The Role of Mirror Neurons in Movement Suppression

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Declaration of conjoint work

I, Ganesh Vigneswaran confirm that the work presented in this Thesis is my own, It was completed without assistance except:

1) Critical stages of the surgical procedures were performed by Professor R N Lemon and members of his research group.
2) The experimental work described in 3, 4 and 6 was performed as part of an on-going research program in Professor R N Lemon's laboratory. This included advice and discussion with members of the research group including Dr Alexander Kraskov, Dr Roland Philipp and Dr Stephan Waldert.
3) Some of the analysis on M43 was based on previously collected data by Professor R N Lemon and collaborators.
4) The experiments described in Chapter 5 were performed as a collaboration with Professor Patrick Haggard and Dr Marco Davare.
5) Spike discrimination software was supplied by Dr Alexander Kraskov.
6) Chapters 3 and 6 include text and figures from first author published work (Vigneswaran et al., 2013, Vigneswaran et al., 2011).

Signature ……

Abstract

The characteristic feature of mirror neurons is that they modulate their firing rate during both a monkey's own action and during observation of another individual performing a similar action. Some premotor (F5) mirror neurons have also been shown to be corticospinal neurons, meaning that spinal targets are also influenced during action observation. Simultaneous electromyography (EMG) recordings from hand and arm muscles provide important evidence that the activity of these cells cannot be explained by any covert movement on the part of the monkey. The question arises as to how output cells (pyramidal tract neurons, PTNs) that are classically involved in the generation of movement can be modulated without any resulting movement. Since there are many more PTNs in primary motor cortex (M1) compared with F5, it is important to assess whether PTNs in M1 also have mirror activity.

We recorded activity of identified PTNs in areas M1 and F5 of two macaque monkeys during action execution and observation of a skilled grasping action. We found evidence of modulation of PTNs in M1 during action observation in over half the recorded units. However, the depth of modulation was much smaller during action observation compared with action execution. In a separate analysis we investigated whether it is possible to assign mirror neuron activity to different cell types on the basis of extracellular spike duration. Surprisingly, we found considerable overlap between identified pyramidal cells and putative interneurons and provide evidence

that spike duration alone is not a reliable indicator of cell type in macaque motor cortex.

In a separate series of studies we used non-invasive transcranial magnetic stimulation (TMS) in human volunteers to measure the corticospinal excitability during the same task.

Taken together, although we found evidence of modulation of PTN activity during action observation in M1, the level of activity was greatly reduced during action observation and may not be sufficient to produce overt muscle activity.

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List of Abbreviations

AbDM – Abductor digiti minimi

ABPL – Abductor pollicis longus

ADL – Antidromic latency

AIP – Anterior intraparietal area

APB – Abductor pollicis brevis

BA – Brodmann area

BRR – Brachioradialis

CM – Cortico-motoneuronal

CMA – Cingulate motor area

CST – Corticospinal tract

cTBS – Continuous theta burst stimulation

DO – Displacement onset

ECR-L – Extensor carpi radialis longus

ECU – Extensor carpi ulnaris

EDC – Extensor digitorum communis

EEG – Electroencephalogram

EMG – Electromyogram

F5 – Premotor cortex

FCR – Flexor carpi radialis

FCU – Flexor carpi ulnaris

FDI – First dorsal interosseous

FDP – Flexor digitorum profundus

fMRI – Functional magnetic resonance imaging

HOFF – Hold offset

HON – Hold onset

HP – Homepad

HPP – Homepad press

HPR – Homepad release

ICMS – Intra cortical micro stimulation

IFC – Inferior frontal cortex

IPL – Intraparietal lobule

ISI – Interstimulus interval

LFP – Local field potential

M1 – Primary motor cortex

MEP – Motor evoked potential

MRI – Magnetic resonance imaging

OBJ-H – Human object

OBJ-M – Monkey object

PMd – Dorsal premotor cortex

PMv – Ventral premotor cortex

PT – Pyramidal tract

PTN – Pyramidal tract neuron

S-E – Screen for execution

S-O – Screen for observation

SD – Standard deviation

SEM – Standard error of the mean

SICF – Short intracortical facilitation

SICI – Short intracortical inhibition

SII – Secondary somatosensory cortex

SMA – Supplementary motor area

STA – Spike-triggered average

STS – Superior temporal sulcus

TMS – Transcranial magnetic stimulation

UID – Unidentified neuron

Chapter 1: Introduction

1.1 THE MOTOR SYSTEM: NEURAL CONTROL OF THE HAND

1.1.1 The importance of the hand and hand movements

The capacity to use the hand to grasp and control objects is continually under the influence of precise visual guidance. Visuomotor control of prehension and manipulation of objects is extremely well-developed amongst humans and is crucial to the way we interact with the environment (Lemon, 1993). Hand function is one of most highly evolved aspects of human biology, and as such is also vulnerable to disease and injury (Jackson, 1884). The importance of the hand is highlighted by the fact that quadriplegic patients ranked the regaining of arm and hand function as higher than recovery of stance, locomotion, bowel and sexual function (Anderson, 2004).

