the hold period. Figure 3.2D shows an M1-PTN with a steady baseline firing rate, which completely silenced during both execution and observation hold period, before showing some rebound at the end of the hold period, particularly during execution.

3.4.5. Corticomotoneuronal cells during action observation

Figure 3.3 shows an example of a CM cell in M48. Clustered spikes from different stages of the recording are shown in Figure 3.3A, and the SpTA revealed a clear thenar PSpF at around 10ms (Figure 3.3B). During action execution, the cell showed strong persistent activity during PG and a brief increase in activity during WHG (Figure 3.3C, top panel), whereas during action observation, there was a negligible change in firing rate (bottom panel). The profile of EMG activity during PG and WHG execution (Figure 3.3D) exhibited a strong
3.4. Results

similarity with the firing rate of the CM cell. Figure 3.4 shows another example of an M1-PTN CM cell recorded in M49, with example spikes shown in Figure 3.4B. This cell had a PSpF in the abductor pollicis longus (AbPL) muscle, and some sign of a weak synchrony PSpF in the 1DI muscle (Figure 3.4B). This cell was also strongly modulated during action execution, particularly for PG, whereas during observation, firing initially decreased from baseline, before showing some rebound after the Go cue, but remained suppressed relative to the initial baseline period (Figure 3.4C). Again, EMG activity during action execution showed a clear, slightly delayed, correlation with the firing rate of the neuron (Figure 3.4D).
3.4. Results

Figure 3.2. Example mirror neurons in M1 and F5. Raster and histogram representations of single neuron activity during execution (top panels) and observation (bottom panels). Activity is aligned to object displacement (DO). Rasters are split by grasp (PG and WHG, objects shown in inset) and condition for easier visualisation, although trials were presented in a pseudo-randomised order during recording. (A-C). Facilitation responses during execution and observation in two M1-PTNs and F5 unit, respectively. (D), M1-PTN showing pronounced suppression of activity during both execution and observation. Units in (A), (C) and (D) were recorded in M48, (B) was recorded in M49. Single trial events are indicated on raster plots (LCDon, Object Cue, Go, HPR, HO, HOFF, HPN), and median times relative to alignment are shown on histograms. Event colours are as shown previously (Figure 2.1C): LCDon - grey; Object Cue - orange; Go - green; HPR &HPN - magenta; HO & HOFF - cyan). For histograms, firing rates were calculated in 20ms bins and boxcar-smoothed (200ms moving average).
### 3.4. Results

Figure 3.3. Example cortico-motoneuronal cell in M48. (A). Spike waveforms of identified M1-PTN at beginning, middle, and end of recording. (B). Spike-triggered averages of EMG activity (n = 11,305), showing clear post-spike facilitation (PSpF) in the thenar muscle, with an onset of 10ms. Dashed horizontal lines indicate ±2SD from the baseline mean. (C). Activity of the identified CM cell during execution and observation, for PG and WHG, aligned to object displacement onset (DO) (20ms bins, boxcar-smoothed with 200ms moving average) (D). EMG activity in the thenar muscle for execution of each grasp, and observation across both grasps, during the session in which the unit in (C) was recorded, aligned to DO. Event markers shown median event times relative to alignment for PG and WHG execution, respectively. Event colours in (C) & (D) are as shown previously.
3.4. Results

Figure 3.4. Example cortico-motoneuronal cell in M49, presented in the same manner as Figure 3.3. (A). Spike waveforms of identified M1-PTN. (B). Spike-triggered averages of EMG activity (n = 17,519), showing post-spike facilitation (PSPF) in AbPL. (C). Activity of the identified CM cell during execution and observation. (D). EMG activity in the AbPL muscle during the same session.
3.4. Results

3.4.6. Population-level activity during execution and observation

Although the connections of most of the recorded neurons were unknown, it is clear that action execution and observation produced different and complex response patterns both within and across units. For each neuron and task condition, we first assessed the statistical significance of changes in firing rate across relevant task intervals. For PG, 222/304 neurons (73.0%) showed significant modulation relative to baseline during the movement periods of execution (1-way ANOVA and post-hoc comparisons; see subsection 3.3.2). 117/222 (52.7%, 38.5% of total) were also modulated during action observation movement periods, and were therefore classed as MirNs. For WHG, 235/304 (77.3%) modulated during execution movement, and 110 of these (46.7%, 36.2% of total) were MirNs. Grasp specificity was frequently apparent during execution, whereas observation responses, when present, were often more comparable across the two objects (Figure 3.2), consistent with the broad congruence found in many MirNs (Gallese et al., 1996).

The extent of modulation during action observation may differ across premotor and motor cortex at the population level, with important implications for the effect of this activity on downstream targets. The heatmaps in Figure 3.5A-C show the time-resolved net normalised firing rate during execution and observation across the three MirN sub-populations, and Figure 3.5D-F show the averages during execution and observation for the facilitation-facilitation and facilitation suppression units. Within each sub-population, we found both facilitation and suppression responses relative to baseline during execution and observation, and the relationship between activity in the two conditions was variable. For the commonest group of identified MirNs, net normalised activity of facilitation-facilitation MirNs (those which increased their activity during execution and observation) was generally larger during execution movement than observation (Figure 3.5D-F, top panels). Population activity during the hold period of execution was substantially larger than that during observation in M1-PTNs (Wilcoxon sign-rank tests with Bonferroni correction for multiple comparisons, $p = 0.0011$ for PG, $p = 0.011$ for WHG), but not in M1-UIDs or F5 (all $p > 0.05$). To examine potential differences in time-varying pattern of activity during action execution and observation, we computed the correlation between execution and observation activity across all MirNs during different task epochs (Figure 3.6). During ObjCue, when trials were identical from
the monkey's perspective, all populations as expected showed a strong, significant correlation ($r > 0.8$, $p < 1 \times 10^{-13}$) between the two conditions (Figure 3.6A, top row). Contrastingly, activity patterns during the early stages of the reach were markedly different (Figure 3.6A, middle row). This was particularly the case in M1-PTNs, which showed no significant relationship between execution and observation activity at this stage of the task ($r = 0.23$, $p = 0.2$). M1-UIDs and F5 populations were also less well correlated during this period than before the Go cue, although the correlations remained significant ($p < 0.0005$). During the Hold period, activity patterns across the conditions became significantly correlated again during the grasp/hold stages of the task ($p < 0.0001$, Figure 3.6A, bottom row). We also compared the observed correlation values to a null distribution created by shuffling the observation vector so that within-unit relationships were lost. Correlations during the early reach period were significantly greater than all values in the null distribution for M1-UIDs and F5 (both grasps, $p = 0$), but not M1-PTNs (both grasps, $p > 0.1$, Figure 3.6B). For WHG, M1-PTN execution and observation activity during Late React and Late Reach epochs were also uncorrelated ($p > 0.05$). Direct comparison of the correlation coefficients during early reach (Fisher z-tests with Bonferroni correction for multiple comparisons) did not reach significance, likely because of the relatively low sample sizes. To assess the temporal stability of cross-condition similarity, we performed a cross-temporal pattern analysis using time-resolved PSTHs, by computing the correlation between net normalised activity at each timepoint with that of every other timepoint (Figure 3.7). The diagonal of this matrix therefore roughly corresponds to the epoch-based correlation values above. Activity prior to the Go cue, and during the hold period, was generally well correlated across the two conditions in all three populations and for both grasps. F5 neurons showed stronger correlations between the object cue and later hold periods, which was not apparent for M1-PTNs, indicating that the pattern of activity in these two periods was more consistent in F5.
3.4. Results

Figure 3.5. Mirror neuron population activity. (A-C). Heatmaps of net normalised activity in MirNs of each sub-population. Neurons are split into facilitation-facilitation, facilitation-suppression, suppression-facilitation, and suppression-suppression categories based on the sign of their modulation during action execution and observation relative to baseline. Horizontal black lines indicate splits between categories. Within each category, neurons are sorted based on the latency of their absolute peak response during execution (peak calculated between GO and HO+0.5s). Asterisks denote units shown in Figure 3.2. (D-F). Population averages for F-F (top panel) and F-S categories (bottom panel) separately for each sub-population.
3.4. Results

Figure 3.6. Relationship between execution and observation activity. (A). Net normalised activity during PG observation plotted against execution for the ObjCue, Early Reach, and Hold epoch, for MNs in each population (M1-PTNs left, M1-UIDs middle, F5 right). Pearson correlation coefficient $r$ and corresponding $p$-value are shown in the lower right of each panel. Line of best fit for significant correlations are shown in red, dashed black traces mark $y = x$ line. (B). Summary of Pearson correlation coefficients between execution and observation for each MirN population and PG task epoch. Dashed coloured lines denote 5 and 95th percentile correlation coefficients derived from 1000 shuffles for each population.
3.4. Results

We next used PCA to examine the nature of time-varying patterns of activity across action execution and observation in each sub-population within a movement subspace. PCA identifies a few dominant modes, or dimensions of neural activity within the full dimensional space which capture the majority of the variance in the data. The activity of the same neurons recorded during a different behaviour or time period can then be compared to the first based on the similarity of the covariance across neurons, which will result in similar or different projections upon the defined dimensions. This holds advantages over unweighted averaging of neural activity in different conditions, which also reduces dimensionality, but altogether sacrifices information regarding the relationships between different neurons and conditions. We defined a movement subspace empirically for each sub-population, using trial-averaged activity during execution reach and grasp, and then visualised evolution of execution (green) and observation (purple) trajectories across the first 2 axes of this execution movement subspace (Figure 3.8). PG activity prior to the Go Cue was similar and overlapping for the two conditions and showed little variance in the movement subspace, reflected by the minimal evolution of the trajectories until this point. After the Go cue in execution, activity in each population then progressively evolved through different stages of the trial through HPR and DO, as indicated by the arrows, spanning the movement subspace (Figure 3.8A). During action observation, M1-PTNs (Figure 3.8A, top) and M1-UIDs (Figure 3.8A, middle) showed a highly collapsed trajectory, suggesting little similarity between population activity in execution and observation. F5 population activity, on the other hand, followed a qualitatively similar, but smaller trajectory to that seen during execution, with ordered progression through stages of the task (Figure 3.8A, bottom). For each population, we quantified the level of variance captured on these axes for both execution and observation. While the PCA method ensured that three dimensions captured the majority of the variance (>90%) of the execution data for all 3 populations (Figure 3.8B), captured observation variance was relatively low for both objects (5-10% for M1 populations, 20-25% for F5 for both objects). The ratio of this variance, to the maximum possible variance which could be captured within the observation data constituted a measure of alignment (Figure 3.8C, purple lines, see Methods). To quantify the significance of this overlap relative to what could be expected simply by chance, we compared this alignment to a null distribution of alignment calculated from pairs of random orthonormal dimensions. During movement,
we found that only F5 showed an alignment between observation and execution greater than expected from chance for both grasps (PG alignment: 0.20, p = 0.003, WHG: 0.21, p < 1 × 10⁻⁵, upper-tailed permutation test). In M1-PTNs and M1-UIDs, on the other hand, alignment was not significantly different to chance (both grasps p > 0.1). Qualitatively similar results were obtained when data was projected onto an observation-defined subspace (Figure 3.9), with alignment significantly greater than chance for both grasps in F5 (p ≤ 0.0003), but not in M1 populations (all p > 0.05).

Figure 3.7. Cross-temporal correlation between execution and observation. Correlation across time between MirNs in each sub-population during execution and observation for PG (top), and WHG (bottom), M1-PTNs (left), M1-UIDs (middle), F5 (right). Green colours indicate low correlations, darker red shades indicate high correlation. Coloured ticks along left and bottom of each panel denote average event times across conditions, colour codes as in Figure 3.2 & Figure 2.1C.
3.4. Results

Figure 3.8. Execution and observation activity within a movement subspace. (A). Traces showing the evolution of M1-PTNs, M1-UIDs and F5 population activity within a 2-D movement subspace (defined by movement execution activity) across the whole trial during PG execution (green) and observation (purple) conditions. Larger coloured circles on each trajectory mark key events (Go, HPR, DO, labelled) in trial sequence which were used for multiple alignment of neural activity, and arrows on trajectories indicate direction of time. (B). Cumulative variance captured by the first three principal axes, separately for each sub-population. Exe-E (green), execution variance in execution subspace; Obs-E (purple), observation variance in observation subspace; Obs-O (dashed purple), observation variance in observation subspace. (C). Alignment index of observation activity in the movement subspace, for each population separately. Coloured horizontal line denotes observed alignment indices. Execution alignment index is equal to 1 by definition (not shown). Black points show alignment values from the null distribution.
3.4. Results

Figure 3.9. Execution and observation activity within an observation subspace. Same as Figure 3.8, except for a subspace defined by activity during movement observation. (A), Traces showing the evolution of M1-PTNs, M1-UIDs and F5 population activity within a 2-D subspace defined by observation activity during PG execution (green) and observation (purple) conditions. (B), Cumulative variance captured by the first three principal axes, separately for each sub-population. Exe-O (green), execution variance in observation subspace; Obs-O (purple), observation variance in observation subspace (identical to dashed purple lines in Figure 3.8B); Exe-E (dashed green), execution variance in execution subspace identical to green lines in Figure 3.8B). (C), Alignment index of execution activity in the observation subspace, for each population separately (green), and values from null distribution (black points).
3.4. Results

3.4.7. Movement suppression during action observation

Given that the patterns of neural activity show a clear divergence after the Go cue in the two conditions, we considered whether the activity during action observation contained signatures of movement suppression. We therefore examined the activity of the same populations of neurons during cued action withholding, and compared this to the responses during action observation. Figure 3.10A shows four single neurons recorded during PG execution, observation, and NoGo conditions. The activity of the M1-PTN and M1-UID (top panels) was clearly different for movement and non-movement around 100-150ms after the Go/NoGo cue, but showed comparatively little difference between observation and NoGo. By contrast, the activity of the M1-PTN in the lower left panel, which is the same neuron as shown in Figure 3.2A, was clearly different for all three conditions. The F5 neuron (Figure 3.10A, lower right) discharged in a similar way for execution and observation, first decreasing then increasing activity, while increasing activity in the NoGo condition. Using all neurons with at least 10 trials recorded per task condition, we trained a maximum correlation coefficient classifier to decode condition for each cortical population (Figure 3.10B). Across all three populations, the decoder was able to distinguish condition with high accuracy from 100-150ms after the Go/NoGo cue was given. We hypothesised that this could be largely driven by very reliable decoding of execution, which often shows greater variation in firing rates, and therefore also trained and tested the decoder with observation and NoGo conditions only (Figure 3.10B). This revealed a slower rise in accuracy, which was also different between the three populations. F5 showed significant decoding between these two conditions 300ms after the imperative cue, whereas for M1-UIDs and M1-PTNs, this was delayed until 400 and 450ms, respectively. Similar results were observed for decoding of WHG (in the 3-condition case, significant decoding occurred after 100-150ms, whereas when decoding between Observation and NoGo, significant decoding was again delayed; F5 - 300ms, M1-UIDs - 400ms, M1-PTNs 550ms). We also trained and tested the decoder on the other condition pairs (Execution-Observation, Observation-NoGo), and these also always produced strong decoding from 100-150ms after the Go/NoGo cue. Lastly, we performed a second PCA (Figure 3.11) this time defining each population’s subspace using observation activity after the Go cue (see Methods). We then projected each condition’s activity onto this subspace,
which allowed us to compare the overlap of the execution and NoGo conditions with the observation subspace separately, in an analogous way to the analysis presented in Figure 3.8 and Figure 3.9. In M1-PTN and M1-UID populations (Figure 3.11, first two rows), NoGo trajectories (orange) show a closer similarity to observation ones (purple). Although the M1-PTN population trajectory during NoGo condition showed smaller variance, its evolution over time was similar to the observation population trajectory, with the “trough” of both trajectories occurring at a similar time in advance of the average time of experimenter HPR (orange circles). By contrast, execution activity (green) showed quite different patterns to observation. In F5 (bottom row), the execution and NoGo trajectories both showed little variance, suggesting that neither condition overlap strongly with the observation subspace. Quantitatively (Figure 3.11C), M1-PTN NoGo activity overlapped with observation activity during this period significantly more often than chance (p = 0 for both grasps), and the raw alignment value was much larger for NoGo than for execution (PG: 0.31 vs. 0.06, WHG: 0.44 vs. 0.18). M1-UID NoGo activity also overlapped significantly with observation relative to the chance (both p = 0), whereas execution activity did not (both p > 0.1). By contrast, F5 NoGo and execution activity showed low levels of overlap with observation during this period, although this was significant relative to chance for WHG (0.03 and 0.03 for PG, both p > 0.3; 0.11 and 0.09 for WHG, both p < 0.0001).
3.4. Results

Figure 3.10. Activity during NoGo. (A). Example single-neuron responses during execution, observation, and NoGo. Each subplot shows a raster and histogram representation of single-neuron activity during PG execution (green), observation (purple), and NoGo (orange), with single alignment to the Go/NoGo cue (vertical black lines). Rasters and histograms are compiled from a randomly selected subset of 10 trials in each condition. For histograms, firing rates were computed in 20ms bins and boxcar-smoothed with a 200ms moving average. Event markers (LCDon, Object Cue etc.) are as shown previously (Figure 3.2). (B). Classification accuracy of maximum correlation coefficient classifier decoding between PG execution, observation, and NoGo conditions within each population. Grey trace and shading shows mean±1SD of decoding accuracy following permutation shuffling, and coloured bars along bottom show period of consistent significant decoding for each population. (C). As for (B) but decoding between observation and NoGo only.
3.4. Results

Figure 3.11. NoGo activity within an observation subspace. (A), Traces showing the evolution of M1-PTNs, M1-UIDs and F5 population activity during PG execution (green), observation (purple) and NoGo (orange) conditions within the first 2 dimensions of an observation subspace spanning the 100-400ms after the Go cue. Each trajectory show the -100 to +400ms period around the Go/NoGo cue (green/red circles). Average HPR time (across execution and observation) is also shown on each trajectory An arrow on observation trajectories indicates the direction of time. (B), Cumulative variance captured by the first three principal axes for execution, observation, and NoGo, separately for each sub-population. (C), Alignment indices of execution and NoGo activity in the observation subspace shown as coloured lines, for each population separately (Execution - green, NoGo - orange). The alignment index for observation activity is equal to 1 by definition (not shown). Scattered points show alignment values from the null distributions for execution and NoGo separately.
3.5. Discussion

In this study, we investigated the relative contributions of F5 and M1 populations during the execution, observation, and withholding of grasping actions. We found that the modulation depth and profile of activity in F5 MirNs was more similar between execution and observation. In M1 populations, particularly M1-PTNs, although many neurons did modulate during both execution and observation, the magnitude and pattern of activity was distinct in these conditions, and observation activity shared parallels with activity when the monkeys simply withheld their own movement.

Previous interpretation of mirror activity has mostly been made in the context of known motor properties of the areas and pathways in question. F5 is critical for goal-directed visual guidance of the hand (Godschalk et al., 1981; Weinrich & Wise, 1982; Rizzolatti et al., 1998; Fogassi et al., 2001), and contains a ‘vocabulary’ of motor acts (Rizzolatti et al., 1988), supporting internal representation of different grasps (Murata et al., 1997; Raos et al., 2006; Umiltá et al., 2007; Spinks et al., 2008; Fluet et al., 2010; Schaffelhofer & Scherberger, 2016). F5 makes only a limited contribution to the CST (Dum & Strick, 1991; He et al., 1993), but is anatomically (Muakkassa & Strick, 1979; Godschalk et al., 1984; Matelli et al., 1986; Dum & Strick, 2005), and functionally (Cerri et al., 2003; Shimazu et al., 2004; Schmidlin et al., 2008; Kraskov et al., 2011) strongly interconnected with M1. M1 provides a major contribution to the CST and exerts a direct influence over distal hand musculature, which is probably exploited by executive commands necessary for control of skilled hand movements (Kakei et al., 1999; Brochier et al., 2004; Lemon, 2008).