1.1.2 Classical investigation of brain control of hand movements

Our understanding of the brain architecture that mediates grasp and its online visual control has been based on the combination of three classical approaches:

1) Electrical stimulation
2) Neuroanatomical tracing studies
3) Lesion studies, including clinical neurology

Electrical stimulation started with ideas of galvanic stimulation and also repetitive faradic stimulation (see Phillips, 1969) . Leyton and Sherrington furthered these techniques using minimal faradic stimuli and were able to localise the areas of cortex

involved with different muscles in the body including the frontal eye fields (Phillips, 1969). These approaches were refined for the study of monkey motor cortex maps (Woolsey et al., 1952) and human motor cortex by Wilder Penfield (Penfield, 1959). Using electrical stimulation of the brain in awake epileptic patients, Penfield discovered a map of the human motor cortex, which still holds value today, although often misinterpreted as demonstrating a fine somatotopy that is hard to reconcile with modern evidence, which shows a more complex organisation of highly overlapping, multiple representations of movements and muscles (Schieber, 2011) . This complexity has been revealed with other mapping techniques, including transneuronal retrograde labelling of cortico-motoneuronal (CM) cells in monkey (Rathelot and Strick, 2006) and fMRI studies (Sanes et al., 1995) of human hand and digit movements. The highly distributed, overlapping representation of muscles may be an optimal solution for flexible combination and recombination of muscles to provide a highly diverse motor repertoire for the skilled hand (Schieber, 2001).

1.2 GRASP

1.2.1 The neuroanatomy of the 'visuomotor grasping circuit'

The original 'visuomotor grasping circuit' of Jeannerod et al. (1995) comprises area AIP (anterior intraparietal area) of posterior parietal cortex (BA7), area F5 of ventral premotor cortex (BA6) and primary motor cortex (M1; BA4). The following discussion concentrates on the neuroanatomy of these three key structures. Later sections will deal with the fact that, since the original paper by Jeannerod et al. (1995) it has been proposed that there are multiple parietal-frontal pathways that mediate reaching and grasping in macaque monkeys ((Davare et al., 2011) review; see below).

1.2.2 "Grasp Zones"

Parietal cortex grasp zone: area AIP

Although it is likely that areas in the superior parietal lobule, such as BA 5 and 2, are also involved in the grasp circuit (Gharbawie et al., 2011), most attention has been focused on area AIP in the anterolateral bank of the intraparietal sulcus. AIP had strong local connections with the intraparietal lobule (IPL), SII and lateral intraparietal cortex (Borra et al., 2008). AIP has long range reciprocal connections with the premotor cortex (especially area F5p) and in addition receives inputs from the lower bank of the superior temporal sulcus (STS) areas TEa/TEe and the middle temporal gyrus (Borra et al., 2008). In the same experiment, a connection from AIP to the prefrontal areas 46 and 12 was found.

AIP has been shown to be fundamental for grasping as inactivation results in impairment of grasping actions with the contralateral hand, most noticeably precision grip (Gallese et al., 1994). IPL lesions result in mis-reaching of the contralateral arm and failure to make correct grasping actions (Haaxma and Kuypers, 1975).

F5 grasp zone

Area F5 can be divided into 3 distinct areas, areas F5a (anterior) F5p (posterior) and F5c (convexity) based on immunoreactivity (distribution of SMI-32 and calbindin). These different anatomical areas within F5 might have different functional roles in grasping, but this is yet to be elucidated (Belmalih et al., 2009).

Area F5p found in the inferior bank of the arcuate sulcus (posterior part) is characterised by sparse large layer V pyramidal cells. In addition there are other identifying characteristics; a barely noticeable layer II, homogenous layer III, Layer V is sublaminated (Va housing densely populated small pyramidal cells in contrast to Vb containing many medium sized pyramidal cellsin addition to the sparse large Betz cells), and layer VI has a radial organisation and is homogenous.

Area F5a, in the anterior part of the inferior bank is mostly populated with densely packed medium sized pyramidal cells as well as being less myelinated and a lower intensity of SMI-32 immunoreactivity compared with F5p. In contrast the calbindin reactivity is much higher. Unlike the other subdivisions of area F5, F5a has strong connections with prefrontal cortex (BA 46).

Area F5c, on the convexity of the gyrus adjacent to the inferior limb of the arcuate sulcus, has a poorly laminated appearance due to the homogeneity of the cell population as well as having a high SMI-32 reactivity in layer III and numerous apical dendrites. In terms of functional analysis, it is important to stress that all three subdivisions, (including F5c in which mirror neurons are thought to be primarily located) are densely interconnected (Gerbella et al., 2011).

Gharbawie and colleagues used two injections in the F5 grasp zone and showed that over 50% of the connections were with frontal motor cortex regions (Gharbawie et al., 2011). F5 gives rise to corticospinal projections, mostly from area F5p; it also makes numerous reciprocal cortico-cortical connections with the primary motor cortex (M1), again mostly from area F5p (Gerbella et al., 2011). A large proportion of the connections were from SMA, 20% from AIP (mostly from SII /PV) and 28% were

from posterior parietal cortex. They also found small contributions from dorsal premotor cortex (PMd), ventral cingulate motor area and parietal operculum. In agreement with this finding, others have shown that there is a strong input from SII and area PF (Godschalk et al., 1984, Matelli et al., 1986).

Area F5 is connected with area F6 (pre-supplementary motor area, pre-SMA) and also with the prefrontal cortex (area 46) (Rizzolatti and Luppino, 2001).The prefrontal cortex is also richly connected with another mirror neuron area, AIP (Rizzolatti and Luppino, 2001). These frontal inputs might coordinate and selectively modulate the selection of neurons involved in voluntary actions according to the intentions of the agent (Rizzolatti and Sinigaglia, 2010).