3.5.1. M1 observation activity is dissimilar to execution activity

We first confirmed that, although both F5 and M1 neurons can show mirror responses (Figure 3.2), F5 mirror activity during observation is more comparable in amplitude to execution activity (Figure 3.5). This is in line with previous reports of F5 MirN activity, suggesting a similar representation of grasp irrespective of whether the action is executed or observed (Gallese et al., 1996; Kraskov et al., 2009; Bonini et al., 2010). By contrast, M1 was first thought to completely lack MirNs (Gallese et al., 1996; Nelissen et al., 2005), and although several studies have now shown that neurons in this area, including PTNs,
3.5. Discussion

can show mirror responses, this activity is often relatively weak (Dushanova & Donoghue, 2010; Vigneswaran et al., 2013). Here, we found that M1-PTNs which increased firing during both execution and observation i.e. classical MirNs, showed a 3-4 times reduction in activity during observation relative to execution (Figure 3.5D), quantitatively comparable to previous reports (Dushanova & Donoghue, 2010; Vigneswaran et al., 2013). When examining the correlation of population-level activity across execution and observation, we found differences across different task periods, with M1-PTN MirNs showing a particularly weak correlation between the two conditions during the early stage of movement (Figure 3.6). Low-dimensional subspaces capturing variance associated with movement execution also captured meaningful observation variance in F5, but not in M1-UID and M1-PTN populations (Figure 3.8), and similarly, an observation subspace captured execution variance in F5, but not M1 populations (Figure 3.9). It should be noted that the alignment of uniformly random orthonormal subspaces is dependent on the dimensionality, rather than structure, of the data, and therefore constitutes a relatively low bar for significance testing. However, an alternative method which seeks to circumvent this by constraining random subspaces to the covariance structure of the full dataset (Elsayed et al., 2016) is biased towards identifying orthogonality between two different subspaces, due to regression to the mean within the null distribution. The finding that execution and observation are more overlapping in F5 is consistent with recent work demonstrating MirN activity in PMv and M1 during execution of reach and grasp to be associated with a series of hidden states, which were recapitulated during observation in PMv, but not M1 (Mazurek et al., 2018). Overall, although M1 neurons can be active during both execution and observation, the pattern of this activity at the population level was quite different between the two conditions. In a classical gating model of CS control where increased activity in excitatory pyramidal cells drives movement, the net disfacilitation of M1-PTNs during observation provides a plausible substrate for inhibiting movement, given their anatomical and functional proximity to the spinal output (Kraskov et al., 2009; Vigneswaran et al., 2013). However, suppression of PTN activity has also been reported during movement execution tasks involving the arm, wrist and hand (Evarts, 1968; Kraskov et al., 2009; Quallo et al., 2012; Vigneswaran et al., 2013; Soteropoulos, 2018), and was observed in the present task (Figure 3.2D). Suppression during movement could drive downstream inhibitory spinal circuits, given that PTNs not only make direct
3.5. Discussion

connections with motoneurons via the CM system (Lemon, 2008; Rathelot & Strick, 2009), but also connect to sINs within the spinal cord (Kuypers, 1981), and tightly timed suppression of muscle activity is essential for skilled movement (Quallo et al., 2012). An alternative, but not mutually exclusive, possibility, is that population activity at the cortical level evolves within a dynamical system, which implicitly gates downstream circuitry (Kaufman et al., 2013, 2014; Elsayed et al., 2016), although this framework is not yet reconciled with the known anatomy of neuronal sub-populations. Under the assumption that the balance of excitation and inhibition at the motor cortical level is fundamental for movement generation and suppression, then it should be expected that the respective patterns of activity during execution and observation should be reflected in the resultant behaviour. From both a representational and dynamical systems viewpoint, M1 activity during execution and observation, particularly in PTNs, may be sufficiently dissimilar so as to ensure movement is only produced in the former condition. Interestingly, in examples of M1-PTNs which were identified as CM cells (Figure 3.3 & 3.4), strong execution activity was contrasted with very limited, or even suppressed, activity during action observation. Suppression responses in CM cells were also reported by Vigneswaran et al., (2013), although more quantitative analyses are needed to clarify whether these cells, with their direct excitatory influence over spinal motoneurons, form a distinct population in terms of their role in action observation.

3.5.2. Correlates of movement suppression in M1 observation activity

The dissociation between execution and observation appeared most prominent around the time of movement onset, in line with previous suggestions regarding the role of MirNs in movement suppression (Kraskov et al., 2009; Vigneswaran et al., 2013). In the present study, we used a pseudo-randomised trial sequence, and Go/NoGo and execution/observation information was provided simultaneously on each trial (Go/NoGo cue; Figure 2.1B). This contrasts with most action observation studies in which block-designs are used, and may provide a more ethological framework for assessing functions of the CST in movement suppression. We identified movement-related cortical neurons responding to both observation and NoGo conditions to varying degrees (Figure 3.10A). A decoder trained to discriminate between three conditions exceeded chance and reached plateau 100-150ms after the Go/NoGo cue (Figure 3.10B), presumably
3.5. Discussion

the time necessary for visual information about trial type to become available to motor areas. A second decoder trained to distinguish only between observation and NoGo took longer to exceed chance performance, particularly for M1 neurons, indicative of similar activity patterns in the two conditions (Figure 3.10C). This was corroborated by analysis of the evolution of activity within an observation subspace after the Go cue, which captured significant NoGo variance in M1-PTNs, but less so in F5 (Figure 3.11). Taken together, these results suggest a greater overlap between observation and NoGo neural states in M1 than F5. The task design itself, with observed actions taking place within the monkey’s reach, may introduce some similarity between observation and NoGo, such that the strategy adopted by the monkeys is to treat the observation Go cue and NoGo cue similarly. Observed actions occurring in peri-personal space often modulate MirN responses differently to when the action is beyond the monkey’s reach (Caggiano et al., 2009; Bonini et al., 2014a; Maranesi et al., 2017), suggesting the capability to interact with observed actions is an important aspect of mirror activity. A further important point is the difference between F5 and M1, which indicate that while M1’s priority is to distinguish movement from non-movement from an egocentric perspective, F5 maintains a more similar representation across executed and observed actions, independent of the acting agent’s identity. These results suggest the formulation of a simple model framework, in which the movement execution and suppression-like features of the unfolding action observation response in M1 (and F5) reflect a balance of the activity patterns seen during the execution and NoGo conditions. This balance could be determined by inputs from upstream areas within the MNS, and prefrontal areas responsible for encoding general features of action and self v.s. other encoding, as well as intrinsic dynamics within premotor and motor cortex. State-space analyses provide a useful tool for analysing these temporal dynamics during different stages of action execution, observation, and withholding. Future investigations which more widely sample grasping execution state space (i.e. recording from more neurons but also using a much more extensive range of movement and grasping conditions) may be able to address this, and also relate neural correlates of suppression during observation and NoGo conditions to the withholding of movement during instructed delay periods (Kaufman et al., 2014; Bestmann & Duque, 2016; Elsayed et al., 2016; Hannah et al., 2018a; Soteropoulos, 2018). Single-trial analyses may also hold particular relevance for examining the switching of neural state between initiation and suppression of
3.5. Discussion

movement in the context of an action execution-observation task. Our dataset, with small samples of recorded cells per session, was not well suited to this type of analysis.

The timing and kinematics of monkey and experimenter movements were clearly different, which could explain why similarity between execution and observation decreased during the reaching phase, however, there are several reasons this is unlikely to be a dominant factor. Firstly, correlations between execution and observation already began to decrease during the late reaction period, i.e. before any movement had occurred (Figure 3.6B). At the single-neuron level, firing rates showed little correlation with movement speed (inversely proportional to movement time given constant distance between hand and objects) (see also Vigneswaran et al., 2013). Furthermore, given that many sessions involved simultaneous recording of units in F5 and M1, timing reasons could not explain differences between the sub-populations. The targeting of recordings to F5, an area with a preponderance of grasp-related activity (Rizzolatti et al., 1988; Gallese et al., 1996; Raos et al., 2006; Umlitā et al., 2007; Michaels et al., 2018), and the M1 hand area, may also contribute to closer similarity between execution and observation during grasp and hold, rather than reach periods of the task. However, we did not impose strong online selection criteria regarding the proximal vs. distal related activity of recorded cells (in particular, all stable and well-isolated PTNs, once identified, were recorded for a full set of trials), and rICMS at some recording sites elicited movements of proximal muscles. This is also consistent with a developing body of literature involving anatomical tracing, stimulation mapping and task-related activity which questions the simple segregation of dorsal and ventral premotor cortex into reaching and grasping areas, respectively (Raos et al., 2003, 2004; Dum & Strick, 2005; Stark et al., 2007a; Lehmann & Scherberger, 2013; Takahashi et al., 2017). On the other hand, although there is now ample evidence that cells in dorsal premotor areas, or within proximal limb representations in M1, do mirror reaching movements (Cisek & Kalaska, 2004; Dushanova & Donoghue, 2010; Papadourakis & Raos, 2019), to our knowledge, the anatomical identity of these MirNs, and their potential influence on downstream targets, has not been directly tested, and could be of particular relevance for initiation or suppression of reaching movements.

Overall, the results of this study point to a functional distinction in premotor and motor cortex respectively regarding the representation of executed and observed grasping actions.
3.5. Discussion

F5 neurons appear more engaged in the encoding of the upcoming grasping action, such that execution and observation activity remain similar over a longer time course. Contrastingly, M1 populations, and M1-PTNs in particular, show a more flexible dissociation through the task, first distinguishing movement from non-movement, which may then allow subsequent grasp-related mirror activity to evolve in the absence of self-movement.

3.5.3. Conclusions

In this study, we confirm that F5 activity is closer in amplitude and profile during action execution and observation, whereas M1 showed a particularly weak relationship in activity between the two conditions. The M1 neural state during observation diverges from the execution state in the lead-up to movement onset, and contains signatures of an action withholding state at this time. Functionally, the different patterns of activity between execution and observation in the two areas could support a context-dependent dissociation between grasp-related visuomotor transformations and the recruitment of descending pathways for elaboration into actual performance of skilled grasp. The increasing capabilities for wide-scale simultaneous recordings from many neurons, and accompanying inactivation and manipulation experiments, should help to shed further light on the transfer of information through premotor and motor areas for the representation and organisation of goal-directed actions and the observation of these actions.
4. Modulation of spinal excitability during action observation

4.1. Abstract

Neurons in the primate motor cortex, including identified PTNs projecting to the spinal cord, respond to the observation of others’ actions, yet this does not cause movement in the observer. Here, we investigated changes in spinal excitability during action observation by monitoring short latency EMG responses produced by single shocks delivered directly to the PT. Responses in hand and digit muscles were recorded from two adult rhesus macaques while they performed, observed or withheld reach-to-grasp and hold actions. We found modest grasp-specific facilitation of hand muscle responses during observation of hand shaping for grasp, which persisted when the grasp was predictable but obscured from the monkey’s vision. We also found evidence of a more general inhibition before observed movement onset, and the size of this inhibition effect was comparable to the inhibition after an explicit NoGo signal. These results confirm that the spinal circuitry controlling hand muscles is modulated during action observation, and this may be driven by internal representations of actions. The relatively modest changes in spinal excitability during observation suggest net CS outflow exerts only minor, sub-threshold changes on hand motoneuron pools, thereby preventing any overflow of mirror activity into overt movement.
4.2. Introduction

MirNs are found in the ventral premotor cortex (PMv) and primary motor cortex (M1), as well as other parts of the primate motor cortical network. These cells modulate their firing during both the execution of grasping actions and the observation of similar actions performed by others (Gallese et al., 1996; Rizzolatti et al., 1996a; Kilner & Lemon, 2013). Activity in this latter condition occurs in the absence of any detectable muscle activity or movement in the observer, despite the finding that even PTNs, which project directly to the spinal cord, can be MirNs (Kraskov et al., 2009; Vigneswaran et al., 2013). The extent to which cortical mirror activity influences the excitability of spinal motor networks is not well understood.

PMv, where MirNs were first discovered, has limited direct projections to the lower cervical spinal cord (He et al., 1993; Borra et al., 2010), but MirNs in PMv could also exert influence at the spinal level via strong anatomical (Muakkassa & Strick, 1979; Godschalk et al., 1984; Matelli et al., 1986; Dum & Strick, 2005) and functional (Cerri et al., 2003; Shimazu et al., 2004; Schmidlin et al., 2008; Prabhu et al., 2009; Kraskov et al., 2011) connectivity with M1. M1 itself makes a major contribution to CS projections to the cervical cord, including via its direct cortico-motoneuronal (CM) projections to hand muscle motoneurons (Porter & Lemon, 1993; Rathelot & Strick, 2006; Lemon, 2008). Importantly, many mirror PTNs, particularly in M1, either show sharply reduced activity during action observation compared with execution, or actually suppress their activity relative to baseline (Vigneswaran et al., 2013). Together, these features could feasibly produce a “cancellation effect” to prevent mirror activity from inducing movement (Kraskov et al., 2009, 2014; Vigneswaran et al., 2013). However, it is difficult to predict the spinal impact (or lack thereof) of cortical PTN activity without knowing their targets and actions, which could include both excitation and inhibition at the spinal level (Soteropoulos, 2018).

In humans, TMS has been useful for non-invasively probing net excitability changes during action observation. TMS over M1 during observation of grasp results in an increased amplitude of MEPs in hand and arm muscles (Fadiga et al., 1995; Gangitano et al., 2001; Cattaneo et al., 2009; Alaerts et al., 2010a; Sartori et al., 2012, and see p.52), consistent with the congruence of MirN responses during action execution and
4.2. Introduction

observation. SICI paradigms have suggested that excitability changes during action observation are primarily cortical in origin (Strafella & Paus, 2000). Monitoring of H-reflexes in upper limb muscles provides a measure of spinal excitability changes, and several studies have reported fluctuations in H-reflex amplitude during action observation with a profile similar to that of muscle activity occurring during execution of the same actions (Baldissera et al., 2001; Borroni et al., 2005; Montagna et al., 2005). H-reflexes may be confounded by pre-synaptic inhibition, and are difficult to elicit in intrinsic hand muscles (Mazzocchio et al., 1995; Knikou, 2008), which are of particular relevance for grasping by virtue of their strong CM connections (Porter & Lemon, 1993; McKiernan et al., 1998). The net balance of cortical and spinal effects underlying excitability changes during action observation is uncertain due to variability across subjects and tasks (Naish et al., 2014; Hannah et al., 2018b).

Stimulation of the pyramidal tract (PT) produces antidromic responses which can be used to identify PTNs, and also produces orthodromic descending volleys which exert direct and indirect actions on spinal motoneurons. PT stimulation has advantages for probing spinal excitability compared with stimulating the cortex or peripheral afferents, as the evoked descending volley is unaffected by cortical interactions, and is not thought to be subject to the spinal pre-synaptic inhibition affecting peripheral afferent inputs (Jackson et al., 2006). Direct, monosynaptic excitation of hand muscle motoneurons from this volley gives rise to a short-latency MEP in these muscles (Olivier et al., 2001; Cerri et al., 2003), and the amplitude of the MEP should reflect post-synaptic excitability of the motoneurons during different phases of action observation, including any descending effects originating from MirNs. A more complete understanding of the pattern and extent of action observation activity not just within cortical networks, but also in the downstream spinal circuitry controlling hand muscles, could provide new insights into mechanisms for the generation and suppression of grasping movements.

Here, we assessed changes in excitability during action observation in the primate spinal cord via direct stimulation of the PT in two rhesus macaques and comparison of the modulation of short-latency evoked responses in hand muscles. MirNs have previously been shown to continue to modulate when the grasp is obscured (Umiltá et al., 2001), and also been implicated in the suppression of movement (Kraskov et al., 2009, 2014;
4.3. Methods

Vigneswaran et al., 2013) and inaction representation (Bonini et al., 2014b). We therefore also examined excitability changes when the availability of visual information of the observed grasp was altered, or an explicit stop signal was provided in place of the implied movement suppression required during passive observation.

4.3. Methods

Animals, surgical procedures, and basic elements of experimental task design have been previously described in chapter 2.

4.3.1. Experimental task

Monkeys performed three observation trial types, embedded within the execution-observation-NoGo task design of the basic task (Figure 4.1). On Visible Observation trials, the LCD screen remained transparent until experimenter homepad return (HPN). On Hidden Grasp trials, the LCD screen became opaque 0.15s after HPR, so that the monkey knew the upcoming grasp (via the Object and Go Cues), and saw the initial part of the experimenter reach, but could not see the grasp itself. On Hidden Cue&Grasp trials, object cues were provided to the experimenter via different LEDs (not visible to the monkey), and the screen became opaque at the moment of the Go cue. The monkey therefore could not see the grasp, and also received no information about which object would be grasped. The hold and reward tones were retained for all trial types, and the relative proportion of trials for the 5 conditions was 20:6:6:3:5 (Execution, Visible Observation, Hidden Grasp, Hidden Cue&Grasp, NoGo). The delay periods for this experiment were identical for the two monkeys (LCDon, Baseline: uniformly distributed between 0.25-0.45s, Object Cue: distributed between 0.8-1.2s, NoGo 1.0s).

4.3.2. PT stimulation

We delivered single bipolar, biphasic stimulus pulses (each phase 0.2ms) between the two chronically implanted electrodes continuously during task performance (300µA every 500ms in M48, 350µA every 600ms in M49). PT stimulation in the left medullary pyramid elicited MEPs in the right hand (Figure 2.5A). Stimulus current was monitored at
all times, and evoked EMG responses at rest, when present, were of moderate amplitude and sub-maximal. The stimulus frequency reflected a compromise between obtaining a sufficient number of responses within each session, avoiding any summation across consecutive responses, and ensuring that the monkey tolerated the stimulation and maintained good task performance.

4.3.3. Recording

Stimulation was delivered to the left medullary pyramid during task performance over 5 separate sessions in each monkey. Monkeys performed roughly 25 trials each in the Visible and Hidden Grasp observation conditions, 12 trials in the Hidden Cue&Grasp observation condition, 20 NoGo trials, and 80 execution trials, per session and object. We simultaneously recorded EMG activity from muscles in the right arm and hand, the timing of all task events and PT stimuli (25 kHz), and analog object displacement and homepad pressure signals (5 kHz), as described in subsection 2.3.1 (p.77). In separate sessions, we recorded eye movements from each monkey’s right eye using a non-invasive ISCAN camera system (ETL-200, 125Hz), which was located 25cm away from, and 17cm above the objects. No explicit fixation criteria were imposed on the monkeys during the task.
Figure 4.1. Experimental task design. (A.) Initial sequence for different trial conditions, shown for precision grip (PG). Object and Go cues were not visible to the monkey on Hidden Cue &Grasp. On Hidden Grasp trials, the LCD screen became opaque 0.15s after experimenter movement onset. (B.) Schematic of homepad and object displacement signals, and corresponding task events on Go trials (as in Figure 2.1C). Hidden Cue&Grasp and Hidden Grasp arrows denote time at which LCD screen became opaque on these trials. Numbered intervals 1-5 denote epochs used for statistical analysis of MEPs.
4.3. Methods

4.3.4. Data analysis

Eye movements

The location of each object was estimated from a smoothed (24ms moving average) x-y map of eye movements on execution trials. First, maximally visited locations in the 400ms intervals (± 200ms) around Go and HPR were identified. Monkeys typically fixated on the target object LED around the Go signal, and around the object at HPR. The range of horizontal and vertical eye positions visited at least half as often as these maximally visited locations were then used to form a rectangular window estimate for each object’s location. These windows included the objects and their corresponding LEDs on the monkey side, and subtended a visual angle of approximately 5°. The two windows were confirmed to be non-overlapping in the x-direction for each session, but given the unequal position of the two objects relative to the camera, were allowed to have different sizes in the y-direction. Heat maps of gaze behaviour produced for each session clearly indicated well-separated locations of the relevant LED and object during the ObjCue and execution reach-to-grasp periods, respectively. Within a session, we aligned data to different task events and defined, for each condition and timepoint separately, a dwell likelihood, the proportion of trials in which the monkey’s gaze fell within either the window for the target object, the other object, or neither window. A dwell likelihood equal to 1 for a given 24ms time window thus indicated that the monkey’s gaze fell within the corresponding spatial window at that time window on every trial, whereas a dwell likelihood of 0 meant that the monkey never looked within the window at that time point. The resulting dwell likelihoods were then averaged across sessions, and expressed as mean±SEM at each time point. In addition, we measured the proportion of “look” trials in which the monkey looked at the target object for at least 120ms (5 consecutive bins) during different 300ms epochs of the trial.

Motor-evoked potentials

To quantify MEP amplitude modulation across the task, we initially grouped stimuli times across each session into 300ms-long bins aligned to salient task events. 1. -300 to +300ms around LCDon (2 bins), 2. -1200 to +300ms around LEDon (5 bins) 3. -300 to +300ms around HPR (2 bins) 4. -300 to 1500ms around DO (6 bins). Responses to stimuli falling
4.3. Methods

outside any of these bins (e.g. in the inter-trial interval), and the corresponding evoked responses, were excluded from further analysis. For the NoGo condition, only the first 2 alignments (7 bins) were defined. As we recorded EMG in a range of hand and arm muscles, we were able to quantitatively exclude MEPs which may have been influenced by voluntary EMG. To do so, we used the absolute average of all raw pre-stimulus EMG segments (100ms period immediately before the stimulus) in the two bins around LCDon (-300ms to +300ms) to determine muscle-specific thresholds. Any stimulus during passive periods of the task (all observation and NoGo bins, execution bins before the Go cue) for which the average absolute pre-stimulus EMG in any muscle exceeded 5 standard deviations (S.D.) of the mean were discarded. If any trial contained more than 2 contaminated MEPs, all MEPs from that trial were discarded. This resulted in 2.3±0.3% (mean±SEM) of MEPs being discarded in M48, and 1.6±0.8% in M49, with no more than 4% discarded in any session. A stricter threshold of 3S.D. above the mean resulted in higher discard rates (M48: 7.8±0.8%, M49: 7.6±0.1%), but did not qualitatively change any of the results. For each muscle and MEP, the peak-to-peak (peak-to-trough) amplitude was extracted from the raw unrectified EMG traces in the 5-14ms period after stimulus delivery. Peak-to-peak amplitudes were then averaged within each bin and session, and these averages were normalised to the average amplitude across conditions of the 300ms bin beginning at LCDon.