M1 and F5 are heavily interconnected with each other anatomically and functionally (Godschalk et al., 1984, Cerri et al., 2003, Shimazu et al., 2004, Dum and Strick, 2005) and thus they can influence each other and indirectly hand motoneurons in the spinal cord. Stimulation of F5 with single pulses fails to evoke excitatory post synaptic potentials in hand motoneurons (Shimazu et al., 2004), but can modulate M1 output activity , and this is thought to be the main pathway through which neurons in F5 could exert an effect on hand motoneurons (Cerri et al., 2003, Boudrias et al., 2009). Reversible inactivation of F5 leads to degraded grasp (Fogassi et al., 2001), and disorganised voluntary movements similar to an apraxic state (Fulton and Sheehan, 1935).

M1 grasp zone

M1 lacks granular cells (agranular frontal cortex) (Rizzolatti and Luppino, 2001). In the cebus monkey, Dum and Strick (2005) showed that the M1 digit representation receives its strongest inputs from the digit representations in the PMv and PMd; PMv contributes ~20% of the total cortico-cortical input labelled by tracer injection in the centre of the M1 hand area. It is interesting that the majority of neuroanatomical studies of cortico-cortical connections, starting with Pandya and Kuypers (1969) up to the more recent investigations of Luppino and colleagues (e.g. Gerbella et al., 2011) have found that the vast majority of posterior-parietal input to motor cortex is through premotor cortex and SMA.

Gharbawie and colleagues found that within motor cortex M1, 80% of the connections were with frontal motor cortex regions (PMd (more rostral), PMv (more caudal). They also report that M1 receives medial connections from SMA and CMAd. AIP connections were 7% of the total inputs (over areas 3a and SII/PV). In addition there is a small input from insular cortex. The posterior parietal cortex (PPC) contributed to 12% of total connections (Gharbawie et al., 2011). Prior to the above study, there has been no previous neuroanatomical evidence suggesting a direct connection between areas AIP and M1 and so further investigation is required to validate these findings.

1.2.3 Visuomotor Grasping Circuit in the Human Brain

Importantly, non-invasive studies using techniques such as fMRI and TMS suggest that the cortical network sub-serving grasp is similar in humans. AIP, PMv and M1 are

all involved in the human grasping circuit (Davare et al., 2011). The functional properties of the circuit in humans will be discussed in section **1.2.6**.

1.2.4 Descending pathways in the control of grasp

Descending pathways from motor areas of the cortex are crucial to the understanding of how premotor and also motor areas can influence the motoneurons and muscles involved in skilled grasp. These pathways consist of those that influence the spinal cord via their influence over brainstem motor pathways and those that comprise the corticospinal tract, which is particularly well-developed in primates (Lemon, 2008). The corticospinal tract arises from a wide variety of cortical areas in the monkey, including M1, dorsal and ventral premotor cortices (PMd and PMv), the SMA, and cingulate motor areas. The tract terminates widely in the spinal grey matter at all levels (Rizzolatti and Luppino, 2001, Dum and Strick, 2005).

The cortico-motoneuronal (CM) system is a component of the corticospinal tract which involves those fibres which exert direct monosynaptic action on spinal motoneurons (Bernhard and Bohm, 1954). CM connections are numerous in the macaque. In a recent anatomical study, CM cells innervating the spinal motoneurons of single muscles (ADP, EDC or ABPL) were retrogradely labelled with rabies virus. Each single muscle exhibited widespread labelling within the primary motor cortex, resulting in a large amount of overlap between representations of different muscles (Rathelot and Strick, 2006).

The size of the labelled cortico-motoneurons was also interesting, as most cells were small in diameter (70-90%), in contrast to the large Betz cells of layer V (the

characteristic feature of M1 cortex). Electrophysiological studies thus far have been biased towards recordings from the larger cells (Vigneswaran et al., 2011), and the function of the smaller cells (which are far more numerous) has yet to be elucidated (Rathelot and Strick, 2006). In addition to this study, another anatomical study suggests that corticospinal neurons in SMA provide relatively weak input to motoneurons directly, compared with M1 (Maier et al., 2002). Electrophysiological studies show that only a few motor responses evoked from SMA had latencies as short as the shortest ones from M1; suggesting that there is only a small direct CM input to motoneurons from SMA. The vast majority, however, had latencies that were 8-12ms longer than those from M1. This is consistent with terminations onto interneurons of the intermediolateral zone of the spinal cord (lamine V-VIII) (Boudrias et al., 2009). However, Rathelot and Strick (2006) could not find any labelled cortico-motoneuronal cells outside area 4 (M1) and area 3a (S1). This suggests that corticospinal neurons from secondary motor areas, including F5, do not have CM connections and agrees with other electrophysiological studies on F5 projections (Shimazu et al., 2004).

While PMv has a low-threshold motor representation of the hand and digits (e.g. Godschalk et al., 1995), it does not give rise to many corticospinal projections (only 4% of the total frontal lobe corticospinal projection (Dum and Strick, 1991) and these terminate mostly in the upper cervical segments of the spinal cord (He et al., 1993). This established view has recently been re-examined by Borra et al. (2010), which examined in detail, both brainstem and spinal targets of specific regions of PMv. Although these authors found some sparse projections from area F5p to the lower

cervical cord (segments C6-T1, which contain the motor nuclei controlling the muscles acting on the hand and digits (Jenny and Inukai, 1983)) the corticospinal projections seem to be mainly focused on the upper cervical segments (cf. He et al, 1993). There were no projections beyond T6. It is puzzling that despite the very high incidence of neurons in F5 with activity related to the ipsilateral hand, there are few projections to the ipsilateral grey matter.