Statistical analysis

To assess modulation during observation, we performed planned comparisons of MEP amplitude across five epochs of interest for Visible and Hidden Grasp observation conditions via 2-way ANOVA (factors EPOCH × GRASP (PG, WHG)). The five epochs were as follows: 1. Baseline (0 to 300ms from LCDon), 2. ObjCue (-300 to 0ms before Go/NoGo), 3. Reaction (0 to 300ms from Go/NoGo cue), 4. Hand Shaping (-300 to 0ms before DO), and Grasp (0 to 300ms from DO). The Hidden Cue&Grasp condition was identical for both objects and contained a lower number of trials per object, and showed no significant differences in the 2-way ANOVA. We therefore pooled MEPs from trials for both objects in this condition and assessed modulation via a 1-way ANOVA across the same five epochs. We performed an additional 2-way ANOVA (factors CONDITION × GRASP) to compare directly across the 3 observation conditions and 2 objects during the Hand Shaping epoch. To assess modulation of MEPs during NoGo in relation to execution
and observation, we compared 2 epochs (ObjCue and Reaction) across 3 conditions (Execution, Visible Observation, and NoGo) and 2 grasps via 3-way ANOVA (EPOCH × CONDITION × GRASP). For all analyses, significant ANOVA results were followed by post-hoc tests, with Tukey-Kramer correction for multiple comparisons. P-values ≤ 0.05 (corrected) were considered significant.

**Figure 4.2. Eye movements during action observation.** (A). Likelihood of gaze on target object in M48 (left) and M49 (right). Traces + flanking shaded regions show proportion of trials, for each condition and timepoint, in which monkey’s gaze fell into a window around the target object (mean±SEM, 4 sessions). Data is multiply aligned to different task events (LCDon, ObjCue, Go/NoGo, HPR, DO, hold offset (HOFF), and HPN, colour conventions as in Figure 4.1B). (B). Proportion of trials, for each condition, in which monkey gaze fell within the target object window for a sustained period of at least 120ms in different 300ms epochs during the task (mean±SEM, 4 sessions).
4.4. Results

To assess modulation of spinal excitability during action observation, we recorded MEPs elicited by direct stimulation of the medullary pyramid while monkeys passively observed a human experimenter performing cued reach-grasp-and-hold movements. These observation trials were randomly interleaved with execution trials, in which the monkeys performed the same movements themselves, and NoGo trials, where the monkeys were explicitly cued to refrain from movement. Furthermore, on a subset of observation trials, a controllable LCD screen was used to modulate visual information provided to the monkeys about the upcoming grasp.

4.4.1. EMG and behaviour

EMG activity recorded during sessions without stimulation showed distinct patterns for the different grasps during action execution, whereas during (Visible) action observation and NoGo conditions, there was no systematic EMG activity (Figure 3.1, p.87). For analysis of stimulation sessions, any stimulus-aligned sweeps contaminated by voluntary EMG activity in non-movement stages of the task were removed before analysis. Monkey reaction and movement times across all trials were considerably faster than the human experimenter (see Table 4.1; all monkey-human comparisons \( p < 0.0001 \), Mann-Whitney U-test). Monkey reach times were generally longer for PG than WHG, due to the trapezoid object’s location contralateral to the reaching (right) arm (Table 4.1, both within-monkey comparisons \( p < 0.0001 \), Mann-Whitney U-test).

4.4.2. Eye movements

In separate sessions, we recorded eye movements in each monkey to assess whether monkeys attended to the observation task, and verify that the opaque screen successfully abolished the visual information available to the monkey about the upcoming grasp. For each trial and time point we defined whether the monkey’s gaze fell within a window around (a) the target object for the given trial, (b) the other (non-target) object, or (c) outside of both windows. Figure 4.2A shows, for each condition, time point, and monkey, the likelihood that gaze fell within the target object window. A value of 1 indicates that the
4.4. Results

<table>
<thead>
<tr>
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<th>M48</th>
<th>M49</th>
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<tr>
<td>Monkey</td>
<td>Human</td>
<td>Monkey</td>
</tr>
<tr>
<td>RT</td>
<td>0.31 ± 0.07</td>
<td>0.42 ± 0.06</td>
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<tr>
<td>MT (PG)</td>
<td>0.42 ± 0.11</td>
<td>0.51 ± 0.07</td>
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<tr>
<td>Movement time</td>
<td>0.33 ± 0.07</td>
<td>0.45 ± 0.10</td>
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Table 4.1. **Behaviour during PT stimulation sessions.** Number of trials across all sessions. Human trials include Visible Observation trials only. Square brackets show trial numbers for PG and WHG, respectively. Reaction time (RT, across objects), and movement time (MT) in seconds for each grasp (mean ± S.D. across trials, 5 sessions per monkey).

monkey’s gaze was within the target object window at that particular time point on every trial. These values are averaged across sessions, so that the error reflects the variability across sessions, and the mean value itself reflects variability in gaze within a session. Both monkeys reliably began to look at the target object over the course of the Object Cue period, except during Hidden Cue&Grasp trials where they did not exceed chance (0.5), and looked away after the Go cue (when the screen went off). During Visible and Hidden Grasp observation, M48 maintained gaze until shortly after experimenter HPR, and tended to remain longer and view the actual grasp and hold on Visible Observation trials. In these conditions, M49 looked away earlier, before occasionally returning gaze to the target object during the reach and grasp. On execution trials, monkeys maintained gaze until DO (M48) or late reach (M49), frequently looked away while maintaining stable hold, and tended to return gaze to the object in the lead-up to the end of the hold. In the NoGo condition, both monkeys’ gaze behaviour showed a similar pattern to observation trials, viewing the target object during the ObjCue period and then deviating shortly after the NoGo cue. Both monkeys also returned to the target object towards the end of the trial (1s after the NoGo cue), possibly in expectation of the extinguishing of the LED marking the end of the trial and subsequent reward. In Figure 4.2B, we quantified the proportion of “look” trials, where the monkey’s gaze was persistently upon the target object, for different task epochs (the same epochs as used for MEP analysis, as well as Reach (0-300ms from HPR), and Late Hold (-300 to 0ms before HOFF). This analysis largely recapitulates the results of the dwell likelihood analysis, showing that M48 regularly looked at the target
object during the observation of hand shaping, whereas M49 tended to look away earlier. In both monkeys, the proportion of “look” trials during grasp was higher for Visible observation than Hidden Grasp. On Hidden Cue&Grasp trials, monkeys directed their gaze to the target object at chance levels during the Cue period, and hardly looked at the object at all in the later stages of the trial.

Figure 4.3. Motor-evoked potentials elicited by PT stimulation. (A). Single sweeps of 1DI MEPs recorded in M48 during hand shaping, randomly selected across a single session. Dashed vertical lines denote 5-14ms window used to extract peak-to-peak amplitudes. (B) Same as (A), but for AbDM in M49. Long-latency responses (at ∼15ms latency) in M49 during action observation were not included in peak-to-peak analysis window to focus on direct corticospinal effects. (C). Responses to stimulation in 5 muscles in M48. Traces show mean±SEM across MEPs during PG hand shaping epoch (300ms leading up to DO) from a single session (note different scales for each muscle). (D). Same as (C), but for M49.

4.4.3. Motor-evoked potentials

Overall, each stimulation session lasted 30-40 minutes, resulting in 3000-4500 PT stimuli being delivered. Stimuli were binned in 300ms epochs according to their timing relative to
task events and sweeps contaminated by pre-stimulus EMG were rejected (<4% of binned MEPs per session). Within the epochs used for statistical analysis, there remained an average of 13.5±0.4 MEPs per epoch, object and observation/NoGo condition in M48, and 11.2±0.2 in M49. MEP counts for each condition and epoch separately are presented in Table 4.2. Figure 4.3A shows single sweep responses to PT stimulation in the 1DI muscle (M48) during the observation grasp interval, with a latency of 9-10ms, consistent with monosynaptic activation of spinal motoneurons (Olivier et al., 2001; Cerri et al., 2003). In several muscles in M49, we observed some unusually long-latency effects during passive periods of the task, which sometimes dominated, but were also often present in tandem with short-latency responses (Figure 4.3B). For the purposes of this study, we focused on the short-latency responses typical of direct (monosynaptic) excitation of spinal motoneurons. As we did not attempt to optimise the stimulation intensity for each muscle, the amplitude of MEP responses was also variable across muscles. Figure 4.3C and D show examples of averaged MEPs from five muscles in each monkey. For further analysis, we concentrated on distal muscles (1DI, thenar complex and AbDM) most relevant for the grasp, due to the known stronger CM connections to these muscles compared with proximal muscles (Porter & Lemon, 1993; McKiernan et al., 1998; Morecraft et al., 2013). The AbDM muscle in M48 was excluded because of a high level of noise in the recorded signal.

Figure 4.4. 1DI MEPs during different task conditions and epochs from single session in M48. (A), Baseline (0-300ms from LCDon), B, PG Hand Shaping (300ms leading up to DO). C, WHG Hand Shaping. Baseline MEP amplitudes are fairly similar across all conditions. Execution trials are contaminated by voluntary EMG activity and are considerably larger during PG and WHG Hand Shaping (note different scale for execution MEPs).
### 4.4. Results

<table>
<thead>
<tr>
<th></th>
<th>M48</th>
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<tbody>
<tr>
<td></td>
<td>Execution</td>
<td>Baseline</td>
<td>ObjCue</td>
<td>Reaction</td>
<td>HandShaping</td>
</tr>
<tr>
<td>PG</td>
<td></td>
<td>41.6 ± 3.5</td>
<td>40.0 ± 2.9</td>
<td>42.0 ± 4.1</td>
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<tr>
<td>Obs: Visible</td>
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<td>14.0 ± 1.0</td>
<td>15.0 ± 0.8</td>
<td>13.4 ± 0.7</td>
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<tr>
<td>Obs: HiddenGrasp</td>
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<td>12.2 ± 0.2</td>
<td>14.0 ± 0.9</td>
<td>14.0 ± 0.9</td>
<td>13.2 ± 0.5</td>
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<tr>
<td>Obs: HiddenCue&amp;Grasp</td>
<td></td>
<td>15.2 ± 1.0</td>
<td>14.4 ± 1.0</td>
<td>16.4 ± 1.3</td>
<td>16.4 ± 1.1</td>
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<tr>
<td>NoGo</td>
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<td>12.0 ± 1.2</td>
<td>12.6 ± 1.0</td>
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<td>WHG</td>
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<td>15.6 ± 1.0</td>
<td>17.0 ± 1.3</td>
<td>14.4 ± 1.2</td>
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<tr>
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<td>13.6 ± 0.9</td>
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<tr>
<td>Obs: HiddenCue&amp;Grasp</td>
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<td>15.2 ± 1.0</td>
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<tr>
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<td>38.0 ± 2.4</td>
<td>41.8 ± 2.9</td>
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<tr>
<td>Obs: Visible</td>
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<td>12.2 ± 1.5</td>
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<td>Obs: HiddenCue&amp;Grasp</td>
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<td>13.0 ± 1.6</td>
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<tr>
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<td>39.0 ± 0.8</td>
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<tr>
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<tr>
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<td>12.0 ± 1.5</td>
<td>11.4 ± 0.7</td>
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<tr>
<td>NoGo</td>
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<td>10.2 ± 0.7</td>
<td>10.8 ± 0.4</td>
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</table>

Table 4.2. Average number of MEPs (mean±SEM, 5 sessions) per analysis epoch and condition, for each monkey and grasp separately (top: M48, bottom: M49). Hidden Cue&Grasp counts are pooled across grasps.

### 4.4.4. Task-dependent modulation of spinal excitability

To examine the temporal modulation of MEP amplitude during the different conditions, we calculated the mean peak-to-peak amplitude of MEPs within 300ms bins across different stages of the task. Figure 4.4 shows examples of averaged 1DI MEP responses obtained during Baseline and Hand Shaping task periods on execution and observation trials for PG and WHG. For PT stimuli during Baseline, the resulting MEPs were unsurprisingly comparable across the different task conditions (Figure 4.4A). By contrast, during Hand Shaping for PG (Figure 4.4B), clear differences emerged in the amplitude of MEPs across the different observation conditions; these changes were less pronounced for WHG (Figure 4.4C). While MEP amplitudes increased in the Visible Observation and Hidden Grasp conditions, they remained close to baseline in the Hidden Cue&Grasp condition.
4.4. Results

Execution MEPs, shown on a different scale, were much larger at this point and contaminated by voluntary EMG activity.

We next examined the profile of modulation across time, conditions, and sessions. For responses in 1DI of M48 (Figure 4.5A), facilitation of spinal excitability during the object presentation period was apparent in all conditions except for Hidden Cue&Grasp observation (green), where LED cues regarding the upcoming trial were not visible, and the MEPs remained close to baseline. MEPs following the imperative Go/NoGo cues then suppressed back to baseline levels or somewhat below, except during execution. Following this, unobstructed observation of PG produced an increase in 1DI MEPs (orange trace), which was particularly prominent during final hand shaping leading up to the experimenter’s grasp. When the grasp was obscured but the monkey had seen the object and Go cues (Hidden Grasp condition), a very similar facilitation profile was observed, with a 50% increase in MEP size in the lead up to grasp. During the Hidden Cue&Grasp condition, on the other hand, when the grasp was obscured and the monkey did not know which object was being grasped, MEPs did not modulate to the same extent. Observation of WHG also showed some facilitation effects around the time of the grasp, but these were weaker than for PG, particularly during the Hidden Grasp condition. During execution, 1DI MEPs were substantially increased from the lead-up to HPR until the end of the trial, reflecting the corresponding increase in spinal excitability during the monkey’s own movements. We observed some qualitatively similar modulation in the AbDM muscle of M49, with an increase in excitability during the observation of hand shaping and grasp for PG, although these effects were generally weaker (Figure 4.5B).

To assess quantitatively the modulation of MEP size during action observation, we compared MEP amplitudes across five salient epochs in the observation trial sequence: Baseline, Object Cue, Reaction, Hand Shaping and Grasp (2-way ANOVA, EPOCH × GRASP). In M48, there was a main effect of EPOCH during Visible Observation and Hidden Grasp conditions in 1DI (both p < 0.0001), and thenar (p < 0.0001 and p = 0.0068, respectively). Post-hoc comparisons across epochs revealed that 1DI MEPs in these two observation conditions were larger during Hand shaping relative to Baseline (35-40%, both p < 0.005, indicated by orange and purple asterisks) and Reaction (50-65%, both p < 0.0003) epochs (Figure 4.5A). The Hidden Grasp condition also showed an
interaction of epoch and grasp ($p = 0.045$), and 1DI MEPs during hand shaping for PG were significantly larger than those during WHG ($p = 0.021$). Visible Observation and Hidden Grasp MEPs during the ObjCue epoch were also facilitated relative to those during Baseline and Reaction in 1DI (all $p < 0.05$). In M49, short-latency responses in the 1DI muscle of M49 were generally weak, but more apparent in AbDM (Figure 4.5B). Nonetheless, a 2-way ANOVA (EPOCH $\times$ GRASP) revealed significant main effects of EPOCH during Visible Observation and Hidden Grasp conditions in 1DI ($F_{4,40} > 2.9$, $p < 0.05$), Thenar ($F_{4,40} = 4.9$, $p < 0.003$), and AbDM ($F_{4,40} = 4.3$, $p < 0.006$). In all three muscles, MEPs during Hand Shaping in Visible and Hidden Grasp conditions were 20-35% larger than those in the Reaction epoch (all $p < 0.03$), although modulation relative to Baseline was limited. Across both monkeys and all three muscles, the Hidden Cue&Grasp condition showed no modulation across epochs (all $p > 0.3$).

Given these results, we next assessed whether visual or predicted information about the observed grasp affected spinal excitability, by comparing directly across the three observation conditions within the Hand Shaping epoch (2-way ANOVA, CONDITION $\times$ GRASP). In 1DI of M48, this revealed a significant main effect of condition ($F_{2,24} = 5.70$, $p = 0.009$) and grasp ($F_{1,24} = 5.84$, $p = 0.024$), since PG MEPs were larger than those during WHG. During observation of Hand Shaping, Visible and Hidden Grasp MEPs were not different from each other across objects ($p = 0.61$), but were larger than those in the Hidden Cue&Grasp condition, by almost 50% in the case of PG ($Visible: p = 0.0085$, Hidden Grasp: $p = 0.07$, post-hoc tests collapsed across grasps). In the thenar muscle, there was also a main effect of condition ($F_{2,24} = 6.38$, $p = 0.006$), and MEPs in Visible and Hidden Grasp conditions were not different to each other ($p = 0.53$), but again larger than those during Hidden Cue&Grasp ($p = 0.0052$ and $p = 0.06$ respectively, post-hoc test collapsed across grasps). MEPs in M49 showed no significant differences between observation conditions.
4.4. Results

Figure 4.5. Time-dependent modulation of MEP amplitude during action observation. (A). Normalised MEP amplitude in 1DI of M48, averaged within 300ms bins across the trial for PG (top) and WHG (bottom), then across sessions (mean±SEM, 5 sessions). Execution data is cut off on y-axis to better show modulation during other conditions. Vertical markers in centre show average event times across sessions for five conditions, as indicated by legend. Conventions for events as in Figure 4.1B, with additional markers for the time that the screen became opaque on hidden observation trial types. Marked time points 1-5 mark bins used for planned comparisons of MEP modulation (Baseline, ObjCue, Reaction, Hand Shaping, and Grasp). Orange and purple ** indicate significant differences (p < 0.005) relative to baseline, # indicates significant difference between Visible Observation and Hidden Cue&Grasp conditions during Hand Shaping (p < 0.01, collapsed across grasps). (B). Same as (A), but for AbDM in M49.
4.4. Results

![Graph](image)

**Figure 4.6.** Normalised MEP amplitudes (mean±SEM) in intrinsic hand muscles around the imperative stimulus. Panels show normalised amplitudes across sessions for ObjCue and Reaction timepoints during Execution, Observation, and NoGo, averaged across objects. Top row: M48, lower row: M49 (n=5 sessions per monkey). Execution MEP amplitude typically increased once movement initiation processes had begun (Reaction), whereas Observation and NoGo MEPs during this period were suppressed relative to ObjCue. * p < 0.05, ** p < 0.005, *** p < 0.0001, significant difference compared to relevant ObjCue. Observation and NoGo were significantly different to Execution during Reaction in all cases (p < 0.0001), but not from each other (all p > 0.7).

Finally, to assess evidence of suppression associated with action observation and action withholding, we compared MEP amplitudes in the two bins before and after the Go/NoGo cue (ObjCue and Reaction) across three conditions (Execution, Visible Observation, and NoGo), and two grasps (Figure 4.6, 3-way ANOVA). All muscles showed significant Condition × Epoch interactions (all p < 0.0001). There was no difference between any of the three conditions during the ObjCue period (all p > 0.9). During the Reaction period, which was before any movement, execution MEPs were consistently larger than Observation and NoGo MEPs, and larger than ObjCue MEPs (all p < 0.0001). Observation MEPs showed a 15-30% suppression across monkeys and hand muscles during the Reaction interval relative to ObjCue, which was frequently significant (p < 0.05). NoGo MEPs tended to show a similar suppression of 10-30%, and observation and NoGo MEP amplitudes during Reaction were comparable (all p > 0.7).
4.5. Discussion

During action observation in macaques, there is modulation in the activity of CS outputs from both premotor and primary motor cortex (Kraskov et al., 2009; Vigneswaran et al., 2013). Similar inferences have been made from human TMS studies probing CSE (Fadiga et al., 1995; Gangitano et al., 2001; Montagna et al., 2005; Urgesi et al., 2006; Cattaneo et al., 2009; Bunday et al., 2016), yet these changes do not result in overt movement in the observer. Although spinal circuitry represents the final common path for the neural signals generating or suppressing muscle activity and movement, our understanding of the influence of action observation on this circuitry remains limited.

4.5.1. Observation of grasp produces facilitation at the spinal level

During the observation of grasp, we found facilitation in the 1DI muscle (Figure 4.4 and Figure 4.5A), consistent with previous results reporting sub-threshold modulation in human CSE during action observation (Fadiga et al., 1995; Gangitano et al., 2001; Montagna et al., 2005; Urgesi et al., 2006; Cattaneo et al., 2009; Bunday et al., 2016). In monkeys, there is an overall disfacilitation of CS outputs during action observation (Kraskov et al., 2009; Vigneswaran et al., 2013), but the net effects of this disfacilitation at the spinal level were not tested directly, since the spinal targets of these CS MirNs were largely unknown. The results presented here demonstrate that grasp-related mirror activity in descending pathways can produce sub-threshold increases in net excitability within spinal circuitry. This facilitation was most prominent during the observation of hand shaping prior to grasp, and just after the grasp itself. Previous findings in humans suggest that CSE modulations peak at the time of observed maximal hand aperture (Gangitano et al., 2001), while dynamic stages of the action are ongoing (Urgesi et al., 2006; Urgesi et al., 2010), or at the time when grasp is achieved (Gueugneau et al., 2015), and the peak of the observation response in macaque MirNs often occurs prior to, or around, the grasp (Vigneswaran et al., 2013). Facilitation in the 1DI muscle was more pronounced for observation of PG rather than WHG (Figure 4.5A), although this effect was relatively weak. Grasp and muscle-specific changes in CSE occur during action execution (Lemon et al., 1995; Davare et al., 2008, 2009), and action observation (Catmur et al., 2007; Sartori et al., 2012; McCabe et al., 2015; Bunday
et al., 2016), and at the cortical level, many macaque MirNs show congruence between the executed and observed action most effective in activating them (Gallese et al., 1996). The index finger and 1DI muscle are important for PG execution (Muir & Lemon, 1983; Bennett & Lemon, 1996; Quallo et al., 2012), and 1DI MEPs are modulated during execution in a time-locked, grasp-specific manner (Davare et al., 2008, 2009). The relatively larger modulation of spinal excitability during PG in the 1DI suggests that action observation activates some of the same spinal circuitry which is used when the monkeys perform the grasp themselves. However, the evidence for specificity of CSE modulation during action observation is mixed (Naish et al., 2014), and congruence in cortical MirNs is often broad (Gallese et al., 1996). Additionally, the PG and WHG performed by the monkeys in the present task were complex, multi-phasic actions. As such, recording of muscle activity during execution verified that the modulation during observation was complementary to the activation profile of the muscles during execution, demonstrating greater 1DI activation during PG execution than during WHG. Facilitatory effects during observation of grasp were generally weaker in M49, with the AbDM muscle showing modest facilitation during Hand Shaping relative to Reaction, but not compared to the Baseline (Figure 4.5B). One possible explanation for the weaker effect in this monkey may have been the small amplitude of short-latency responses, with several muscles showing late responses with latencies of 15-16ms, i.e. at least 5ms longer than the short latency MEPs (Figure 4.3B,D). These long-latency effects were present during action observation, when ongoing EMG activity was absent, and disappeared from MEPs evoked during action execution. They were probably mediated by pathways other than the fast CM component of the CST, which might include reticulospinal pathways (Riddle et al., 2009; Baker, 2011) or propriospinal pathways (Isa et al., 2006; Isa, 2019) activated by CS collaterals. However, the additional latency suggests rather indirect, oligosynaptic actions.

A further factor potentially influencing grasp-related facilitation effects during action observation is the behavioural strategy adopted by the two animals, as reflected in their eye-movement patterns (Figure 4.2). Monkeys were allowed to gaze freely during all conditions, which we believe provides a more ethological way to study action observation. M49 consistently diverted gaze from the target object shortly after the Go/NoGo cue which indicated that no response was required, and showed a small tendency to return around the time of the grasp, possibly reacting to the experimenter’s movement. On the other hand,
4.5. Discussion

M48 more often maintained gaze on the target object after the experimenter’s movement had begun. A previous study suggested that gaze behaviour during action observation can modulate mirror neuron activity (Maranesi et al., 2013), although almost half of recorded PMv MirNs in that study showed gaze-independent modulation during observation. A recent TMS study using simple, single finger movements found that gaze fixation at the point of movement facilitated MEPs relative to free gaze (D’Innocenzo et al., 2017). Given the more complex movements used in the present task, and trial-to-trial variability of free gaze behaviour, further interpretation of the relationship between eye movements and excitability changes in motor pathways during naturalistic action observation, if present, would require simultaneous recordings. From the current data, we speculate that M48 spent more time collecting information about the forthcoming action, presumably for updating of an internal model for better prediction of the upcoming observed grasping action (Kilner et al., 2007).

4.5.2. Available visual information modulates responses

A secondary aim of this study was to probe the spinal response during action observation when the amount of visual information available to the monkey was altered. Previously, F5 MirNs have been shown to continue to modulate their response even when the grasp was obscured (Umiltá et al., 2001; Kraskov et al., 2009), or only auditory cues are available (Kohler et al., 2002), suggesting that mirror activity is not simply a passive visual response, but an unfolding of the internal representation of the action, reflecting a prediction about the action goal (Kilner et al., 2007). Here we found spinal facilitation was still present in the 1DI of M48 when the view of the grasp was obscured, but the type of grasp was known to the monkey i.e. perfectly predictable. This facilitation showed the same pattern as seen during visible observation (Figure 4.5), with a preference for PG, in which the 1DI muscle is intimately involved (Muir & Lemon, 1983; Maier et al., 1993; Quallo et al., 2012). Importantly, the pattern of eye movements in the Hidden Grasp, but not Hidden Cue&Grasp condition, was similar to the Visible Observation condition, suggesting that the monkeys frequently anticipated the target object even though it was not visible, but only if they had seen the object cue. Interestingly, the modulation in the Hidden Grasp condition had a tendency to remain for longer than that seen in the Visible Observation condition. In this latter condition, the monkeys can accurately predict the end stage of the grasp, which
may lead to an earlier attenuation of excitability changes (Urgesi et al., 2006; Urgesi et al., 2010). This persistence of action observation modulation in the absence of direct visual input suggests that, even at the spinal level, the internal representation of an upcoming action may be sufficient to modulate excitability. Previous evidence for comparable changes in CSE during hidden action observation is limited, with changes relative to full vision observation and a resting baseline either weak or not tested (Villiger et al., 2011; Valchev et al., 2015; Cretu et al., 2019).

In our task, information about the general trial structure (via hold window and reward sounds) was available to the monkey on both visible and hidden trial types, and auditory cues have been shown to elicit mirror responses (Kohler et al., 2002; Alaerts et al., 2009b). These sound cues were not grasp-specific, but may have been sufficient to trigger a more general mirror response in the well-trained monkeys, accounting for some of the non-specific facilitation later in the trial (Figure 4.5).

4.5.3. Suppression of excitability during withholding of movement

Alongside the grasp-related facilitation during action observation, we were also able to assess spinal excitability at the stage prior to reaching and grasping, at the time when the monkey had to initiate the movement on execution trials, or remain still on observation trials. In both monkeys, we found evidence for suppression of MEPs during this period (Figure 4.6) which was comparable in amplitude to when they explicitly suppressed their movement after being cued with a NoGo signal. This suppression following the Go/NoGo cue is consistent with the notion that action observation implicitly requires movement suppression (Kraskov et al., 2009, 2014; Vigneswaran et al., 2013), and suggests that the neural substrate for this suppression overlaps with that involved in the explicit suppression of movement. This finding contrasts with a previous study, which found many MirNs which also responded to an observation-NoGo condition, but not when the monkey suppressed its own movement (Bonini et al., 2014b). These MirNs were recorded in F5, which may show a different relationship between observation and NoGo conditions, than areas closer to the spinal output (see also chapter 3). The different findings in our experiment may also have arisen due to the use of an interleaved task design and relative timing of the NoGo cue. The monkeys had to decide at matched time points on a
trial-by-trial basis whether to generate or suppress movement, which is different from the block design used by Bonini et al., (2014b), where it was clear from the outset of all trials within a block whether the monkey would be executing or observing actions. The Go/NoGo cue was then provided as an auditory cue prior to object presentation, meaning that, unlike in our study, the action or inaction could be predicted in advance (Maranesi et al., 2014). During the ObjCue period preceding the Go/NoGo cue, we observed a consistent increase in excitability across all conditions in which the object cue was provided in M48, with relatively little change in M49. Premotor and motor cortex are known to show anticipatory activity prior to intended movements (Tanji & Evarts, 1976; Weinrich & Wise, 1982; Alexander & Crutcher, 1990; Riehle & Requin, 1993; Churchland et al., 2006a,b) and preparation-related changes also occur at the spinal level (Prut & Fetz, 1999; Fetz et al., 2002). Suppression of CSE during movement preparation has been hypothesised to have important roles in response selection and impulse control (Hasbroucq et al., 1999; Duque & Ivry, 2009; Duque et al., 2010; Greenhouse et al., 2015; Lebon et al., 2016). In premotor area F5, a substantial proportion of neurons also become active during object presentation, in a manner reflecting upcoming grasp (Murata et al., 1997; Raos et al., 2006; Umiltá et al., 2007). In our task, the non-cued object was still present within the monkey’s field of view for the duration of the trial, and the absence of object specificity during this pre-movement stage suggests this response is distinct from grasp-specific facilitation reported in F5 neurons during object presentation (Raos et al., 2006). We therefore consider it more likely that the different levels of modulation during ObjCue in the two monkeys arose due to qualitatively different strategies, as eye movement recordings suggest that M48 was more attentive during action observation than M49 (Figure 4.2). Alongside possible inhibitory mechanisms at cortical and sub-cortical levels, attentional and motivational factors of the specific monkey may influence activity in cortical and spinal motor circuitry during action observation.

4.5.4. Conclusions

Here, we used direct stimulation of the PT to probe the modulation of spinal excitability during preparation, observation, and explicitly cued suppression of reach-to-grasp actions. The MEPs elicited by PT stimulation reflect post-synaptic mechanisms in alpha motoneurons, and PT stimulation therefore offers a useful and unique method for probing
4.5. Discussion

excitability of spinal circuitry controlling the hand in the awake, behaving animal. Our results confirm that the motoneuron pools innervating the hand can undergo sub-threshold modulations during action observation. We found an increase in excitability at the spinal level around the observation of hand shaping and grasp, particularly in the 1DI muscle during PG, suggesting that the same spinal circuits which are recruited during PG performance are modulated when the same action is observed. This excitability was still apparent when the grasp was obscured but predictable, indicating that predictive activity of cortical MirNs can reach and excite motoneurons in the spinal cord. This increase in excitability was preceded by a relative suppression in the lead-up to the observation of movement. This was comparable to the suppression when the monkey withheld movement, and may support the suppression of movement during action observation. Our previous findings showed that descending activity from PTNs, particularly in M1, is attenuated during observation, and we inferred from those results that hand motoneurons would receive less excitation than during execution. Here we used a more direct assessment of spinal motoneuron excitability and the relatively modest effects, and balance of excitation and inhibition, provide further evidence that the net effects of motor cortical output during the different stages of action observation must be limited such that spinal motoneuron pools remain sub-threshold to movement. Future studies might consider more direct measures of spinal modulation during action observation, including single motor unit responses to PT stimulation, as well as extracellular or intracellular recording from spinal sINs. These would shed additional light on the excitatory and inhibitory effects of cortical MirN activity on the spinal cord machinery, and could yield further insights into the physiological mechanisms underpinning the generation and suppression of grasping movements.
5. Additional properties of mirror neurons

5.1. Abstract

Recent literature has drawn attention to some dissimilarities between execution and observation activity, particularly in M1, and also highlighted a number of contexts which modulate MirN activity. Here, in three experiments involving extracellular recording in F5 and M1 during the same sessions as the basic observation task, we examined several contextual factors which could modulate MirNs activity, and where possible, compared contextual modulation in F5 and M1 populations. Although imitation has been put forward as a function of MirNs, requiring the monkey to imitate the observed action did not produce a reliable change in mirror activity relative to activity during basic observation. This may have been because the cognitive difficulty of the imitation task was insufficient. In a second task, observing an action while simultaneously engaged in execution revealed superimposition of observation activity on execution activity in F5, but less so in M1. This suggests that while F5 can represent executed and observed actions simultaneously, observation-related activity may need to be particularly suppressed in M1 in order to prevent interference with the ongoing motor program. Finally, using a hidden observation paradigm, we found that both F5 and M1 MirNs continued to modulate if the observation grasp was obscured but known to the monkey (via object cues), but generally did not modulate if no contextual information was provided. This is in line with previous evidence from F5 MirNs and human studies showing that predictable information about the goal of an observed action can be sufficient to modulate MirN activity.
5.2. Introduction

The original discovery of MirNs emphasised the similarity in F5 activity between execution and observation conditions, and attributed this similarity to a general representation of action, independent of the acting agent. Nonetheless, some neurons in F5 do not modulate during action observation (Gallese et al., 1996), or exhibit differences in activity between execution and observation (Kraskov et al., 2009, and see chapter 3). These differences are even more pronounced in M1 (Vigneswaran et al., 2013), and results in the previous chapters have shown that the temporal profile of M1 and CS mirror activity may help to dissociate representation of grasping actions from movement initiation. To assess sensory or contextual factors which may modulate F5 and M1 neural activity during action observation, we recorded the activity of MirNs under different experimental conditions, and compared this activity to that of the same neurons during the basic observation task.

In Experiment 1, monkeys had to extract information from the observed action in order to correctly choose the target object for subsequent execution. Action recognition and imitation have both been attributed as functions of the MNS, and there is evidence from fMRI and TMS that the intention to imitate observed actions can modulate mirror activity (Iacoboni et al., 1999; Rizzolatti et al., 2001; Buccino et al., 2004; Hardwick et al., 2012). We hypothesised that, if MirNs are involved in encoding the observed action for imitation, that the requirement to imitate (and therefore actively attend to) the experimenter’s action would alter MirN activity relative to observation in the basic mirror task.

In Experiments 2 and 3, we also tested in one monkey whether observing an action while simultaneously engaged in execution, or altering the level of available visual information about the final grasping action, modulated MirN activity. Although visual information is typically available during both action execution and action observation, somatosensory and proprioceptive inputs are very different in these two conditions. Observing an action before or during the production of a compatible or incompatible action measurably affects behaviour (Brass et al., 2001; Kilner et al., 2003; Blakemore & Frith, 2005) and perception (Hamilton et al., 2004). For example, Kilner et al., (2003) found that observation of arm movements perpendicular to the one being performed (e.g. observing up/down movements
5.3. Methods

while performing left/right movements), increased the end point variance in the vertical axis, perpendicular to that of the performed movement. This was hypothesised to occur due to superimposition, or interference, of the motor plan associated with the observed action onto the motor plan already engaged in production of the executed action (Kilner et al., 2003; Blakemore & Frith, 2005; Stanley et al., 2007; Capa et al., 2011). In experiment 2 we examined whether, at the single-neuron level, activity related to action observation in our grasping task could be superimposed on execution activity.

There is evidence that F5 MirNs continue to modulate their activity when grasp is obscured from the monkey’s view (Umiltá et al., 2001; Kraskov et al., 2009). There is also some evidence from TMS studies that CSE modulations take place even when grasp is obscured or visual input is reduced, and that these are comparable to modulation during full vision observation (Alaerts et al., 2009d; Villiger et al., 2011; Valchev et al., 2015; Cretu et al., 2019). However, M1 neurons often demonstrate less abstract relationships than PMv to motor output, with many encoding movement within an intrinsic reference frame (Kakei et al., 1999). Although non-invasive evidence suggests that mirror activity in M1 is not affected by reduced visual input, the responses of MirNs in M1 to obscured action observation have not yet been examined. In Experiment 3, we therefore recorded and compared MirN activity while varying the level of visual and contextual information regarding the grasping action performed by the experimenter.

5.3. Methods

The same monkeys used previously also participated in these experiments, and general training methods, surgical preparations, recording and pre-processing methods have been described in chapter 2. Recordings for Experiment 1 were made from both monkeys, but for technical and timing reasons, recordings for Experiments 2 and 3 were only made from one monkey (M49). Experiments 1 and 2 were performed in separate blocks during the same sessions as recordings of the basic mirror task, while Experiment 3 was embedded within the basic mirror task performed by M49. All units were therefore recorded during basic observation trials as well as during these additional experiments. Spike waveforms for experiments 1 and 2 were manually cross-checked against those in the basic observation task to ensure satisfactory consistency. The general goal of analyses was to
5.4. Experiment 1: Observation for imitation

assess modulation during basic execution and observation to identify MirNs, and then compare the activity of modulated units in the basic observation condition to that during the extended experiments. Specific data analysis procedures are presented in the following sections. Unless otherwise stated, the alpha value for all statistical tests was set to 0.05. In separate sessions, we assessed the gaze behaviour of the monkeys using the same non-invasive ISCAN system and analysis procedures previously described (p.126).

5.4. Experiment 1: Observation for imitation

5.4.1. Experimental task

Monkeys performed both execution and observation trials in a similar, interleaved manner as during the basic task, involving both PG and WHG actions. However, in contrast to the explicit object cue provided during basic observation, on observation for imitation trials all four LEDs surrounding the objects (Figure 2.1A) were illuminated, the subsequent go cue for the experimenter was not visible to the monkey, and no reward was given after the experimenter returned to their homepad. After a delay (0.3s in M48, 0.2-0.5s in M49), green LEDs at both object locations instructed the monkey to make a reach-to-grasp and hold movement to the object they had just observed being grasped by the experimenter (Exe, Imitation). Grasping the incorrect object resulted in an error and the trial was aborted. The lack of LED cues regarding the observed grasp ensured that the monkey needed to attend at least a portion of the experimenter’s reach-to-grasp movement to correctly repeat the action. Units were recorded for at least 10 observation trials (with successful imitations) for each object. All other experimental procedures were identical to the basic mirror task (subsection 2.1.3, p.70). When comparing activity across basic and imitation tasks, the following nomenclature was adopted. “Basic” execution and observation referred to activity during the basic mirror task. “Cued” execution referred to the simple execution condition in the imitation task, which was identical to Basic execution. “Imitation” observation refers to the observation trials in the imitation task, and Imitation Execution refers to the subsequent execution of the same action by the monkey.
5.4. Experiment 1: Observation for imitation

Figure 5.1. Observation for imitation trial schematic. Schematic of observation for imitation trial types, showing object displacement signals for cued execution (Exe, Cued) trials (equivalent to execution trials in the basic task), and observation trials (Obs, Imitate), followed by imitation execution (Exe, Imitate). Shaded regions below observation trace indicate epochs used for firing rate analyses. The React and Hold epochs were each divided into two periods of equal duration (Early and Late).

5.4.2. Data analysis

To identify MirNs, firing rates on each trial were initially calculated during three epochs 1.) LCDon-Cue (Baseline, Bsln), 2.) HPR-DO (Reach), 3.) 0-500ms from HO (Hold) during the basic observation task. These firing rates were subjected to a one-way ANOVA (factor EPOCH with 3 levels), followed by post-hoc comparisons, with Tukey-Kramer correction. Neurons were considered modulated for a given condition if the ANOVA produced a significant effect, post-hoc tests demonstrated a significant effect during Reach or Hold relative to Baseline, and if there was a minimum firing rate range of 5 spikes s$^{-1}$ across conditions. A neuron was defined as an MirN if it was significantly modulated during both execution and observation, independently for each object.

To assess MirN population activity across the two observation tasks, we first computed firing rates across a wider range of epochs, as shown in Figure 5.1. The React (Go-HPR and Hold (0-700ms from HO) periods were each divided into two segments of equal duration (Early and Late). We subtracted average baseline activity across conditions within each task, and soft-normalised firing rates (division by maximum absolute rate across execution and observation in the imitation block + constant factor 5). For visualisation of population averages, net negative activity (below baseline) was rectified to avoid the cancellation of facilitation and suppression effects. Epoch activity during basic observation and imitation
5.4. Experiment 1: Observation for imitation

for observation were compared across the population via Pearson correlation. To assess the level of object specificity during the two types of observation, we took the absolute difference in normalised activity between PG and WHG for each epoch and unit, and then averaged these values across the population.

5.4.3. Results

In M48, we recorded 63 units which modulated their activity during action execution, and 50 of these (79.4%), were classed as MirNs (45 for PG, 38 for WHG). In M49, 54 of 141 execution-modulated units (38%) were MirNs (38 each for PG and WHG).

Figure 5.2 shows responses of neurons recorded in M48 during cued (basic) and imitation execution and observation conditions. Both M1-PTN MirNs (A & B) showed highly similar activity profiles across basic and imitation observation. (A.) exhibited an increase in activity after experimenter HPR followed by sustained hold period activity above baseline, while (B.) showed an initial increase during the object cue period, followed by a further increase peak before grasp, before gradually returning to baseline. At the end of imitation observation and the appearance of the Go cue, the first M1-PTN (A.) immediately ramped up activity for imitation execution (left panel), and this activity was greater for both grasps compared to basic execution activity. The second M1-PTN, shown in Figure 5.2B, showed small differences in activity between basic and imitation execution, with a slight increase during the early hold period in basic PG and during the lead-up to the Go cue for basic WHG. The unit was most active during the reach and grasp stages of WHG, reaching up to 50 spikes s⁻¹, and this activity was very similar for basic and imitative execution. Finally, the F5 unit in Figure 5.2C exhibited a slightly increased rate of firing during the reach and grasp periods of imitation execution compared to cued execution. During observation, firing rate during all conditions peaked just prior to grasp, but was relatively reduced for imitative observation relative to observation in the basic task.
5.4. Experiment 1: Observation for imitation

Figure 5.2. Example neural responses during observation for imitation. Responses of three recorded units in M48 during execution (left), and observation (right). (A & B). PTNs in M1, C. F5 unit. Basic execution and observation are shown in red and blue for PG and WHG, as previously. Imitation execution and observation are shown in darker shades (PG-imi and WHG-imi). Colours for event markers for single trials and median times on histograms are as indicated previously, and in Figure 5.1. Average times for Go, HPR, HO, HOFF, and HPN appear separately for the basic and imitation tasks. Note that imitation observation in the right panels, and imitation execution in the left panels partly overlap e.g. the high firing at the start of imitation execution in (A, left panel) shows the same activity as during imitation observation in the right panel.