1.2.5 The map of outputs in M1

Activation of descending motor pathways is thought to be an important component of the cortical control of grasp. This idea is very much derived from the earliest stimulation studies, in which movements or muscle activation was evoked by cortical stimulation. The somatotopical organisation of the evoked outputs has been particularly carefully researched using single pulse intra cortical micro stimulation (ICMS) and compiling post-stimulus averages of EMG activity recorded from many different muscles while a macaque performed a reach and prehension task (Park et al., 2001). The physical spread of the 15 µA stimulus current used would only have spread around 105-245 µm and the authors claimed that this approach was the best for detailed examination of the output map, with the use of single shocks limiting the indirect, trans-synaptic effects of the stimulus.

These authors reported a central area representing the more distal muscles of the forelimb whilst it was surrounded by a "horseshoe" shaped zone of muscle representation of more proximal muscles such as deltoid. The authors go on to explain that this might allow for functional synergies between proximal and distal muscles of the forelimb.

In stark contrast, Graziano and colleagues investigated a similar hypothesis with a much stronger stimulus (100 μA) and applied this for a longer duration (500ms) (Graziano et al., 2002). They found that complex movements (e.g. "the left hand closed in a grip posture with the thumb against the forefinger the forearm supinated such that the hand turned toward the face, the elbow and shoulder joints rotated to bring the hand smoothly to the mouth, and the mouth opened.") when the stimulus was administered to the precentral cortex. They argue that this duration and intensity might be the intensity required for the behavioural and physiological response during voluntary movement (e.g. Georgopoulos et al., 1986, Reina et al., 2001). This is highly controversial research, as it is difficult to argue that 'natural' discharge within M1 is as highly synchronised and intense as that evoked by long trains of ICMS.

1.2.6 Functional properties of cortical circuits involved in grasp

Grasping an object requires a sensory-motor transformation of the object's properties (size, shape, orientation) to an appropriate pattern of hand and digit movements necessary for efficient grasp. This involves processing of the object's precise location with respect to the hand and the integration of the object's intrinsic properties. These 'intrinsic properties' will be transformed into an appropriate motor command that will coordinate a reach and grasp phase to lead to a successful and meaningful grasp.

Jeannerod et al. (1995) first proposed that this transformation involved a visuomotor grasping circuit, comprising anterior intraparietal (AIP) area, premotor cortex (F5)

and primary motor cortex (M1). This is also referred to as the dorso-lateral pathway. The visuomotor grasping circuit has been suggested to carry out three main functions: sensorimotor transformations, action understanding and action selection during execution (Rizzolatti and Luppino, 2001).

AIP. Classically, antero-lateral parts of the intraparietal sulcus carry and integrate more grasp-related information by processing intrinsic features of the object to guide hand shaping, in contrast, the antero-medial regions which might have a role in the reach and transport phase of the grasp by processing the extrinsic properties such as spatial location. Area AIP contains neurons that respond to different grasps. They are typically visual neurons; they respond to object presentation but do not have a motor component, and are silent when monkeys grasp in the dark. In addition there are visuomotor neurons, which respond not only to object presentation but also during the actual movement (Sakata et al., 1995, Murata et al., 2000).

F5. It was suggested that F5 provides a motor 'vocabulary' for the motor cortex to select (Rizzolatti et al., 1988, Umilta et al., 2007). It has been shown that different types of grasp modulate neuronal activity within this circuit at both the single neuron level (Umilta et al., 2007) as well as local field potentials (LFPs) (Spinks et al., 2008).

Understanding how grasp as opposed to reaching is coded in neuronal activity has led to much research using objects that require different types of grasps, such as precision grip, whole hand grasp and a hook or ring grip (Umilta et al., 2007, Lemon, 2008, Spinks et al., 2008). These detailed experiments have shown that macaque premotor cortex area F5 contains 'canonical' visuomotor and motor neurons that are selectively active for specific objects that afford specific grasps (Raos et al., 2006,

Umilta et al., 2007). In agreement with F5 neuronal firing rates having different firing rates for different grasps, it has been shown that F5 neuronal activity can be used to correctly decode six different grip types. This can even be accurate using the activity during the visual presentation of the object and not just during the movement (Umilta et al., 2007, Carpaneto et al., 2011). In a similar study, grasp information was decoded (precision grip vs power grip) best in area F5 (90.6%) whilst wrist orientation was better decoded in AIP, although maximum decoding performance was achieved when using neural activity recorded from both areas simultaneously (Townsend et al., 2011).

In addition, it has also been shown that LFPs (represent the net inhibitory and excitatory synaptic activity in a large neuronal population) recorded in the same area might also carry this same information (Spinks et al., 2008). In future applications, this information might be used to control a BMI (brain machine interface). However, it is unlikely that movement onset is encoded within F5 since many cells do not fire at movement onset, rather, this might be coded more downstream in area M1 (Carmena et al., 2003).