Figure 5.3 & 5.4 show the population averages, for M48 and M49, respectively, of MirN activity in three sub-populations during all conditions, for PG and WHG. Overall differences in activity between the two observation conditions were small and inconsistent.
5.4. Experiment 1: Observation for imitation

Figure 5.3. Population activity in M48 during basic observation and observation for imitation tasks for three different MirN sub-populations. Left panels PG, right panels WHG. “Basic” refers to the basic mirror task, “Cued” and “Imitate” refer to the conditions in Experiment 1 (see subsection 5.4.1 for details). * indicates significant difference between Exe, Imitate activity and other execution conditions at Late React stage in F5 (Wilcoxon sign-rank tests, p < 0.05). Note that the ObjCue period in the Exe, Imitate trace corresponds to the pause period before the Go cue to imitate.
5.4. Experiment 1: Observation for imitation

Figure 5.4. Population activity in M49 during basic observation and observation for imitation tasks for three different MirN sub-populations. Left panels PG, right panels WHG. All plotting conventions are the same as Figure 5.3. In M49, most neurons in the observation for imitation task were only recorded during observation in the basic task, so no Exe, Basic trace is presented.
5.4. Experiment 1: Observation for imitation

Execution activity in the basic task and cued condition in the imitation task was also very similar (Figure 5.3), although in M48, activity during imitative execution was greater than either of the other execution conditions, and this difference was significant during the Late React period (Wilcoxon sign-rank test, $p < 0.05$). A direct comparison of the modulation in the two observation conditions confirmed that population activity in the two conditions was highly correlated for both grasps (Figure 5.5). Given that the overall level of activity in the basic observation and observation for imitation conditions appeared very similar, we next assessed the absolute differences in activity between the objects in the two conditions (Figure 5.6). During basic observation, object differences evolved during the ObjCue and React periods, and remained at a similar level or decreased during reach and hold. During imitation observation, object differences remained small initially, when the object was not known to the monkey, and only appeared during the early Hold period in M48, once the object for the current trial could be unequivocally identified from the experimenter’s movement (Figure 5.6, bottom panels), but were never noticeably larger than those during the basic task. Execution in the basic mirror task, and both cued and imitation execution in Experiment 1, all showed similar profiles of object selectivity (Figure 5.6, top panels). Finally, we examined the gaze patterns of the monkeys in this imitation task, to assess whether the requirement to imitate encouraged the monkeys to pay greater attention to the experimenter’s actions (Figure 5.7). Both monkeys gazed at the target object at chance levels during the object cue period, and showed some tendency to divert gaze towards the target object around the time of observed grasp during the imitation observation condition. However, the overall likelihood of gaze at the target object at this time was not different from gaze during the basic task (Figure 4.2, p.128). Despite this, prior to the end of the experimenter’s return to the homepad (HPN), and during the subsequent pause period, probability of gaze towards the target object rapidly increased, indicating that the target object had been correctly inferred from the observed action, as expected since error rates were low. Gaze patterns during cued and imitative execution were generally similar, although the proportion of trials with persistent gaze at the time of grasp was higher for imitation, compared to cued, execution in both monkeys (Figure 5.7B).
5.4. Experiment 1: Observation for imitation

Figure 5.5. Net normalised activity of each MirN in M48 during basic observation and observation for imitation Reach and Early Hold periods, shown for PG (top) and WHG (bottom). Correlation coefficients and corresponding p-values were calculated across all three sub-populations.

Figure 5.6. Absolute difference in activity between objects during basic observation and observation for imitation at different task stages, in M48 (left), and M49 (right). Execution, top panels; observation, lower panels. Colour conventions as in Figure 5.3 & 5.4.
5.4. Experiment 1: Observation for imitation

5.4.4. Discussion

Imitation is a key component of social behaviour and learning in non-human primates and humans, and its neural implementation has previously been linked to the MNS and related areas (Ferrari et al., 2009; Iacoboni, 2009). In this experiment, we tested whether the activity of MirNs is modulated by the requirement for monkeys to imitate the observed action. Overall, we found few differences between MirN activity during simple action observation, and action observation for imitation, both in terms of overall firing rates, and the difference in activity between different objects. This contrasts with previous fMRI studies, which found a relative increase in activity during observation for imitation vs. observation with no subsequent action (Iacoboni et al., 1999; Buccino et al., 2004). On the other hand, a comparison of changes in CSE during observation for attention and observation for imitation noted facilitation in the former, but not the latter condition (Hardwick et al., 2012), suggesting an increased inhibitory influence in the imitation condition in order to suppress premature movement.

In M48, we found that MirNs, particularly in F5, showed a somewhat higher level of activity during imitation execution than execution cued by object-specific cues. This was most apparent during the reaction period, prior to any movement, although eye movement recordings indicated that persistent target object gaze was higher for imitative execution at the time of grasp. Evidence from fMRI and MEG also indicates that areas of the human MNS are more strongly activated during imitative, rather than non-imitative or symbolically cued, action execution (Iacoboni et al., 1999; Nishitani & Hari, 2000). Importantly, our experiment did not involve imitating actions while they were being observed, but relied on the monkey recalling the appropriate object from working memory after the delay period (since no explicit object cues were available), which is more similar to the design of Buccino et al., (2004), in which a pause period separated the observation of actions and subsequent imitation. Speculatively, the increased activity in F5 in the lead-up to imitative execution could therefore reflect activation of the appropriate motor program for the upcoming performed grasp.

Our experimental design was a simple stimulus-response paradigm, involving actions which were complex in nature, but which the monkeys were already well trained to
perform. As such, it did not require learning in the manner of true, intentional imitation (Buccino et al., 2004; Bien et al., 2009). The MNS has been hypothesised to underlie automatic, neonatal imitation (Ferrari et al., 2009), based on the direct matching of observed and executed motor acts within the observer's motor system, as well as being involved in a more indirect, acquired pathway for imitation, which includes interactions with prefrontal regions encoding action goals and contexts (Ferrari et al., 2009). As this latter pathway develops, the requirement to suppress automatic imitation increases, and this may be subserved by a reshaping of the MNS and the emergence of suppression responses to action observation. The possible role of MirNs in acquired imitation is also aligned with associative learning accounts of MirN development, since brief sensorimotor experience can increase or decrease the imitative compatibility effect, in which reaction times are shorter for the imitation of compatible, rather than incompatible actions (Brass et al., 2001; Heyes et al., 2005; Press et al., 2007).

Figure 5.7. Eye movements during observation for imitation. (A). Likelihood of gaze on target object in M48 (left) and M49 (right). (B). Proportion of trials for each condition in which the monkey's gaze (M48 (left) and M49 (right), fell within the target object window for a period of at least 120ms in different 300ms epochs during the task (mean±SEM, 4 sessions)
5.4. Experiment 1: Observation for imitation

Given the evidence for the role of MirNs in imitation in the human literature, it is somewhat surprising that we did not find any systematic difference in activity between basic observation and observation for imitation in our task, although there are a number of plausible explanations for this. Differences between human and monkey MirNs, and species-comparative differences in imitative behaviour, may account for some of the overall lack of effect we observed. Although this possibility cannot be ruled out and may be worthy of further exploration, the simple nature of the task, which did not require true imitation, argues against this. If one assumes that MirN activity was involved in extracting the necessary information for this task, then it may have done so equally well in the basic observation paradigm, even though the monkey did not have to use the information in that scenario. A corollary of this is that the observation for imitation task was not sufficiently cognitively challenging as to be treated differently by the MNS. This could have been because overtraining minimised any original differences between the two forms of observation, because there were only two objects, or because the monkeys had at least 1s during the experimenter grasp and hold period in which to extract the information needed to repeat the grasp. This latter point could also explain the eye movement results - although the gaze towards the target object increased at the point of grasp in the imitation task once its identity was clear, the overall dwell likelihood and proportion of look trials were not substantially different from the basic task. A number of possible modifications to the paradigm could address these issues, and increase the chance of observing a modulatory effect on MirN observation activity by the requirement to imitate. Providing information for a shorter period of time, increasing the number of objects, or requiring monkeys to grasp the same objects in different ways depending on which grasp they had just observed would likely increase the chances of observing grasp or object-related differences in MirN activity. An increase in the cognitive difficulty of the task would also eventually increase the proportion of trials in which the monkey grasped the incorrect object (which were low in the current data). This would then enable a comparison of neural activity on correct and error trials, which could provide further insight into the neural bases of imitation at the single-cell level.
5.5. **Experiment 2: Observation during action execution**

5.5.1. **Experimental task**

Two types of trial were presented to the monkey. On execution-only (“single-hold”) trials, monkeys were presented with the same series of LED cues as during execution trials in the basic task, and made reach-grasp and hold movements after the green LED, using their right hand. The target object was always the sphere. On “double-hold” trials, trials initially began in the same way, with the monkey cued to make a reach-to-grasp movement towards the sphere. Once the object was displaced, the experimenter received a go cue (not visible to the monkey), and made a similar reach-to-grasp movement to the trapezoid object, using their right hand. The experimenter displaced the object into the hold window, before releasing and returning to the homepad. The monkey release their hand after the experimenter, and also returned to the homepad. Figure 5.8 shows the events in the task sequence on double-hold trials.

![Figure 5.8. Active observation trial schematic.](image)

*Figure 5.8. Active observation trial schematic.* Schematic of a double-hold trial, showing object displacement signals for the monkey (top) and experimenter (bottom). Epochs 1-5 used for analysis are shown below the experimenter trace. The monkey executed reach-to-grasp and hold movements as previously described in the basic mirror task. On some trials, the experimenter received a GO cue at monkey hold onset (HO), and briefly grasped and held the other object. Experimenter reach and hold periods roughly overlapped with the middle and late stages of the monkey hold (epochs 4 and 5). On single-hold trials, the monkey performed the same action, and the experimenter did not move.
5.5. Experiment 2: Observation during action execution

5.5.2. Data analysis

Units were recorded for at least 10 observation trials in the active observation task, and 10 trials during passive observation of PG. Spike counts during both the basic and active observation tasks were computed within five task epochs (Figure 5.8), as follows; (1) LCDon-ObjCue (Baseline), (2) HPR-DO (Reach), (3-5) 0-1.05s from HO, split into three non-overlapping bins of 350ms (Early, Mid, Late Hold), and converted to firing rates by dividing by epoch length. For active observation trials, firing rates were also computed in the intervals between experimenter HPR and DO (Reach 2), and experimenter HO-HOFF (Hold 2). The monkey’s mid-hold period overlapped in timing with the experimenter’s reach (mean±SEM experimenter HPR across sessions: 313±32ms after monkey HO), while the monkey’s late-hold period overlapped with the experimenter’s hold (mean±SEM experimenter HO across sessions: 884±83ms after monkey HO). Firing rates during the basic observation task were subjected to a one-way ANOVA (factor Epoch with 5 levels), followed by post-hoc tests with Tukey-Kramer correction for multiple comparisons. Neurons were considered modulated for a given condition if the ANOVA produced a significant effect, post-hoc tests demonstrated a significant effect during any of epochs 2-5 relative to Baseline, and if there was a minimum firing rate range of 5 spikes s⁻¹ across WHG execution and PG observation conditions. We selected these two conditions because they were most relevant for comparison to the active observation task, in which monkeys always executed WHG actions and observed PG actions. A neuron was defined as an MirN if it was significantly modulated during both of these conditions in the basic observation task.
5.5. Experiment 2: Observation during action execution

To assess population activity in the basic and active observation tasks, we averaged firing rates in each interval across trials. Task activity was baseline-corrected by subtracting the average activity across conditions (WHG execution and PG) during Baseline, and soft-normalised (divided by maximum absolute across conditions + a constant factor 5). Baseline activity and the normalisation factor were calculated based on basic task activity. For the active observation task, we also extracted observation-related activity as the difference between single- and double-hold activity during each epoch, and normalised this by the same normalisation factor. The relationship between passive and active observation activity was assessed via Pearson correlation. Specifically, we compared activity during the reach period in passive observation to that of the mid-hold period in active execution and activity during early hold in passive observation with that during the late-hold period in active observation. We also directly compared the reach and early hold periods during passive observation to the equivalent reach and early hold periods during
5.5. Experiment 2: Observation during action execution

active observation (i.e. aligned to the experimenter movement). This analysis was performed across all units modulated during action execution, and also for each neuronal sub-population separately (M1-PTN, M1-UID, F5).

![Graph A](image1)

![Graph B](image2)

**Figure 5.10. Population activity during active observation.** (A). Mirror neuron population activity (n=12) during active observation. Net normalised activity was rectified to examine absolute difference in firing rate between execution (single-hold), and observation (double-hold). (B). Same as (A), but for all execution-modulated units, split by cell population.
5.5.3. Results

We recorded 36 units in M49 which modulated their activity during action execution (10 M1-PTNs, 11 M1-UIDs, and 15 units in F5). 12 of these units (33.3%) also modulated during passive observation, and were therefore considered as MirNs. Figure 5.9 shows raster and histogram plots for two example units (top: M1-PTN, bottom: F5), recorded during active (left panels), and basic (right) observation tasks. Both units had a steady, low baseline firing rate. The M1-PTN increased its activity after HPR on WHG execution trials, peaking at grasp, before a second peak around HOFF. During passive observation, the unit showed a steady increase in firing during the hold period, which was relatively small in amplitude. Activity on single- and double-hold trials was largely similar, although double-hold trials showed a brief increase in activity after experimenter HPR, when the monkey began to observe movement of the experimenter hand towards the trapezoid object. The F5 neuron had a large single peak in activity around HPR during execution, and weak, broad modulation during passive PG observation. During active observation, there was a substantial additional peak during observation of the experimenter’s reach (∼30 spikes s⁻¹) above the level of hold activity on single-hold trials. In both examples, the weak mirror activity seen during passive observation appears to be superimposed to varying degrees upon execution activity during the active observation tasks, once the single- and double-hold trials diverged behaviourally at the point of experimenter HPR (note that the experimenter hold period during double-hold trials was curtailed - see Figure 5.8, which accounts for the more dynamic peak in observation-related activity during active observation).

The population average of the 12 MirNs demonstrated similar levels of activity in the single- and double-hold conditions (Figure 5.10A), and splitting all execution-modulated units into the three sub-populations also showed that single- and double-hold modulation profiles were highly overlapping (Figure 5.10B). As seen in the single-neuron examples, activity during the middle of the execution hold (approximately the reaching period for the experimenter’s action) on double-hold trials was marginally greater than the equivalent period on single-hold trials, and this was particularly the case in F5, although this difference was not significant (Wilcoxon sign-rank test, p > 0.05). To assess the relationship between passive and active observation modulation in more detail, we correlated normalised activity during the reach and early hold periods in the passive
5.5. Experiment 2: Observation during action execution

observation condition, with the net activity during active observation (after subtraction of activity during single-hold), in the middle and late stages of the monkey hold (roughly corresponding to experimenter reach and hold periods) (Figure 5.11). Across all units, there was a significant correlation between modulation in passive and active observation in these two periods ($r = 0.61$, $p < 0.0005$, and $r = 0.33$, $p = 0.047$, Figure 5.11A). After splitting by population, there was a significant correlation between modulation in passive and active observation at the mid-hold and late-hold periods in F5 ($r = 0.80$, $p < 0.0005$, and $r = 0.59$, $p = 0.02$). M1-PTNs showed negative correlations, although correlations were not significant in either M1 population ($p > 0.1$) (Figure 5.11B). Correlation values were qualitatively similar when comparing passive observation to activity in the experimenter reach and hold periods (Reach2 and Hold2, F5; $r = 0.76$, $p = 0.001$, and $r = 0.63$, $p = 0.012$, respectively). To assess whether M49 actively attended the experimenter’s action on double-hold trials, we monitored eye movements, and quantified gaze behaviour, both at individual timepoints, and in terms of persistent gazing within defined task epochs (“look” trials). Figure 5.12A and B shows the dwell likelihood and look proportions across the trial on the target object aligned to the monkey’s movement (grey and orange), and on the experimenter object aligned to the experimenter movement on double-hold trials (purple). The gaze behaviour on single- and double-hold trials was largely similar, and M49 only showed an infrequent tendency to gaze at the experimenter object around the times at which the experimenter grasped or released the object. It was also apparent that the monkey exhibited a weak tendency to return gaze to his own object (which was always the “target” object) during the double-hold periods (bump in orange trace in Figure 5.12B around 1.3-1.4s).
5.5. Experiment 2: Observation during action execution

5.5.4. Discussion

In this experiment, we tested whether observation of action under circumstances, where the motor system was already engaged in producing another action, induced additional modulation in F5 and M1 neurons. The activity of individual units was largely similar in profile when the monkey executed an action alone, or when they also observed the experimenter grasping an object - in both cases, execution-related activity predominated. Small increases in absolute modulation during the middle and late hold periods on
5.5. Experiment 2: Observation during action execution

double-hold trials, corresponding to the period when the monkey observed the human experimenter’s movement were largely driven by changes in activity in F5 neurons, consistent with the greater proportions and levels of mirror activity in F5 compared to M1 (Gallese et al., 1996; Kraskov et al., 2014; Mazurek et al., 2018). Indeed, when we compared the degree of modulation in neurons recorded during both passive and active observation (after subtraction of execution activity), F5 showed the clearest positive correlation between the two, particularly during the middle of the hold period, when the monkey observed the human experimenter reaching towards the other object.

Figure 5.12. Eye movements during active observation. (A). Dwell likelihood at each timepoint during trial sequence, aligned to multiple task events. Exe,Targ: Single-hold execution, target (monkey, sphere) object; Obs,Targ: Double-hold execution, target object, Obs, Oth: Double-hold execution, other (i.e. experimenter, trapezoid) object. Target object traces are aligned to monkey actions, whereas other object dwell likelihood is aligned to experimenter events. Mean±SEM, 4 sessions. (B). Proportion of trials for each condition in which monkey gaze fell within target object window for at least 120ms in 300ms task intervals. Colour conventions as in (A).

Previous studies in the human literature have demonstrated a modulatory effect of action observation on self-action performance and perception (Brass et al., 2001; Kilner et al., 2003; Hamilton et al., 2004). For example, observation of compatible finger movements shortened reaction times, compared to incompatible movements, for execution of the same response (Brass et al., 2001; Heyes et al., 2005; Press et al., 2007). In our study, action observation was overlapping with execution but started afterwards, and this design was implemented so that observation occurred while EMG and unit activity was relatively stabilised in the hold period, and changes in firing rate related to observation could be assessed more easily. During simultaneous execution and observation, Kilner et al., (2003) reported that observation of incongruent (perpendicular) arm movements while performing
one’s own (e.g. observing movements in the up/down plane while performing left-right movements), led to an increased variance in fingertip positions. This was not apparent for congruent movements, or for incongruent movements performed by a robotic, rather than human agent (Kilner et al., 2003). Based on these results, the MNS was hypothesised to underlie the neurophysiological substrate for this “motor contagion”, or interference effect, since the motor program for the observed action will become incorporated into the motor output (Blakemore & Frith, 2005). On the other hand, a contrast effect was found in a perceptual test during simultaneous execution and observation, in which subjects lifting heavy objects judged objects they observed being lifted to be lighter, and vice versa (Hamilton et al., 2004). In our study, the effects of simultaneous observation on M1 cells was more limited than in F5, and less comparable to modulation under during passive observation. Although seemingly in contradiction with the findings of motor contagion in human studies, given M1’s proximity to the motor output, the requirement in our task to maintain a relatively stable hold may have suppressed potential effects at the level of M1 and the CS output. Large arm movements may also be more susceptible to variation based on congruency with observed actions, since precision is not a key requirement, and observation in this design occurs during the dynamic stage of action execution. In the context of precision movements, it may be particularly important for changes in activity in M1 and the motor output to be minimal during simultaneous execution and observation in order to avoid disruption of the ongoing motor program, which would have a negative effect on behaviour. During passive observation, motoneurons are below threshold, and the system state is therefore less vulnerable to disruption by action observation-related activity.

The present dataset was limited in size, which meant we were not able to analyse the activity of MirNs exclusively in detail during the different conditions. In addition to obtaining an expanded dataset, future recordings should compare activity during active observation across congruent or incongruent actions in order to gain a better understanding of how multiple simultaneous actions are represented in the MNS as they are being executed and observed. It may also be useful to compare activity in situations where execution of movement occurs after (as in Experiment 1), or in synchrony with, observed compatible or incompatible movements, as this design may offer additional avenues for observing potential behavioural effects of congruency, which can then be linked with functional properties of MirNs.
5.6. Experiment 3: Observation under different visual conditions

Eye movement recordings indicated that the experimenter’s action drew relatively little attention from the monkey. Although mirror activity does not necessarily require ongoing visual input, the gaze results contrast with previous work in humans, which has identified proactive gaze during action observation (Flanagan & Johansson, 2003), which is reduced if observing incongruent movements (Costantini et al., 2012). This may partly be due to monkey v.s. human differences, and M49, the monkey used for this experiment, showed little tendency to observe the human grasp on passive observation trials in the basic task (Figure 4.2). The observed action may draw more gaze attention if the monkey’s own hand is obscured from view, or takes place before the monkey’s action, as in Experiment 1.

Overall, the results of this experiment provide preliminary evidence that, in situations where actions are concurrently performed and observed, M1 may attenuate mirror responses to the observed action to prevent possible contamination of the motor output, whereas F5, given its less direct influence over the spinal cord, may be more flexible in maintaining multiple representations related to both executed and observed actions.