M1. It has long been known that M1 shows marked activity during grasp, and that some neurons are specifically active for particular types of grasp (Muir and Lemon, 1983). A recent study compared the activity of neurons in M1, including PTNs, with that in F5. All populations showed well-tuned activity for a particular set of six different grasps (Umilta et al., 2007). The authors calculated a 'preference index' (PI) for each neuron, which represents a quantification of the grasp-specific tuning (a high PI value reflects high selectivity for grip). While 47% of neurons recorded in F5 had a

value between 0.6-1, this was only true for 22.6% of M1 neurons. While F5 neurons showed significant tuning during the period in which the monkey was required to observe the object, but not grasp it, this grasp-selective activity in the presentation period was not seen in M1 neurons. Grasp-specific discharge in M1 first emerged once the monkey was cued to reach and grasp. These results are consistent with the theory that F5 provides the motor 'vocabulary' to the motor cortex (providing the goal of the action and the way in which it would be executed) (Rizzolatti et al., 1988, Umilta et al., 2007).

According to this theory, it is the corticospinal projections from M1 (approximately 50% of total corticospinal projection from the frontal lobe) (Dum and Strick, 1991) which constitute the major pathway through which the hand and digits are controlled (Lemon and Griffiths, 2005, Lemon, 2008).

1.2.7 Dorso-medial and dorso-lateral pathways for reach and grasp?

MIP (medial intraparietal area) and V6A (both are part of the anteromedial grasp network) house neurons that fire for reaching movements and the intention to move (Eskandar and Assad, 1999, Andersen and Buneo, 2002). Snyder et al. (1997) reported, using a dissociation task in monkey, that the activity of many of the MIP neurons they recorded from fired during a reaching movement and that when both a reach and a saccade were planned, delay-period activity reflected the intended reach and not the intended saccade (Snyder et al., 1997). These ideas developed into a theory whereby reach and grasp depended on different (dorso-lateral and dorso-medial) parieto-frontal circuits (Jeannerod et al., 1995, Caminiti et al., 1996).

More recently, the idea that the dorso-medial and dorso-lateral pathways carry, respectively, information about reaching and grasping has been challenged. Neurons related to both reach and grasp have been described in posterior parietal area V6A, traditionally part of the 'dorsal-medial' system. A large proportion of V6A neurons showed selectivity for one or more grips through unique firing rates (Fattori et al., 2010). The authors validated their conclusions by ruling out other factors like different visual inputs, reach modulation and also wrist orientation (Fattori et al., 2009, Fattori et al., 2010). Evidence from human studies suggest that putative human AIP also has a role in categorising motor acts depending on whether the motor act involves movements away from the body or movement towards the body (Jastorff et al., 2010).

Another area that has been shown to conflict with the classical model is area F2 of the macaque (or more generally PMd), it has been shown that there is grasp information coded here (Raos et al., 2004, Stark et al., 2007), although it is not surprising giving PMd's dense anatomical connections with M1 and PMv (Dum and Strick, 2005, Boudrias et al., 2010).

At the opposite end of the spectrum, it has been shown that in classical grasp areas e.g. AIP (Baumann et al., 2009) and F5 (Fluet et al., 2010) 'grasp' neurons might be influenced by orientation of the wrist as well as by grasp itself. For example, in F5 these authors showed that object orientation (27%) and grip type (26%) were equally encoded after the cue was given to the monkey. However, it is important to note that during the actual movement period, orientation was less represented than grip type (Fluet et al., 2010). This brings to light the problems with classifying the 'reach' and

transport phase from the 'grasp' phase and ultimately shows that there is a great deal of overlap between the two phases.

1.2.8 The cortico-cortical transfer of information related to grasp

Although it is now clear from the foregoing sections that AIP contains neurons that respond to the features of a graspable object and that PMv might code for a repertoire of possible goal directed grasps, it is important to stress that these characteristic properties are not intrinsic to the respective areas, but are more likely to have been determined by the various inputs and outputs of other components of the grasping circuit/network (Davare et al., 2011).

PMv and the M1 hand area are richly interconnected (Muakkassa and Strick, 1979, Godschalk et al., 1984, Matelli et al., 1986, Dum and Strick, 1991, Dum and Strick, 2005). In the macaque, area F5 (and especially area F5p) is strongly interconnected with the M1 hand representation (Matelli et al., 1986, Gerbella et al., 2011). It is likely that these connections define some of the functional properties of F5 and M1 (Schmidlin et al., 2008).

The transfer of information has been investigated using techniques which require perturbing or stimulating the circuit whilst recording from another component of the network (Cerri et al., 2003, Shimazu et al., 2004, Prabhu et al., 2009). Studies carried out in monkeys show that by applying a conditioning stimulus (with a single stimulus subthreshold for any overt motor effects) in area F5 they are able to bring about a strong facilitation of outputs from M1 (measured by the effect on hand muscle EMG

responses to a M1 test stimulus). In humans, a similar paired pulse TMS regime was used by Davare and colleagues to show that there is a muscle specific interaction between areas AIP, PMv and M1, which varies according to which grasp the subject is preparing (whole hand grasp vs precision grip) (Davare et al., 2008, Davare et al., 2010).

If needs be, the transfer of information can be extremely fast. In the monkey, the condition-test interval experiments indicated interaction delays of a few milliseconds, and M1 neurons respond to F5 stimulation with latencies of only 1.8-4 ms (Kraskov et al., 2011). In the human TMS experiments of interaction delays were only 6-8 ms, which scale with the human-monkey conduction differences. Indeed, the entire process of obtaining and processing visual information about an object to formulate an effective grasp is extremely fast and has been shown to be around 100-150 ms (Loh et al., 2010). The temporal precision that can be gained from single neuron and TMS studies is far higher than with other more non-invasive techniques such as fMRI which are known to have poor temporal resolution (Kim et al., 1997).