5.6. Experiment 3: Observation under different visual conditions

5.6.1. Experimental task

This task was embedded within the basic mirror task, and has been described previously (see chapter 4). The monkey performed interleaved execution, observation, and NoGo trials. On all trials, the LCD screen became transparent at LCDon. Observation trials were divided into three subtypes - “Visible”, “Hidden Grasp”, and “Hidden Cue&Grasp”. On Visible observation trials, the LCD screen remained on until HPN. On Hidden Grasp trials, monkeys viewed object and go cues, but the LCD screen became opaque 150ms after HPR, obscuring the observation of prehension of fingers and the final grasping action. On Hidden Cue&Grasp trials, no object or Go cues were visible to the monkey, and the screen became opaque at the Go cue. Sound cues related to the hold period and end of the trial were maintained for all trial types. Units were recorded for at least 10 trials per grasp in the Visible Observation and Hidden Grasp conditions.
5.6. Experiment 3: Observation under different visual conditions

Figure 5.13. Example neural responses during visible and hidden observation. Activity during execution (left panels) and observation (right panels) under different visual conditions in an M1-PTN, and two F5 units. Black: Execution, Orange: Visible observation, Purple: Hidden Grasp, Green: Hidden Cue&Grasp. Firing rates are aligned to DO. Top sub-panels are displayed in raster form, with event markers for individual trials. Lower sub-panels show corresponding PSTH (firing rates in 20ms bins, boxcar-smoothed with 400ms moving average). Vertical lines denote median time of events relative to DO, colour codes as in previous figures.
5.6. Experiment 3: Observation under different visual conditions

5.6.2. Data analysis

Neural activity during action execution, visible observation, and NoGo conditions has been presented and analysed in chapter 3. Here, we therefore focus on comparing activity across the different observation conditions. As the object and Go cues was not presented to the monkey on Hidden Cue&Grasp trials, firing rates during this time often diverged from those in the other two conditions. For this analysis, we therefore considered the 500ms period before the Go cue as a condition-specific baseline, which we could then use to compare with activity during the visible or obscured stages of the reaching and grasping action. For each condition, firing rates were initially computed within three task epochs 1.) 0.5s period before the Go cue, 2.) 150ms after HPR until DO, 3.) 500ms from HO, and subjected to a 1-way ANOVA (factor EPOCH with 3 levels), followed by post-hoc tests with Tukey-Kramer correction. Neurons were considered modulated for a given condition if the ANOVA produced a significant effect, post-hoc tests demonstrated a significant effect during reach or hold relative to Baseline, and if there was a minimum firing rate range of 5 spikes s\(^{-1}\) across execution and visible observation conditions. A neuron was defined as an MirN if it was significantly modulated during execution and visible observation for the same object.

For analysis of population activity, we again calculated firing rates in an extended number of epochs, as follows: 1.) ObjCue, 0.5s period before the Go cue, 2.) React (GO-HPR) (3.) Reach (HPR-DO), 4-6.) Hold (0-1.05s period from HO, divided into 3 segments of equal duration (Early, Middle, Late, 0.35s each)). The average activity during Baseline was subtracted for each condition, and the resulting net firing rates were normalised to the maximum absolute value across all conditions. To compare the magnitude of modulation in different observation conditions at the population level, negative net firing rates were rectified. For react, reach and early hold epochs, we compared the differences in firing rates between each pair of observation conditions via Wilcoxon sign-rank tests, with Bonferroni correction for multiple comparisons (9 comparisons).
5.6. Experiment 3: Observation under different visual conditions

Figure 5.14. Population averages of MirNs activity for PG (left), and WHG (right). (A). All MirNs together. Execution, black; Visible Observation, orange, Hidden Grasp, purple, Hidden Cue&Grasp, green. (B). MirNs split by neuronal sub-population. Colour conventions as in (A).

5.6.3. Results

In this experiment, we recorded 90 units which modulated their activity during action execution involving at least one of the two objects. 45 of these (50%) showed significant modulation during the normal (“Visible”) action observation condition, and were classed as MirNs (30 and 32 for PG and WHG, respectively). 40/90 (44.4%) were modulated during Hidden Grasp, and 6/90 (6.7%) were modulated during HiddenCue&Grasp.
5.6. Experiment 3: Observation under different visual conditions

Figure 5.13 shows the activity of three neurons during execution, and during the three observation conditions, for WHG. The first unit, an M1-PTN, increases its level of activity after the execution Go cue, maintaining a sustained level during the reach and early hold, before reducing back to, or below, baseline. In all three observation conditions, there was a minor, sustained increase in activity, which was slightly greater for the Visible observation condition. During execution, the first F5 unit (middle row) shows a gradual decrease in firing from ObjCue until the early hold period, before increasing activity towards 15 spikes s\(^{-1}\) in the later hold and return periods. During observation, the initial gradual decrease in activity is followed by increased firing after the GO cue in Visible and Hidden Grasp conditions, and activity remains high throughout the reach and hold periods, before falling back to baseline. In the Hidden Cue&Grasp condition, the neuron also increases its firing in a similar manner, although this activity starts earlier. The second F5 unit has a clear peak in execution activity just after HPR. In observation, Visible and HiddenGrasp conditions show similar modulation profiles, peaking just before DO, with a more sustained increase in the Visible observation condition. Hidden Cue&Grasp produced a small level of modulation, which was reduced relative to the Visible observation condition in particular.

Figure 5.14A shows the population averages for all MirNs (classified in the visible observation condition), for each object separately. Figure 5.14B shows the same data split into the three neuronal sub-populations. Execution activity for both grasps, particularly during reach, is generally larger than observation activity, particularly in M1 populations. Within the observation conditions, modulation was largest in the visible observation condition, with Hidden Grasp often of a similar, or slightly reduced amplitude. Modulation during Hidden Cue&Grasp was present, but at the population level this was reduced compared to the other observation conditions during most epochs and across sub-populations. We also compared the absolute difference in activity between observation conditions at different stages of the task (Figure 5.15). During the React epoch, after the screen had become opaque in the Hidden Cue&Grasp condition, the difference between Visible and Hidden Grasp activity remained small (red bars), whereas the Hidden Cue&Grasp condition showed a greater difference in activity with either of the other two conditions (Wilcoxon sign-rank test, \(p < 0.05\)). Other differences were not significant.

Recordings of the monkey’s eye movements provided strong confirmation that the object
5.6. Experiment 3: Observation under different visual conditions

could not be inferred on Hidden Cue&Grasp trials, since the monkey did not gaze within the target object window higher than chance, but consistently gazed at the correct object during other trial conditions (Figure 4.2, M49). Additionally, the gaze patterns during Visible and Hidden Grasp conditions were qualitatively similar for most of the trial, although M49 gazed more regularly at the target object during grasp on Visible trials.

![Figure 5.15. Differences in activity between visible and hidden observation conditions.](image)

**Figure 5.15. Differences in activity between visible and hidden observation conditions.** The difference in normalised activity during react, reach, and early hold epochs was calculated for each unit. Data shown as mean±SEM across units. Visible-Hidden Grasp, red; Visible-Hidden Cue&Grasp, blue, Hidden Grasp - Hidden Cue&Grasp, black. The difference between Visible and Hidden Grasp was significantly smaller than the differences of either condition to Hidden Cue&Grasp for both grasps (sign-rank test, p < 0.05).

5.6.4. Discussion

In this experiment, we assessed the activity of MirNs when the visual and contextual information available to the monkey about the observed grasps was altered on a trial-by-trial basis. We found that MirNs tended to fire in a similar way under full vision observation and Hidden Grasp observation. In both cases, the monkey received informative cues about the object for the upcoming grasp, although the actual prehension of the fingers around the object was obscured in the latter case.

Activity during the Hidden Cue&Grasp condition, where monkeys received no visual or
5.6. Experiment 3: Observation under different visual conditions

contextual cues about the upcoming action, was more distinct from the other two conditions, although some neurons still showed some modulation in their firing over the course of the trial. We speculate that these modulations could have arisen due to the well-constrained trial structure of the task, and because the sound cues and overall movement of the experimenter’s body meant that the well-trained monkey could certainly still identify general features of the experimenter action. A modification of this task could therefore assess the activity of MirNs when even the sounds are not provided, or the experimenter does not actually perform a grasping action.

As previously discussed, MirNs in F5 continue to fire as long as the monkey knows that a grasping action is taking place, even if vision of the actual prehension is obscured (Umiltá et al., 2001; Kraskov et al., 2009). Similarly in humans, CSE can be modulated during occluded grasping actions (Villiger et al., 2011; Valchev et al., 2015), in a muscle-specific manner, and this modulation depends on the provision of contextual cues regarding the upcoming action (Cretu et al., 2019). A number of other studies have examined anticipatory changes in MNS activity by varying the cues provided for upcoming full vision observation, and these studies have also found that mirror activity proceeds on the basis of contextual cues, and is then updated based on kinematic cues (Cavallo et al., 2013; Janssen et al., 2015; de Beukelaar et al., 2016). Altogether, these results, and the modulation profiles we observed in this study, fit within a predictive coding framework, whereby MirN activity is not simply a feedback response to visual input of actual action kinematics, but is also strongly influenced by predictions, or inferences, regarding the evolving observed action (Kilner et al., 2007).

In the present data, the overall level of observation activity was somewhat lower in M1, consistent with previous work (Vigneswaran et al., 2013), but we did not observe any systematic differences between the different neuronal sub-populations in terms of distinctions between the observation conditions. Although a more thorough statistical assessment of this will require a larger dataset, these preliminary results suggest that contextual information in the absence of specific kinematics (i.e. the object cue provided in the Hidden Grasp condition), is sufficient to modulate M1 activity in a comparable way to during full vision observation, where contextual cues and kinematics are combined.

Overall, this study confirms and extends previous results showing that mirror activity is
not wholly dependent on ongoing visual input, probably because contextual and observed 
kinematic cues are combined to predict the likely consequences of observed actions (Kilner 
et al., 2007; Janssen et al., 2015; de Beukelaar et al., 2016; Cretu et al., 2019), and this is 
reflected in the activity of MirNs.
6. Corticospinal excitability during action observation

6.1. Abstract

In humans, transcranial magnetic stimulation (TMS) has shown that action observation and withholding modulate corticospinal excitability (CSE), but modulation in these two conditions has never been directly compared. Additionally, observing an action while executing one, a common real-life situation, has been shown to affect behaviour, but the physiological correlates of this have not been examined. In a first experiment, CSE was assessed while fourteen subjects passively observed or withheld reach-to-grasp actions. TMS was delivered over left M1 and MEPs were recorded in three hand muscles. This revealed little significant grasp-specific or general modulation, with the clearest effect being a general increase in excitability relative to a pre-task, but not inter-trial, baseline. Seven subjects were tested in a block without the NoGo condition, and exhibited muscle-specific modulation of CSE, suggesting that the context of the task shifted with the inclusion of the NoGo condition, and this may have played a role in determining the level of CSE modulation during action observation. In a second experiment, CSE was assessed while subjects simultaneously executed and observed grasping actions. We hypothesised that simultaneously observing an action while executing one might modulate CSE beyond modulation during execution only, and do so differently depending on whether the observed action was congruent or incongruent with the one being performed. The results from this experiment were inconclusive, however, as there was little reliable modulation in either condition, although this may suggest that motor output pathways are able to effectively suppress any superimposition of observation-related modulation if a motor program is already ongoing.
6.2. Introduction

In the previous chapters, I examined functional properties of MirNs at cortical and spinal levels in macaque monkeys. In humans, direct recording of MirNs is rarely possible, so transcranial magnetic stimulation (TMS) has proved to be a useful non-invasive method for probing comparative changes in CSE during action observation. Numerous studies have reported muscle-specific increases in the amplitude of MEPs during the observation of grasping actions (Fadiga et al., 1995; Strafella & Paus, 2000; Gangitano et al., 2001; Alaerts et al., 2010a; Sartori et al., 2012; Gueugneau et al., 2015) indicating increased excitability within the motor system during action observation, in accordance with results at the cortical level (Gallese et al., 1996; Rizzolatti et al., 1996a,b).

At first glance, the literature suggests an established consensus on increased CSE during action observation. However, the magnitude, specificity, and time-course of this effect, almost 25 years after its first report, is still debated (see Naish et al., 2014, and subsubsection 1.4.2, p.52 for review). A balance of excitation and inhibition within the CST during action observation (Kraskov et al., 2009, 2014; Vigneswaran et al., 2013) may explain why some studies find negligible modulation, or suppression of CSE during action observation (Lago & Fernandez-del-Olmo, 2011; Villiger et al., 2011; Bunday et al., 2016; Hannah et al., 2018b). Action observation is typically studied in both monkeys and humans with subjects at rest, and TMS paradigms often explicitly instruct subjects to refrain from movement during the observation condition. Explicit or implicit task demands may affect the direction and magnitude of CSE modulation. TMS has also been used to study CSE during inhibition of movement (Sohn et al., 2002), but the relationship between suppression of movement during action observation and other forms of movement suppression has not been tested.

In addition, although visual information is typically available during action observation (although see Umiltá et al., 2001; Kohler et al., 2002; Caggiano et al., 2009; Caggiano et al., 2011), tactile and proprioceptive input is clearly very different between action execution and observation states. During normal behaviour, action observation rarely occurs in isolation, but is often closely followed by, or simultaneous with, execution of similar or complementary actions. The interaction between action execution and action
observation at both the behavioural and neurophysiological levels is therefore of relevance. Previously, the observation of compatible, but not incompatible, finger movements has been shown to confer a reaction time advantage for the execution of finger movements (Brass et al., 2001; Heyes et al., 2005). Observation of incongruous arm movements leads to increased variance in simultaneously executed hand and arm movements due to interference between the motor representations of the executed and observed movement (Kilner et al., 2003). Simultaneous observation and execution also induces a perceptual contrast effect, possibly due to proprioceptive input from the action being executed (Hamilton et al., 2004). Proactive gaze towards the predicted sites of hand-object interaction is a well-known phenomenon during action execution, and also occurs during action observation (Flanagan & Johansson, 2003). The level of proactive gaze is substantially decreased (relative to viewing in a rest condition) if a grasping action is observed that is incongruous with the one being executed (Costantini et al., 2012), suggesting that the recruitment of compatible motor representations supports this gaze behaviour. 1Hz repetitive transcranial magnetic stimulation (rTMS) disrupts participants’ ability to proactively track observed reaching actions when directed at PMv, but not STS, thus providing a causal link (Costantini et al., 2014). Natural motor behaviour during action execution therefore appears to be influenced by concurrent action observation, suggesting that observation activity at the physiological level can be superimposed upon, and possible interfere with, activity related to action execution.

Here, healthy human volunteers participated in two experiments to assess CSE during passive and “active” observation, and action withholding. In the first experiment, subjects executed or passively observed reach-to-grasp actions directed at two target objects, and TMS was delivered at different times to examine the time-course of possible grasp-specific modulation of CSE. To compare modulation during observed action onset to direct withholding of self-movement, on some trials, subjects were explicitly cued to withhold their action (NoGo). In a second experiment performed on the same day, CSE was measured during two action execution states, either when subjects performed a simple reach-to-grasp and hold action alone, or when they observed an experimenter grasping an object while their motor system was already engaged in the maintenance of grasp. The grasp performed by the subjects was either congruent or incongruent to the grasp they observed the experimenter perform.
6.3. Methods

6.3.1. Subjects

14 right-handed subjects (21-41 years old, 8 female) were recruited to participate in two experiments. All subjects were neurologically healthy, with normal or corrected-to-normal vision. They completed a screening test for contraindications to TMS and gave informed consent for their participation, but remained naive to the experimental aims. All experiments were approved by the UCL Local Ethics Committee.

6.3.2. Behavioural tasks

Subjects completed both experiments in the same session, and the order of the two experiments was pseudo-randomised across subjects. Both experiments used the same general experimental set-up described previously (subsection 2.1.3, p.70). Subjects sat opposite an experimenter, rested their hands on two homepads, and viewed two objects through the controllable LCD screen (Figure 6.1), which remained opaque in between trials. Prior to each experiment, subjects were briefly familiarised with the task in a short practice session. All subjects completed both experiments without reporting any discomfort related to the TMS.

Experiment 1

Objects were modified for human grasp (marble and sphere (Figure 6.1, inset), and afforded either PG via opposition of thumb and forefinger (marble), or WHG with all fingers encompassing the object (sphere). Execution, observation, and NoGo trial sequences have been described previously (subsection 2.1.3, p.70). On execution trials, subjects always used their right hand to grasp, rotate and hold the objects, and were instructed to attend the experimenter’s action on observation trials. Single-pulse TMS was delivered on individual trials at one of four time moments. (1) at LCDon (Baseline) on either execution or observation trials, (2) 350ms after the green LED on observation trials (across trials and subjects, this was just before experimenter movement onset (HPR)) (3) at experimenter DO (Grasp) on observation trials (4) 350ms after the red LED on NoGo
6.3. Methods

trials. 20% of trials were designated as catch trials, in which no TMS was delivered, and all trial types were interleaved in a pseudo-random manner. The proportion of execution trials was relatively low compared to the basic task performed by the monkeys (presented in chapter 3) to reduce the length of the session, but were still included to assess muscle recruitment during action execution, and to encourage subjects to remain attentive throughout the task in case they were required to move. To maintain attention, subjects completed the experiment in two shorter blocks, with \(~10\) MEPs per condition per block. To assess whether the presence of the NoGo condition may have influenced the implicit goal of the task and therefore also the modulation of CSE, 7 subjects completed an additional two blocks of the same task, which did not include a NoGo condition, but was identical in all other aspects. These “Go-only” blocks were always completed before the Go&NoGo blocks, and subjects were not made aware of the NoGo condition until these first blocks were completed. Prior to each block, a set of 20–30 baseline MEPs were recorded, with the subjects at rest.

Figure 6.1. Schematic of the experimental box set-up, which was largely similar to the set-up used in monkey experiments. In Experiment 1 and the incongruent block of Experiment 2, subjects were presented with a small marble and a large sphere (shown in inset). In the congruent block of Experiment 2, the large sphere was substituted with a second small marble.
6.3. Methods

Experiment 2

The general task and trial sequence has been described previously (section 5.5, p.161). Subjects performed two trial types. On execution-only (“single-hold”) trials, the LCD on screen became transparent, and after 0.25-0.45s, the marble object was cued via two amber LEDs. After a further delay (0.8-1.2s), a green LED acted as the Go cue for the subject to make a reach-to-grasp movement to the small marble, using their right hand. After rotation into the hold window and stable hold lasting 1s, subjects returned to the homepad. “Double-hold” trials began in the same manner, with subjects executing an identical cued reach-to-grasp movement to the small marble. When object displacement onset (DO) was detected online, the experimenter received a Go cue (not visible to the subject), and made a similar reach-to-grasp movement with their right hand towards the other object. Once the experimenter achieved object displacement, they released the object and returned to the homepad. Subjects were instructed to pay attention to the experimenter’s action (or lack thereof), while continuing to hold the small marble, and only release after the experimenter. TMS was delivered ∼850ms after subject DO on single-hold trials, and at experimenter DO on double-hold trials (typically 0.9-1.0 after subject DO). 20% of trials were again designated as catch trials, with no TMS delivered, and each block therefore consisted of roughly 50 trials, with 20 MEPs per condition. Subjects completed a “congruent” block, in which both subject and experimenter grasped identical small marbles using PG, and an “incongruent” block, in which the subject grasped the small marble in the same way, and the experimenter grasped the large sphere using a WHG (Figure 6.1, inset). The order of congruent and incongruent blocks was pseudo-randomised across subjects.

6.3.3. Recording

Throughout the task, EMG recordings were made from 3 right hand muscles, namely 1DI, AbPB, and AbDM, using surface electrodes mounted in a belly-tendon montage, with a ground electrode fixed to the bony part of the wrist. EMG signals were amplified (x1000), high-pass filtered (3Hz) (Neurolog EMG amplifier NL824, and Neurolog isolator NL820, Digitimer Ltd, UK), and digitised at 5kHz. These were recorded together with the timing of all task events and TMS triggers, homepad pressure, and object displacement signals (5kHz). All data was stored on a laboratory computer for offline analysis.
6.3. Methods

Figure 6.2. EMG activity on a single trial. Raw EMG activity for the three recorded hand muscles, with corresponding homepad and object displacement signals, on a single precision grip execution trial. **HPR**, homepad release; **DO**, displacement onset; **HOFF**, hold offset; **HPN**, homepad return; **IDI**, first dorsal interosseous; **AbPB**, abductor pollicis brevis; **AbDM**, abductor digiti minimi. Scale is the same for all three muscles.

6.3.4. Transcranial magnetic stimulation

Single-pulse TMS was delivered via a Magstim 200 stimulator attached to a standard figure-of-eight coil (70mm diameter). The coil was held tangentially to the scalp with the handle pointing backwards at a 45° angle to the midline, and the motor hotspot was identified as the position over left M1 eliciting the most consistent MEPs in the three muscles. Stimulator output was set to obtain MEPs averaging approximately 1mV in the 1DI muscle, either at rest (Experiment 1), or while the subject maintained contraction of the 1DI by rotating and holding the small marble (Experiment 2).

6.3.5. EMG and behavioural analysis

To visualise average EMG activity during the task, signals from each muscle were high-pass filtered (30Hz, 2nd order Butterworth), rectified, low-pass filtered (500Hz, 2nd order Butterworth), and smoothed with a 100ms moving average. They were then aligned to the Go or NoGo cue on correct trials, normalised, and averaged across trials. For Experiment 1, normalisation was performed to the 99th percentile across all correct trials and timepoints, within each muscle separately. For Experiment 2, normalisation was performed across muscles. For visualisation of trial-averaged homepad and object
displacement signals, signals were smoothed with a 100ms moving average, aligned to the Go or NoGo cue across trials, normalised to the 99th percentile across all trials and timepoints and relevant channels, and averaged across trials.

**Figure 6.3. Average EMG activity during task performance.** Single subject data, shown for PG and WHG execution separately, and across all observation and NoGo trials. EMG activity for each muscle was normalised across conditions. The peaks at LCDon, and in the Observation and NoGo EMG traces are due to rectification and averaging of MEPs. Coloured vertical bars and shading denote median and 25th-75th percentiles of task events, with colours as shown previously (Figure 2.1C). E-HP, experimenter homepad; S-HPl, subject left homepad; S-HPr, subject right homepad; PG, precision grip; WHG, whole-hand grasp.

**Experiment 1**

To quantify the extent to which subjects used each of the three muscles to perform the two grasps, EMG signals were high-pass filtered (0.5Hz, 2nd order Butterworth), rectified, and low-pass filtered (30Hz, 2nd order Butterworth). EMG activity in the -300 to +300ms period around DO was then averaged on all execution trials for each object. This interval was chosen to capture the dynamic period in which hand shaping and grasp occurred. For comparison across subjects with different levels of EMG, values within each muscle were
6.3. Methods

z-scored, and averaged for each object. The resulting values were statistically compared via 2-way repeated-measures ANOVA (rmANOVA), with within-subject factors of grasp (PG, WHG) and muscle (1DI, AbPB, AbDM), followed by post-hoc tests.

Experiment 2

For each subject, we estimated the primary muscle used in maintaining a stable hold during PG. EMG signals were high-pass filtered (0.5Hz, 2nd order Butterworth), rectified, and low-pass filtered (30Hz, 2nd order Butterworth). The average activity in the 300-800ms following DO, corresponding to a 500ms stable hold before any TMS pulse was averaged across trials separately for each TMS condition and congruency (block). For each subject, we then calculated a ratio of 1DI:AbPB EMG activity, such that values >1 indicate greater 1DI activation, and values <1 indicate greater AbPB activation. As a secondary analysis, we also assessed the level of variance in EMG activity during the hold period in different conditions. The variance in the same 300-800ms period was averaged across trials within subject for each condition, and then normalised to the variance on single-hold trials for congruent and incongruent blocks separately.

6.3.6. MEP analysis

MEPs in each experiment were extracted from raw EMG signals in the 15-35ms interval following TMS pulses on all correct trials, and the corresponding peak-to-peak amplitude was calculated. MEPs during both experiments were discarded from further analysis if: (1.) the peak-to-peak amplitude did not exceed 0.05mV, (2.) the peak-to-peak amplitude exceeded 3 standard deviations (S.D.) from the mean across all trials, (3.) the mean rectified EMG in the 100ms preceding the TMS pulse was more than 3S.D. above the mean across all trials. This procedure was performed independently on data from each muscle and subject.

Experiment 1

The AbDM of one subject was discarded from analysis due to a large number of weak responses. Across all other subjects and muscles, the number of discarded MEPs was 5.8±0.4 (≤4%). For comparison across subjects, MEP peak-to-peak values were
normalised to the average peak-to-peak value either of the intra-task Baseline (inter-trial interval (ITI)) responses, or pre-task baseline MEPs, within each subject. Normalised MEPs were averaged for each TMS condition and object. As responses during the task showed a strong facilitation relative to the pre-task baseline (see Figure 6.7), normalisation to the ITI baseline was used for all subsequent analyses. For “Go&NoGo”, and “Go only” blocks separately, MEPs were first compared via 2-way rmANOVA (within-subject factors TMS condition × grasp) for each muscle separately. Significant ANOVA results were followed up with post-hoc tests where appropriate, with Tukey-Kramer correction for multiple comparisons. To test for muscle-specificity, we computed an MEP ratio for each muscle and condition by dividing the average MEPs during PG by those during WHG, and subjected the results to a 2-way rmANOVA (within-subject factors TMS condition × muscle). Greenhouse-Geisser corrected p-values were used in cases of non-sphericity, as determined by Mauchly’s test.

Experiment 2

To examine the effect of concurrent observation and execution on CSE, MEPs peak-to-peak values in double-hold trials were normalised to the average peak-to-peak value across all single-hold trials, separately for congruent and incongruent blocks, and then averaged within these blocks for each subject. A value greater than 1 would indicate that simultaneous observation increases CSE above the level it is at during normal grasping, a value less than 1 would indicate a decrease in excitability, and a value equal to 1 would mean observation produces no additional change in CSE if the motor system is already engaged in action. For congruent and incongruent blocks, the average MEP values, and levels of EMG variance on double-hold trials were subjected to a one sample t-test using a comparison value of 1. As we were interested in whether any effect was specific to the congruency between the executed and observed actions, MEP values in the two blocks were further compared via a paired t-test. As three comparisons were performed, Bonferroni correction was applied ($\alpha = 0.05/3 = 0.017$).
6.3. Methods

**Figure 6.4.** Z-scored EMG activity in the three recorded hand muscles during the hand shaping and initial grasping period (±300ms around DO) of PG and WHG execution (mean±SEM, 14 subjects). **p < 0.01, ***p < 0.0001.

**Figure 6.5.** Raw traces of example MEPs in the 1DI muscle of one subject during observation of grasp. Stimulus artefact is at time 0, MEP onset latency is ~22ms.
6.4. Results: Experiment 1

EMG and behaviour

Figure 6.2 shows raw EMG, homepad, and object displacement signals from a single subject during a PG execution trial, while Figure 6.3 shows the normalised signals in the same subject, averaged across different task conditions. During PG execution, there was pronounced activation in both 1DI and AbPB muscles, beginning after movement onset, and decreasing rapidly after the release of the object. The AbDM on the other hand, was hardly recruited during PG, but showed a clear peak in activation during the hand opening phase of WHG trials, and some tonic activity during the hold period. In contrast to a simpler task, such as index finger abduction, muscle activity was not orthogonalised across the two grasping movements, and some 1DI and AbPB activity is apparent during WHG, although to a lesser extent than that seen during PG. Importantly, EMG activity during the action observation and NoGo conditions was negligible (Figure 6.3, note that the peaks in the traces (and those at LCDon in the execution traces) correspond to rectified MEPs). We calculated the relative activation of the three muscles for each object grasp (Figure 6.4), and a 2-way ANOVA revealed a significant grasp×muscle interaction (p < 10^{-15}). In the 1DI and AbPB muscles, activity during PG was significantly greater than during WHG (both p < 0.01), whereas in AbDM, the reverse was true (p < 0.0001). For both grasps, 1DI and AbPB activity levels were not significantly different (p > 0.9), but were both significantly different from AbDM (all p < 0.0001).

Corticospinal excitability during action observation and withholding

Figure 6.5 shows example MEPs recorded in the 1DI muscle of one subject, and Figure 6.6 shows 1DI EMG traces from one example trial in each condition. To assess CSE in each condition across subjects, we first normalised raw MEPs for each subject. Figure 6.7 shows the average MEP peak-to-peak amplitude during the pre-task baseline, intra-task baseline (ITI), and at the time of PG grasping observation in the three recorded hand muscles during the Go&NoGo block. In a 2-way rmANOVA (TMS condition × muscle), there was a significant main effect of TMS condition (F_{2,24} = 25.6, p < 1.2 × 10^{-6}). Across
6.4. Results: Experiment 1

Figure 6.6. Raw traces of EMG activity in the 1DI muscle on a single trial from each TMS condition. Baseline TMS could be triggered on execution (first row), or observation trials. TMS after the GO cue (second row) or object displacement (third row) occurred on observation trials only. TMS after the NoGo cue shown in the fourth row. Scale is the same for all conditions.

conditions, the ITI baseline and Grasp conditions were both significantly greater than the pre-task baseline (p = 0.0001 and p = 0.002, respectively), and MEPs in the intra-task baseline was also greater than the Grasp condition (p = 0.024). Since there was a general facilitation of CSE across task conditions relative to the pre-task baseline, and we wished to focus on modulation across different periods of the task, all subsequent analyses used the ITI baseline for normalisation.

Average normalised MEP amplitudes across the different TMS conditions and grasps from the three recorded muscles are shown in Figure 6.8. Figure 6.8A shows the results from all subjects in the Go & NoGo block. Overall changes relative to baseline were small (2-way rmANOVA (TMS condition \times grasp), with non-significant trend of suppression across observed grasps ($F_{3,39} \sim 2.5, p \sim 0.1$). In AbPB, there was an interaction of TMS condition\times grasp ($F_{3,39} = 3.81, p = 0.037$), and suppression during PG NoGo was significantly greater than during WHG (p = 0.0038).

MEPs during the Go only block (Figure 6.8B) were also not significantly modulated relative to baseline (all $p > 0.1$). To test for a grasp-specific interaction, we subjected the MEP ratio scores, which quantified the relative amplitude of MEPs for PG and WHG observation in
6.4. Results: Experiment 1

Each muscle, to a 2-way rmANOVA (TMS condition × muscle). In the Go only block (Figure 6.9), we found a significant muscle × TMS condition interaction ($F_{4,20} = 3.84$, $p = 0.018$). There were no differences across muscles at Baseline or Go, but at Grasp, ratios were significantly different between 1DI and AbDM ($p = 0.034$), and between AbPB and AbDM ($p = 0.003$), but not between 1DI and AbPB ($p = 0.24$). This grasp-specific facilitation was not apparent when the NoGo condition was present (all $p > 0.05$).

![Figure 6.7. Baseline MEP comparison.](image1)

Raw MEP amplitudes in the three recorded muscles during the pre-task baseline block, ITI baseline, and observation of precision grip (mean±SEM, 14 subjects)

![Figure 6.8. Group average MEP amplitudes.](image2)

A. Normalised average MEP amplitudes during four conditions and two grasps in the Go & NoGo block (n=14), in each of the three muscles. B. As for (A.), but for Go only block (n=7).

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6.5. Results: Experiment 2

In this experiment, we assessed CSE during congruent or incongruent action observation which occurred while subjects were engaged in maintaining a stable PG hold, and compared this to a condition when subjects only performed a PG, but observed no other action.

EMG and behaviour

Figure 6.10 shows the normalised EMG activity, homepad pressure and object displacement, in one subject across different task conditions. In both single- and double-hold conditions, performance of PG substantially recruited the 1DI muscle, and also the AbPB to some degree. The AbDM, by contrast, was hardly used. Subjects performed single and double-hold trials in a similar manner across both blocks, and the average reaction time and movement time were not different across conditions and blocks (see Table 6.1). Subject reaction and movement times were frequently slower than the experimenter (Mann-Whitney U-tests, p < 0.05), possibly because the experimenter had more experience with the task, and was unencumbered by wires connected to the EMG electrodes.
6.5. Results: Experiment 2

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Table 6.1. Reaction time (RT) and movement time (MT), rounded to nearest millisecond, for subjects and experimenter across different TMS conditions and blocks (mean±SEM, 14 subjects).

**Figure 6.10. EMG activity during active observation.** Average EMG activity in 1DI, AbPB, and AbDM during incongruent block of active observation task in one subject. Left column: single-hold trials. Middle column: double hold trials, both aligned to subject grasp (DO). Right column: double-hold trials, aligned to experimenter DO. Average times of TMS relative to subject grasp are indicated by dashed lines and text in (A) and (B) for single- and double-hold trials, respectively. Vertical bar shows 50% of normalised scale. Event markers as in Figure 6.3, with additional markers on double trials to denote both subject and experimenter events. 1DI, first dorsal interosseous; AbPB, abductor pollicis brevis; AbDM, abductor digiti minimi; E-HP, experimenter homepad; E-Disp, object displacement for experimenter; S-HPl, subject left homepad; S-HPr, subject right homepad; S-Disp, object displacement for subject.
Corticospinal excitability during active observation

Figure 6.11A shows individual MEP sweeps from the 1DI muscle from a single subject, and the average MEPs for each block and TMS condition are shown in Figure 6.11B. In this subject, observing a congruent grasp with the one being performed resulted in a decrease in MEP size in 1DI relative to simple execution trials (single-hold), whereas observing an incongruent grasp produced a slight increase in MEP size. The group results, shown in Figure 6.12 demonstrated considerable variability across subjects. In 1DI, incongruent double-hold showed a trend effect of increased CSE relative to single hold (mean double-hold: 1.10, t(13) = 1.97, p = 0.07). Congruent double-hold also showed a non-significant increase (mean double-hold: 1.09, t(13) = 1.53, p = 0.15), and the two double-hold conditions were not different from each other (t(13) = -0.03, p = 0.98). We hypothesised that the variability across subjects may partly have been due to different levels of recruitment of the 1DI and AbPB muscles across subjects during the execution task, and in order to stratify subjects, we defined the prime mover of these two muscles via a ratio of EMG activity prior to the TMS pulse. 5 of 14 subjects had a 1DI:AbPB ratio > 1 indicating greater use of the 1DI for stable hold (mean±SEM = 1.41±0.20), whereas 9 subjects preferentially recruited AbPB (ratio < 1, mean±SEM = 0.49±0.08). Stratifying subjects on this basis (Figure 6.13) revealed that within the 5 subjects with greater 1DI activity prior to the TMS pulse, there was an increase in CSE during incongruent double-hold trials relative to single-hold trials (mean double-hold: 1.12, t(4) = 5.28, p = 0.006, ). CSE during congruent double-hold was not significantly different to single-hold (mean double-hold: 0.95, t(4) = -0.99, p = 0.38), and a paired t-test between congruent and incongruent was also not significant (t(4) = -2.47, p = 0.07). There were no significant effects in the AbPB muscle across all subjects (Figure 6.12), or across the 9 subjects who preferentially used this muscle for performing the grasp (all p > 0.15, Figure 6.13B, middle). Tests for the preferred muscle across all 14 subjects (Figure 6.13B, right), were not significant (p > 0.05). When examining EMG variance in the period preceding the TMS pulse, the only significant effect was in the stratified AbPB sample, where the incongruent condition showed a significant reduction in variance relative to single-hold (p < 0.05, Figure 6.14B, middle).
6.5. Results: Experiment 2

Figure 6.11. Example MEPs during active observation. (A). Individual motor-evoked potentials in the 1DI muscle during single-hold trials in one subject. (B). Averages from the same subject in single-and double-hold conditions for congruent (left) and incongruent (right) blocks.

Figure 6.12. MEP modulation during active observation. MEPs were normalised to the average MEP amplitude in the single-hold condition, for each block. Circles and connecting lines mark individual subjects. C, congruent; I, incongruent.
6.6. Discussion

In this study, we first assessed CSE during the passive observation of actions, and simple withholding of action. We found grasp-specific modulation of MEPs during the observation of grasp, and this modulation was congruent with the roles of the muscles involved in execution of the same actions. However, MEPs overall did not modulate much from baseline, and this muscle interaction disappeared in the presence of a NoGo condition.

6.6.1. Non-specific decreases in corticospinal excitability

We did not observe an overall facilitation of CSE during the grasp condition relative to baseline. Previous reports of muscle-specific changes in human CSE during action observation have included muscle-specific facilitation relative to baseline (Fadiga et al., 1995; Gangitano et al., 2001; Alaerts et al., 2010a; Sartori et al., 2012; Gueugneau et al., 2015). On the other hand, several other studies have identified a suppression of CSE during action observation (Lago & Fernandez-del-Olmo, 2011; Villiger et al., 2011; Bunday et al., 2016), or no overall effect (Hannah et al., 2018b). In the monkey, there is also considerable evidence for suppression of discharge in PTNs during action observation (Kraskov et al., 2009; Vigneswaran et al., 2013). Neuronal activity in M1 in particular is relatively reduced during observation relative to execution, and CSE suppression or lack of modulation relative to baseline, as measured by TMS is consistent with this line of evidence, and could reflect mechanisms for the withholding of movement (Kraskov et al., 2009, 2014; Villiger et al., 2011). Non-specific suppression of CSE related to movement withholding has also been observed previously in Go/NoGo paradigms (Hoshiyama et al., 1996; Sohn et al., 2002).

It is important to note that the decrease in CSE we observed was relative to the LCDon baseline, whereas task MEPs were generally facilitated relative to the pre-task baseline (Figure 6.7). This increase was not specific to a particular stage of the action observation trial itself, suggesting it is not due to action-observation specific mirroring, but likely arises from a more general attentional-related phenomenon (Wright et al., 2018). This is consistent with recent reports (Bunday et al., 2016; Hannah et al., 2018b; Cretu et al., 2019) and
underlines that baseline choices in TMS action observation tasks, and muscle-specificity tests, should be considered carefully in study design, and when comparing findings across studies (Naish et al., 2014).

### 6.6.2. Grasp-specific modulation

Based on initial results in the Go&NoGo block, we hypothesised that the trend suppression effects we observed (and lack of grasp-related modulation) may have partly been attributable to the task instructions, which emphasised that subjects should not move on observation (and NoGo) trials. The relatively high proportion of these trials, could have pushed MEPs into the inhibition domain associated with the suppression of movement, which is a necessary part of action observation trials (Lago & Fernandez-del-Olmo, 2011; Villiger et al., 2011; Naish et al., 2014; Bunday et al., 2016).

In half of the subjects (n=7), we therefore included a block without the NoGo condition, which was always completed before the NoGo condition was introduced to the subjects for the second block. In this instance, suppression effects relative to baseline were less apparent, and although we did not see an overall facilitation relative to baseline, there was a grasp-specific interaction effect when we compared MEPs during PG and WHG across conditions and muscles. Grasp-specific interactions in cortical and spinal circuitry have been found previously during the execution of action (Cattaneo et al., 2005; Prabhu et al., 2007b; Davare et al., 2009), and action observation (Montagna et al., 2005; Catmur et al., 2007; Bunday et al., 2016; de Beukelaar et al., 2016; Cretu et al., 2019). During observation, the changes in CSE typically match the patterns of muscle activity used to execute the same actions (Montagna et al., 2005; McCabe et al., 2015). In this study, we confirmed that PG execution preferentially recruited the 1DI and AbPB muscles, whereas WHG execution recruited the AbDM muscle. During action observation, we found that the ratio of MEPs during PG and WHG was significantly different between muscles at the grasp, and this difference was consistent with the use of those muscles in performing grasp. This is comparable with previous work showing PG MEPs to be larger in 1DI and AbPB, and smaller in AbDM (and vice versa for WHG), and supports the idea of grasp-specific motor resonance during action observation (Cavallo et al., 2012; Sartori et al., 2012; Bunday et al., 2016; Cretu et al., 2019).
The fact that this motor resonance was stronger in the block when the NoGo condition was excluded highlights that contextual factors relating to the task requirements or design can affect CSE (Villiger et al., 2011; Janssen et al., 2015; Bunday et al., 2016; de Beukelaar et al., 2016; Riach et al., 2018). For example, in the study by Bunday et al., (2016), motor resonance was present when observation involved the hand only, but disappeared when subjects observed whole-person videos, possibly because this introduced competing inputs to the CST which cancelled out at the level probed by TMS. In our task, since the observation condition also required the subjects to refrain from movement, the introduction of the NoGo condition may have shifted the relevant dimension of the task from execution/observation to movement execution/suppression, and consequently attenuated grasp-specific modulation of CSE during the observed actions.

A possible technical reason for the relatively weak modulation relative to baseline during observation of grasp could have been sub-optimal timing of the TMS stimulus. In our experiment, TMS pulse for the grasping condition was delivered at the time of object contact, when the fingers have closed around the object, because modulation is often highest at this point (Montagna et al., 2005; Vigneswaran et al., 2013; Gueugneau et al., 2015; McCabe et al., 2015). Other studies, however, have found greater modulation of CSE to be associated with larger finger aperture, which necessarily occurs earlier than the grasp itself, during hand shaping (Gangitano et al., 2001; Urgesi et al., 2006). This explanation is unlikely however, since tasks showing higher modulation related to finger aperture are often those using slowed-videos or static images, whereas our task involved a more naturalistic set-up, with actions observed at normal speed in real-time. This is more consistent with the aforementioned studies finding greater modulation at the time of grasp, in line with the pattern of EMG activity in relevant muscles.

Overall, these results suggest that the so-called “Fadiga” effect is rather weak, possibly due to some cancellation of facilitation and suppression effects within the net readout provided by TMS (Hannah et al., 2018b). They also provide further evidence that detection of grasp-specific motor resonance can be influenced by task requirements or contexts.
6.6. Discussion

6.6.3. Corticospinal excitability during active observation

In a second experiment, we sought to examine the effect of action observation on CSE in the presence of ongoing motor activity due to the subject’s own actions. This is a common real-world situation in which action observation occurs, yet possible similarities or distinctions between this form of observation and passive observation have rarely been studied. Across all subjects, we did not observe a significant change in CSE between when subjects performed an action alone, or when they performed an action and simultaneously observed an action in front of them. This was true for both congruent and incongruent conditions, which both showed a weak but non-significant increase in excitability relative to the single-hold condition (Figure 6.12). This non-specific, limited increase could have been due to a general effect of increased attention in the double-hold trials, since an additional action was taking place in view of the subject.