Reversible inactivation of the hand area of macaque primary motor cortex (M1) while simultaneously stimulating ventral premotor cortex (F5) demonstrated that the strong conditioning effects of F5 stimulation on motor responses in the hand were probably mediated via F5 connections to M1 and its corticospinal outputs (Shimazu et al., 2004). In one inactivation experiment the authors chronically implanted electrodes into the hand areas of M1 and F5 in 3 macaque monkeys and used muscimol (selective agonist for the $GABA_A$ receptor) to depress activity in area M1. They found that the motor effects evoked by repetitive ICMS in area F5 stimulation

depended on cortico-cortical interactions with M1 since muscimol injected into M1 completely blocked the effects evoked by rICMS (repetitive intra cortical micro stimulation) administered in area F5 (Schmidlin et al., 2008).

Another way of looking at cortico-cortical interaction, without resorting to the use of unnatural electrical stimuli, is to investigate how the LFP in one brain area might have a role to play in the timing of spike generation in another. Kraskov et al. (2010) have shown that the LFP in M1 is more coherent with single unit activity in F5 than units within M1, and vice versa, that is, the LFP in F5 is more coherent with M1 single unit activity than F5 unit activity. It is suggested that LFP activity might act as a means of synchronising activity in these two brain areas, which is likely to enhance the transfer of information between them.

The human fMRI literature has also added to the debate. Using dynamic causal modelling Grol and colleagues (2007) assessed parieto-frontal connectivity and found that grasping large objects increased couplings within the dorsomedial circuit (PMd and V6a), in contrast grasping small objects was coupled mainly with the dorsolateral circuit (AIP and PMv), although these authors argued that there was a large degree of overlap within the circuit for 'grasp' and 'reaching' (Grol et al., 2007). It has also been shown that the ventral and dorsal streams integrate information when needed. The AIP-PMv circuit was shown to be coupled with the lateral occipital complex (Verhagen et al., 2008). This could be important as a means of providing information about the object properties to the dorsal stream such that there is online adaptation of the grasp. It also suggests that the network is plastic and that one

stream might be able to access another in order to ensure that the upcoming grasp is carried out accurately.

Davare used TMS in human volunteers to provide a conditioning stimulus to PMv and revealed that there is a grasp-specific modulation of M1. It was revealed early during the preparation for grasp, just after subjects could see which object was to be grasped, by the presence of larger MEPs (motor evoked potentials) in the muscle that will be used in the upcoming grasp e.g. planning a precision grip for the grasp of an object such as a pen will mean that there is facilitation of the FDI muscle in comparison to ADM, whilst preparation for whole hand grasp will have the opposite modulation, whereby ADM will be facilitated more than FDI (Davare et al., 2008). Paired pulse TMS using a C-T (conditioning – test pulse) interval of 6-8 ms was optimal for this facilitatory effect; MEPs were recorded from FDI and ADM muscles. This experiment shows that, via PMv-M1 interaction, visual information about an object is used to facilitate a specific pattern of muscle activity which is appropriate for the grasp to being carried out.

One way of understanding the function of the AIP-F5-M1 circuit is to perturb its constituent areas of the circuit. Davare et al. (2010) showed, by using repetitive TMS (cTBS) to induce virtual lesions in human AIP, that the normal grasp specific modulation between F5 and M1 that appears during grasp preparation was significantly reduced. This perturbation was not through direct modification of corticospinal M1 excitability but indirectly through PMv and M1. This is because at rest, cTBS to AIP did not modify PMv-M1 interactions. During preparation for grasp,

this interaction was modified and the 'virtual lesions' led to a loss of the grasp-specific pattern of muscle activity of the digits. cTBS over a control parietal brain area did not have this effect.

This result can be explained at the neuronal level in terms of a specific subpopulation of neurons in AIP being activated by the sight of an object. Grasp-related information is then transferred to another population of neurons present in PMv, in PMv the populations of neurons that are activated are specific to the grasp, such that if the upcoming grasp is whole hand grasp, the 'motor vocabulary' present in PMv affords the use of ADM rather than FDI. This information is finally sent to M1 where neurons can control the specific muscles required for grasp. The authors postulate that when AIP is perturbed, the selectivity of grasp specific information is not passed to area PMv and thus it has reduced the selection of a "motor vocabulary" within PMv and leads to less accurate grasp of the object.

Importantly, the perturbation is only present when a grasp is being carried out and not at rest, the authors argue that this might be because the canonical (the cells respond during action execution and respond to the presentation of a graspable object) and object-related neurons in PMv and AIP have low firing rates at rest. There is normally a net inhibitory effect of PMv on M1. A virtual lesion to AIP does not affect this net resting state inhibition because at rest AIP neurons are mainly inactive. However, when subjects prepare to grasp an object, a population of AIP neurons that are tuned to the intrinsic visual properties increase their firing rates and thus selectively provide information to PMv.

1.3 MIRROR NEURONS

1.3.1 What are mirror neurons?

A key set of functions in the human brain concern the understanding of the movements, feelings, moods, intentions and emotions of other human beings. The first step in revealing the mechanisms that underpin such functions was at the level of movements, and in particular exploring the relationship between an individual's own movements and those of others. One way in which we might understand the movement of others might be through representing the motor event in the same brain area that brings about one's own movement. Mirror neurons, which were first discovered in the ventral premotor cortex of the macaque monkey, have been proposed as a mechanism by which this occurs (di Pellegrino et al., 1992, Jeannerod et al., 1995, Gallese et al., 1996, Rizzolatti et al., 1996).