Since subject EMG patterns indicated a range of preferences for 1DI or AbPB in maintaining the stable hold, we further stratified subjects on this basis for a secondary analysis. In the sub-sample of 5 subjects who preferentially used the 1DI for PG, we observed a small, significant increase in CSE during the incongruent observation relative to single-hold condition, and a small increase relative to the congruent condition, which was not significant (Figure 6.13B). An increase in excitability during observation of an incongruent, but not congruent, action could occur because the motor plan associated with the incongruent action is superimposed on the current output (Kilner et al., 2003; Blakemore & Frith, 2005; Stanley et al., 2007; Capa et al., 2011). The limited number of subjects and the small size of the effect however, render this conclusion largely speculative, and in need of further testing. Additionally, the CSE changes in 1DI for the incongruent condition are related to observation of WHG, which is perhaps unexpected for the 1DI based on the results of Experiment 1. The active nature of the task necessitated the use of a lower stimulus intensity compared to the one used at rest, which unfortunately meant MEPs in the AbDM, which was relatively inactive in most subjects, were often very small or absent altogether, and modulation in this muscle could not be reliably quantified in this task.
6.6. Discussion

There are several possible explanations for the limited effects we observed, even in the stratified samples. One factor may have been the level of ongoing EMG activity, which can dramatically affect MEP amplitude. To mitigate this, we delivered TMS during the stable hold period of the subject grasp so that the EMG in different conditions would be approximately equivalent. In practice, it is considerably unlikely that subjects exerted the exact same force while holding the grasp on different trials, although we did not observe a systematic difference in pre-stimulus EMG across subjects and conditions. Additionally, the average time of TMS delivery on double-hold trials was somewhat later than single-hold trials, relative to the subjects’ own hold. The latter was determined by the experimenter’s reaction and movement time, which varied from trial-to-trial, whereas single-hold trial TMS was consistently delivered 800-850ms into the subjects’ hold period. The excitability changes observed during passive action observation are often small, and so
6.6. Discussion

Any effects related to the observation of action in this task may have been masked by the intrinsic variability in MEPs during the subjects’ own actions. In chapter 5, Experiment 2, the modulation of M1 neurons during active observation, above what was observed during execution-only, was small, and not well correlated with activity during passive observation. Given that M1 excitability is a key contributor to observed MEP amplitude, this could explain why we did not find substantial modulation during active observation in this experiment. As discussed in subsection 5.5.4 (p.167), inhibiting additional changes in CSE during simultaneous execution and observation of precision actions may be particularly important for limiting interference between the two motor programs.

![Figure 6.14](image)

**Figure 6.14. Variance in EMG activity** in 300-800ms period after subject DO, normalised to single-hold trials for each block separately. (A), 1DI and AbPB for all subjects (n=14, circles and lines show individual subjects). (B), Variances after stratification of subjects based on preferential recruitment of 1DI (left, n=5) or AbPB (middle, n=9). Right: Modulation in preferred muscle across all 14 subjects (1DI and AbPB).

Future studies could consider online monitoring of EMG activity or training subjects to maintain a much narrower force range during their own action. This would provide a greater degree of consistency in the level of ongoing EMG activity when TMS is delivered, and increase the chances of teasing apart possible effects of concurrent execution and observation on the human motor system. A further factor to consider is that, in all conditions, subjects received visual feedback from their own-hand action, which has been shown to influence MirN activity (Maranesi et al., 2015). Thus, although the experimenter’s subsequent action on double-hold trials may have drawn some of the
subjects’ attention, it may not have been a sufficiently salient cue to induce a further change in CSE, over and above what was already induced by the subjects’ own actions.

Beyond any effect on MEPs, we considered whether the effect of active observation could have manifested in increased variance in the subjects’ EMG activity while performing PG, depending on the congruency of the action they were observing at the time. In the stratified samples, we found a decreased variance in EMG in the incongruent condition, although this was in the AbPB muscle, rather than the 1DI in which we found an effect on MEPs. The direction of the effect also contrasts with the finding that observing incongruent arm movements increase the variance associated with one’s own movements, which presumably occurred to interference within the MNS (Kilner et al., 2003; Blakemore & Frith, 2005; Capa et al., 2011). However, the gross, dynamic arm movements in that study are qualitatively different to the skilled grasp and static hold performed in the task presented here, and thus perhaps offer a wider scope for interference due to conflicting kinematics or action goals within the motor program. In our task, a comparable behavioural signal which could have influenced by congruency was the degree of object displacement. However, the resolution of this signal was not sufficient to quantify whether this effect was present, and maintaining a highly constrained hold position was not instructed or trained - flexibility in subject behaviour was possible as long as they remained within the hold window.

Overall, the results from this second experiment were inconclusive, as we did not observe a consistent modulation of CSE during concurrent observation and execution relative to simple execution in either congruent or incongruent conditions. Further work is needed in order to understand the role of the MNS during simultaneous execution and observation of precise grasping actions.
7. General discussion and summary

The experiments in this thesis have examined the time-course of mirror activity at cortical and spinal levels during the execution, observation, and withholding of skilled voluntary movement. This was achieved using extracellular recording of cortical activity and recording of stimulation-evoked muscle responses in awake, behaving macaques. Additionally, TMS was used to assess changes in CSE during action observation and action withholding in humans. As the results of these individual experiments have already been discussed in the preceding chapters, the aim of this chapter is to provide a more holistic overview and summary involving the links between these results, and to suggest possible directions for future research.

7.1. Movement suppression and grasp representation in the mirror neuron system

The discovery of mirror neurons (MirNs) placed action observation, a behaviour involving no self-movement per se, at the heart of a system dedicated to conscious control of skilled voluntary movement. The anatomical and functional components of this system were discussed in the first part of the Introduction, and a wealth of research has highlighted similarities in activity between action observation and execution in MirNs. Mirror activity encompasses multiple areas of the visuomotor reaching and grasping network, spanning parietal, premotor, and primary motor cortex (see section 1.4, p.41). This activity even includes PTNs, which constitute the final output to the spinal cord, and are strongly associated with the control of movement. Activity in the motor system is often dissociated from movement in contexts other than action observation (Jeannerod, 1994; Schieber, 2011), and given that PTNs are embedded within the motor system, it is perhaps not wholly surprising that they can show mirror responses (Kraskov et al., 2009; Vigneswaran et al., 2013). This previous work has suggested that differences in the extent and time-course of mirror activity at multiple levels of the motor system could underlie the balance between shared representation of action during execution and observation, and the
suppression of overt movement. Distinctions between action execution and observation activity in parts of the MNS could also form the basis for the solution to the “correspondence problem” of assigning actions to oneself or others (Brass et al., 2005; Yoshida et al., 2011; Breveglieri et al., 2019), and the suppression of automatic imitation (Ferrari et al., 2009). The central aim of this thesis was to quantitatively examine this balance via assessments of the extent and time-course of mirror activity in F5, M1, and the CST.

In chapter 3, I showed that MirN activity in M1 during action observation, particularly within PTNs, is distinguished from execution activity both in terms of amplitude and temporal profile. Although M1 MirNs could show grasp-related observation activity, this activity was preceded by a clear dissociation between execution and observation activity in the lead-up to movement onset. The action observation condition also required the monkey to suppress their own movement, and M1 population activity just after the Go cue for the experimenter’s movement followed a similar pattern to activity on trials in which the monkey was simply instructed to withhold movement. In contrast, F5 MirN activity during execution and observation was more comparable in both amplitude and temporal profile, suggesting that premotor MirNs contain a similar representation of action independent of the identity of the acting agent. Recent work has also shown that premotor MirNs encode action execution and action observation states in a similar manner, whereas M1 MirNs do not (Mazurek et al., 2018). These results fit within the known anatomical and functional properties of premotor and motor cortex. Area F5 contains a vocabulary of motor actions (Rizzolatti et al., 1988; Murata et al., 1997; Raos et al., 2006; Umiltá et al., 2007; Schaffelhofer & Scherberger, 2016), and contains MirNs which often fire to a similar degree across execution and observation (Gallese et al., 1996; Kraskov et al., 2009). However, the direct influence of F5 on the cervical spinal cord is limited (Dum & Strick, 1991; He et al., 1993), such that unfolding representations of action can reasonably occur during execution and observation without causing movement. M1 projects strongly to the spinal cord (Dum & Strick, 1991; Porter & Lemon, 1993) and contains muscle and movement-related representations of actions (Kakei et al., 1999), and so the dissociation of signals associated with action execution and observation in this area is likely fundamental for the appropriate generation and suppression of movement. This dissociation may be particularly important for actions occurring in peri-personal space, which provide the
possibility for the observer to interact with the action (Caggiano et al., 2009; Bonini et al., 2014a).

Studying the modulation of PTNs during action observation can provide useful clues into what might be taking place downstream, within the spinal cord (Kraskov et al., 2009; Vigneswaran et al., 2013). However, the output targets of these PTNs are generally unknown, and can include both direct excitatory connections to motoneurons via CM connections, and synapses onto inhibitory sINs or propriospinal neurons. In chapter 4, I addressed this issue more directly, by monitoring the short-latency evoked responses in hand muscles to direct stimulation of the PT as a proxy for spinal excitability. Here, I found a balance of excitation and inhibition during action observation which followed a similar temporal profile to mirror activity in M1. Spinal excitability was suppressed in the lead-up to the observation of movement, and also somewhat after cued withholding of movement, whereas excitability increased in a grasp-specific manner during the observation of grasp. Together with the results from chapter 3, these findings strongly suggest that cortical mirror activity does induce minor changes at the spinal level, but that these may remain sub-threshold to movement via a balance of excitation and inhibition.

The macaque monkey is an essential model for the study of motor system function, because of the significant changes in the structure and function of the motor cortex and CS system in the evolution of primates (Lemon & Griffiths, 2005). Accordingly, a great strength of motor system research has been the comparison of results from non-human primate electrophysiology with neurophysiological and neuroimaging findings in human subjects. In chapter 6, CSE was assessed in healthy human volunteers performing the same basic mirror task. In a task involving only execution and observation, we found some evidence for grasp-specific facilitation at the time of observed grasp, whereas there was weak suppression of CSE after action withholding and around the time of experimenter movement onset in the observation condition. Interpretation of these results may be more challenging for a number of reasons. TMS reflects only the net CS output, the temporal profile of modulation examined was coarser than in the monkey studies, and there was some degree of inter-individual variability. However, the grasp-specific facilitation occurred in the manner expected by the way the subjects performed the grasp themselves, supporting the notion that cortical MirN activity induces motor resonance within the CST.
The trend of CSE suppression after action withholding also parallels the results from the monkey experiments and suggests that a common mechanism may underlie suppression of movement during action observation and other forms of movement withholding.

7.2. Contextual factors which modulate mirror activity

Alongside the assessment of the time-course of action observation activity and its relationships with movement execution and action suppression, we also examined a number of contextual factors associated with action observation which may have altered these relationships.

The presence of a NoGo condition in the TMS task induced some generalised suppression of CSE, and weakened the grasp-specific modulation during observed grasp. The TMS experiment contained a more limited number of time points in which physiological changes could be examined, and there was a relatively low proportion of execution trials relative to the other trial types. In the monkey experiments, differences in modulation of spinal excitability during the observation of grasp between the two monkeys may have been attributable to the relative influence of the NoGo condition on strategy - this influence may have been stronger in M49 than in M48, which could explain the weak modulation around the time of the observed grasp in the former.

Appropriate withholding of movement during action observation may be particularly relevant in the context of imitation, for example in contexts where complementary, rather than imitative action is required, or when imitation must be withheld until an appropriate time. On the other hand, requirements to imitate could enhance motor system modulation by increasing the attention paid to the observed action. However, in an additional cortical recording experiment presented in chapter 5 (Experiment 1), we did not observe clear differences between observation activity in the basic task, and observation activity preceding imitation. Withholding of movement or automatic imitation is also likely to be important in contexts where the motor system is already engaged in an action, as additional movement could interfere with the performance of action. In chapter 5 Experiment 2, we presented the finding that during simultaneous execution and observation, F5 MirNs show slightly increased modulation relative to simple execution, and this increase appears to be
proportional to the level of mirror activity during passive observation. The same relationship was not found in M1, and in human subjects performing the same task, there were no reliable changes in CSE during the execution and observation of congruent or incongruent movements. As discussed in chapter 6, the small size of these changes may have been due to overshadowing by fluctuations in the subject’s own action, or because simultaneous execution and observation are more subject to interference for dynamic stages of action, and the interference at a behavioural level may be more quantifiable in this instance (Kilner et al., 2003; Blakemore & Frith, 2005; Stanley et al., 2007; Capa et al., 2011). We designed the static hold period to be when observation of the experimenter action took place since this allowed for a more stable level of neural and EMG activity upon which to measure changes. However future studies could seek a more principled method for analysing changes during observation in the dynamic period of self-movement in both monkeys and humans.

In chapter 4, we found that obscuring the monkey’s vision of the grasping action did not affect the profile of modulation if the monkey had received contextual cues about the object for the upcoming trial. On the other hand, providing no visual cues about the upcoming action abolished this modulation. In the same task, modulation in cortical populations (chapter 5, Experiment 3) was similar during observation with full vision or when the grasp was obscured but known, but was reduced in the condition where no visual or contextual information was provided. These findings are in line with previous results in macaque F5, and the human CST which have shown that visual information about action kinematics is not necessary to induce mirror activity, if appropriate contextual cues are available (Umiltá et al., 2001; Villiger et al., 2011; Valchev et al., 2015; Cretu et al., 2019). These results have strongly suggested that mirror activity arises from the convergence of bottom-up sensory cues (such as visual input) and top-down modulatory inputs based on prior expectations, which could be combined empirically in a Bayesian manner i.e. weighted by the relative uncertainty in each input (Kilner et al., 2007; Cretu et al., 2019).

This computational mechanism goes some way to addressing the question of how action consequences can be reliably inferred based on incomplete information during action observation, but more systematic testing is required to furnish a truly quantitative understanding of probabilistic combination of information from different sources during action observation, and to identify the neural substrates subserving this integration.
7.3. Future directions

The results presented in this thesis raise a number of questions for potential future research. At the cortical level, the physiological interactions between F5 and M1 during visually-guided grasp have been studied extensively in both monkeys and humans (Davare et al., 2008, 2009; Prabhu et al., 2009), and combined with evidence from cell recordings and inactivation studies, have provided strong evidence for a transfer of information from F5 to M1 for accurate grasp execution. In humans, muscle-specific interactions between PMv and M1 during the grasp phase of action observation have been reported (de Beukelaar et al., 2016), but interactions between F5 and M1 have not been studied at the single-neuron level during action observation. A functional dissociation between F5 and M1 during observation in some contexts could help to explain why observation and execution activity become progressively more distinct between these areas. If M1 mirror activity is “inherited” from F5, then muscimol inactivation of F5 during action observation should presumably reduce or abolish M1 mirror activity. Single-pulse stimulation in one area with recording in the other could be used to assess how functional connectivity between macaque F5 and M1 is altered during action observation. This functional connectivity could be assessed at multiple time points and also across different sub-regions of the two areas. These studies would provide more direct insights into the flexible routing of information through premotor and motor areas for appropriate control of reaching and grasping behaviour.

The increasing capability for recording from large numbers of neurons simultaneously with high-density electrodes has opened up new avenues for understanding how population dynamics evolve across the cortical grasping network, and how these dynamics might drive transitions in the state of the motor system, from movement preparation to execution or withholding (Churchland et al., 2012; Shenoy et al., 2013; Michaels et al., 2018). This dynamical systems approach has shown promise for addressing long-standing concerns and lack of consensus regarding what features of movement or muscle activity are encoded in M1. This has involved detaching representational tuning in single neurons, which may be epiphenomenal, from the ultimate purpose of the motor system, which is movement generation (Fetz, 1992; Scott, 2008; Shenoy et al., 2013). Accordingly, a great strength of the recent action preparation literature has been to account for the common seemingly idiosyncratic changes in tuning of many single-neurons across preparation and movement.
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epochs, and provide a mechanistic explanation for how movement is seeded by the
evolution of the preparatory neural state (Churchland & Shenoy, 2007a; Churchland et al.,
2010, 2012). For now, this framework has largely set aside considerations of neural
architecture and anatomical sub-populations, although the distinctions between locally
connected neurons, PTNs, and even CM cells, are surely relevant and indicative of
different functional roles in movement control (Porter & Lemon, 1993; Soteropoulos,
2018). Although initial comparisons of reach and grasp population dynamics suggest
qualitative differences exist between the two (Suresh et al., 2019), recent work (Mazurek
et al., 2018) and results presented in this thesis have sought to apply this generative
framework to grasping actions, and have provided additional insights into mechanisms of
movement suppression during action observation. Future experiments exploring a wider
repertoire of executed and observed grasps combined with large-scale simultaneous
recordings, will permit the inclusion of “untuned” neurons and robust single-trial analyses,
and advance our understanding of these dynamics. Of course, implicit gating of movement
via population state dynamics, which has been reasoned to occur during preparatory delay
periods (Churchland et al., 2010, 2012; Kaufman et al., 2013, 2014), is not mutually
exclusive to threshold-based, non-linear gating mechanisms (Kraskov et al., 2009, 2014;
Vigneswaran et al., 2013; Soteropoulos, 2018). Further work is needed to understand the
relative contributions of these mechanisms, and dynamical systems approaches also offer
promise for understanding the relationship between action observation and other forms of
motor cortical activity during non-movement, including action preparation and motor
imagery. Ultimately, this work will feed into a unified framework which can
mechanistically explain the structure of motor cortical population activity across the
complete spectrum of behaviours (Gallego et al., 2018).

In tandem with studies of neural covariance and population activity, perturbation
experiments would offer potential avenues for a causal understanding of the role of MirNs
in behaviour, which is sorely lacking at present. Muscimol inactivation has previously
been used to great effect for assessing the relative contributions of AIP, F5, and M1 in
visually-guided grasp (Gallese et al., 1994; Schieber & Poliakov, 1998; Fogassi et al.,
2001). Electrical stimulation and optogenetic toolkits provide the opportunity for tight
spatial, and within-trial temporal control over prospective perturbation periods, and
overcome many of the concerns regarding compensatory and non-focal effects following
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muscimol inactivation, cooling, or lesioning. With all these techniques however, it is first essential to establish an explicit, quantifiable behaviour in which the potential involvement of MirNs can be tested. Additionally, the genetic/molecular identity of MirNs, if it exists, has not been established, which means that tagging these cells exclusively for optogenetic inactivation is not yet feasible.

The activity of PTNs during action observation has been highlighted by recent work (Kraskov et al., 2009; Vigneswaran et al., 2013) and also examined in this thesis. Within this class of cells, although there is some evidence that CM cells can modulate their activity during action observation, no quantitative assessment of CM activity during action observation exists. This is particularly important given that the primary effect of these cells at the spinal level is excitatory, and a recent study demonstrated that they show qualitatively different patterns of activity from non-CM PTNs during movement preparation (another non-movement state) (Soteropoulos, 2018). It is important to note however, that the relationship between activity in CM cells and their target muscles is flexible and unpredictable, both within and across cells (Bennett & Lemon, 1996; McKiernan et al., 2000; Schieber & Rivlis, 2007; Schieber, 2011; Griffin et al., 2015). Thus, CM cells may indeed modulate during action observation (Vigneswaran et al., 2013), but at the population level, this activity could be orthogonal to muscle-relevant dimensions.

In addition to rate coding, PTNs and CM cells can also exhibit changes in their inter-spike intervals, and in their level of temporal synchrony with each other. Thus, while the majority of literature focuses on modulation of firing rates, downstream effects of these cells may reflect a multiplexing of rate and temporal coding (Lemon & Mantel, 1989).

Excitability changes at the spinal level, such as those reported in this thesis, which are induced by cortical mirror activity during action observation, must necessarily be weak since they remain sub-threshold to movement. Although responses to PT stimulation provides a more direct measure than cortical recording of the net changes in this excitability, further experiments are needed to causally tie modulation at the cortical level to corresponding changes in downstream circuitry. Along these lines, intracellular recording from alpha motoneurons, or recording from spinal interneurons during action observation, would provide a novel window into these changes, and provide the likeliest route for understanding the possible functional role of mirror activity in priming or altering
spinal circuits for future actions (Schieber, 2013). In addition, it is not known whether mirror activity extends to other descending pathways beyond the CST, such as the RST. Recent evidence has highlighted a potential role for the RST in the control of distal extremities, in conjunction with the CST (Riddle & Baker, 2010; Baker, 2011; Soteropoulos et al., 2012), and it is therefore a distinct possibility that the interplay between RST and CST inputs at the spinal level could interact during the observation and withholding of grasping movements.
7.4. Summary

In this thesis, I showed that the relationship between neuronal activity during action execution and observation in MirNs in F5 and M1 is quantitatively different. F5 neurons maintain a stable representation of grasp which is similar across executed and observed actions, whereas M1 neurons, and particularly PTNs, show clear distinctions between two key stages of action observation - the initial suppression of self-movement, and the observation of the experimenter’s grasp. Given the proximity of M1-PTNs to the spinal output, this may reflect a compromise between shared representation of action within the motor system, and suppression of unwanted, automatic imitation. This dissociation in M1 also manifests in net excitability changes at the spinal level, with suppression of excitability in the lead-up to observed movement onset, and grasp-related facilitation at the time of observed grasp. In related experiments at the cortical level, we found some evidence that the extent of visual and contextual information modulates the activity of MirNs, and that during simultaneous execution and observation, the activity from each condition alone can be superimposed in F5 MirNs, suggesting that this population can simultaneously represent both executed and observed actions.

Finally, I used TMS to investigate changes in CSE in humans during action observation, withholding, and simultaneous execution and observation. I found that the presence of the action withholding (NoGo) condition, appeared to shift the CS system towards a more general inhibition state on action observation trials. In the absence of this condition, grasp-specific modulation in CSE was apparent, reflecting the use of those muscles during grasp performance. The results from a second experiment, measuring CSE during active observation, were inconclusive, and require further investigation.

Altogether, the results in this thesis confirm that action observation activity in cortical and spinal circuits within monkeys and humans reflects a balance between excitation and inhibition processes, and provide new insights into the time-course of this process. This balance is influenced by the context in which action observation occurs, and may subserve the dual processes of providing a shared representation of movement across executed and observed actions, while simultaneously regulating the overflow of this activity into overt movement.
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