'Classic' mirror neurons are neurons that increase in activity typically when an individual performs a motor action as well as when the action is carried out by another individual (Fabbri-Destro and Rizzolatti, 2008, Kraskov et al., 2009). This property of mirror neurons implies that when a monkey observes a motor action that resembles its own, the action is automatically retrieved and represented into the motor system but not necessarily executed (Rizzolatti et al., 1996). In general the actions involved are transitive i.e. there is an interaction with an object, and the goal of the action is clear (e.g. grasp, tear). The original studies suggested that mirror neurons are less strongly activated by intransitive actions (Gallese et al., 1996).

The encoding of the motor 'goal' is a predominant feature of mirror neuron studies. In an experiment involving mirror neurons in the parietal lobe, 75.6% of mirror

neurons were shown to be modulated by the goal of the action. This led to the understanding that by 'goal coding' our actions, we have 'kinetic melody' (Fogassi et al., 2005), that is, it allows execution of a complete motor act made up of movements involving different effectors (e.g. hand and mouth) without breaks or gaps. Many of the studies reporting mirror neurons have shown them to be specific for the way in which a goal-directed movement is achieved. Gallese et al., (1996) showed that there were mirror neurons activated for each stage of grasping the object, and subsequently named them 'grasping neurons', 'placing neurons' etc. Grip specificity (precision/whole hand) is not a pre-requisite for defining a mirror neuron, as the degree of similarity between grip types required to elicit activation of a mirror neuron can vary (Rizzolatti et al., 2009).

Thus there can be a close resemblance between the observed and executed grasping movements that activate a mirror neuron, such as for the 'strictly congruent mirror neurons' (di Pellegrino et al., 1992). Alternatively, there is only a broad correspondence for ('broadly congruent') or, less often, 'non-congruent' neurons (Gallese et al., 1996). Initial studies suggested that mirror neurons only respond to natural actions that are directly viewed and not to video images (Ferrari et al., 2003), but more recently videos have been used to elicit mirror neuron responses (Caggiano et al., 2011).

Theories as to how the 'mirror neuron system' comes about through associative learning have been developed by Keysers & Perrett (2004). The authors further developed the Hebbian idea that 'neurons that fire together, wire together'. They

argue that neurons can show mirror modulation during observation of movement if there are direct anatomical connections and if the events of an action systematically follow each other, e.g. since vision of reaching is always before grasping during execution they might become strongly linked so that vision of another person executing an action could lead to the neuron firing. More recently, these ideas have been challenged (Hickok and Hauser, 2010, Caggiano et al., 2011). Early studies suggested that when monkeys observed a grasping task being achieved with a tool, as opposed to a biological effector (hand/mouth), the activity was weak or absent altogether (Gallese et al., 1996), but once again this has been challenged (Ferrari et al., 2005), highlighting the controversy surrounding the role of mirror neurons.

1.3.2 In which brain areas have mirror neurons been found?

There have been three main areas of the cortex that have definitively been shown to contain mirror neurons; F5, IPL and AIP of the macaque. More recently, it has been suggested that M1 might also contain classical mirror neurons. However, there is currently a lack of definitive evidence.

F5

Mirror neurons were first discovered over twenty years ago in area F5 (di Pellegrino et al., 1992). In one of the original studies, the neurons responded when the monkey grasped a piece of food presented on a plate, and also when the same piece of food was being grasped by the experimenter (Gallese et al., 1996). The mirror neurons in this area have been mainly found in the convexity (F5c) (di Pellegrino et al., 1992,

Gallese et al., 1996, Rizzolatti et al., 1996). In addition, corticospinal mirror neurons have also been reported in area F5 (Kraskov et al., 2009), meaning that some mirror neurons have direct access to the spinal cord to affect downstream spinal targets.

IPL

IPL (consists of PF, PFG and PG areas) also contains neurons that have mirror properties and have been reported mainly in area PFG (Fogassi et al., 2005, Rozzi et al., 2008). IPL is characterised by different sensory, motor and eye-related behaviours. PF is typically somatosensory, PFG responds to hand and mouth actions and has rich connections with the visual system MST (middle superior temporal) and areas of the STS (superior temporal sulcus), thereby receiving higher-order visual information (Seltzer and Pandya, 1978). The STS contain neurons that respond to biological motion but are inherently not motor (neurons are not activated during the individual's own actions). Therefore they have not been regarded to be part of the mirror neuron system.

AIP

Mirror neurons have been identified within this area (Buccino et al., 2001, Shmuelof and Zohary, 2005) and because it is an area that has purely 'visual neurons' (Sakata et al., 1995), it has been suggested that area AIP plays a major role in proving object grip affordances (Taira et al., 1990, Baumann et al., 2009). AIP also receives connections from the middle temporal gyrus (Borra et al., 2008). This input could provide the mirror areas with information concerning object identity. There are additional connections from AIP to area F5a (Rozzi et al., 2006) and thus there is a

direct connection for the information gathered by AIP to enter the premotor areas and motor grasping network.

M1

M1 has not been generally considered to be part of the mirror neuron system (Gallese et al., 1996, Fogassi et al., 2001). However, there is conflicting evidence from fMRI, TMS (Fadiga et al., 1995, Baldissera et al., 2001, Montagna et al., 2005) and other non-invasive techniques, suggesting that M1 might have mirror properties. In contrast, some single neuron studies in monkey have not found direct evidence of mirror neurons in M1 that modulate their spiking activity (Gallese et al., 1996, Fogassi et al., 2001). Instead, the mirror effects elicited through TMS and other non-invasive methods may be detecting the influence of remote areas on M1 (this can be measured by modulations in LFP), rather than M1 itself. These effects can be as a result of stimulation of M1 neurons that have lower thresholds for activation due to direct synaptic connections with F5 mirror neurons rather than spontaneous spiking activity (Hari et al., 1998).

More recently, there have been reports that M1 might also contain mirror neurons (Dushanova and Donoghue, 2010). This hypothesis might come as a surprise considering that M1 has been classically considered as an output area to directly control the musculature. However, Dushanova and Donoghue (2010) found M1 'view' neurons that not only fired during a visuomotor step-tracking task (a point to point arm reaching task), but also when viewing an experimenter carry out the same task. They also found that the 'view' neurons were spatially intermixed with the

neurons that were only active during actual movement; these neurons were named 'do' neurons.

In the 'view' condition, the experimenter stood next to the monkey and moved the manipulandum to perform the task in an identical fashion. In another session the experimenter's moving hand was contralateral to the monkey's 'moving' arm rather than ipsilateral.

To control for any covert movement that might account for the discharge of the neurons during the viewing tasks the monkeys were required to hold switches using sustained finger flexion. The first 100 ms after the 'Go' cue was excluded from the analysis because it might be confounded with perisaccadic activity (Cisek and Kalaska, 2002). An interesting observation is that firing rates were significantly lower during viewing only compared with when the monkey actually made a movement.

The authors make the distinction between 'mirror' neurons and 'mental rehearsal' neurons. The difference being that mental rehearsal neurons exhibit activity during both execution and observation of a movement but are active at an earlier time moment reflecting rehearsal of an upcoming learned action (Cisek and Kalaska, 2004). Mental rehearsal neurons modulate their firing rates depending on whether the trial is rewarded, by contrast mirror neurons do not. In the study described above the authors report that their neurons were influenced by reward expectancy making them more likely to be neurons involved in mental rehearsal rather than mirror neurons. Surprisingly, they found that the cells fired less when reward was likely. However, the distinction between mental rehearsal neurons and mirror neurons is still controversial and unclear.

1.3.3 STS and the action observation circuit

The STS is not generally considered to be part of the 'classical' mirror neuron system, but certainly has features relevant to the system. This area contains cells that respond to complex visual biological stimuli, but does not appear to have a role to play during self-movement (Rizzolatti and Luppino, 2001, Nelissen et al., 2011). It has relevant neuroanatomical connections: STS provides visual information for the IPL and particularly area PFG, and this has projections to area F5c, a known mirror neuron area. There are no direct connections between the STS and area F5c (Rizzolatti and Luppino, 2001).

In a recent fMRI study in monkey, it has been shown that there are two functional routes active during action observation of grasp; the first links STS to F5 via PFG and the second via AIP. Observation of grasping activated MT/V5 and its satellites, three STS regions (STPm, LST and LB2). They also found that the PFG route was more active when an agent was involved compared with a video, whilst the AIP route was more sensitive to the object (Nelissen et al., 2011).

1.3.4 The different types of mirror neurons

In their landmark study, Gallese et al. (1996) carried out experiments in which a macaque monkey was trained to pick up objects on cue as well as observe a similar action made by the experimenter; they showed that there were mirror neurons activated in each stage of grasping the object, and subsequently named them

'grasping neurons', 'placing neurons', 'manipulating neurons', 'hand-interaction neurons' and 'holding neurons' (Gallese et al., 1996). These authors showed that in most cases there was a clear relationship between the visual action to which they responded and the motor response they coded. Accordingly, they classified some mirror neurons as strictly congruent: in these cases both the observed and executed action were the same in terms of general action (e.g. grasping) and in terms of the way in which the action was executed (e.g. precision grip). For 'broadly congruent' neurons there is similarity but not identity between the observed and executed actions; one type of broadly congruent neuron was activated by the goal of the action, irrespective of the manner in which the goal was achieved. For the final group of 'non-congruent' neurons, no link was found between the observed and executed actions to which the neuron responded (Gallese et al., 1996).

Since these initial studies, further important properties of mirror neurons have emerged:

Mirror neurons fire dependent on location in space

F5 mirror neurons have been shown to contain groups of neurons that modulate their activity dependent on whether the motor act observed was carried out in the monkey's peripersonal (26%) or extrapersonal space (27%). This finding was furthered by peripersonal neurons switching their preference in 43% of cases when a front panel was placed so that the monkey could observe the motor event being carried out in the peripersonal space but would no longer be able to reach the object. Thereby mirror neurons had been coded in an operational manner, supporting the

idea that they also allow the observer to realise whether they can interact with the action/object being observed (Caggiano et al., 2009).

Mirror neurons trigger when the object is just out of sight

In an experiment where the last visual part of the motor event (precision grip of an object) was hidden by an opaque screen, over 50% of identified mirror neurons in F5 continued to discharge. There were enough visual clues to create a mental representation of the event strongly supporting the role of mirror neurons in understanding and possibly predicting actions (Umilta et al., 2001).

Other instances of mirror neuronal activity

The sound of breaking a peanut or tearing a piece of paper being performed by the experimenter is also able to activate mirror neurons (Keysers et al., 2003). However, sound alone often produced a significant but smaller response and highlights the importance of vision in action recognition (Kohler et al., 2002).

View based encoding of actions

Caggiano et al., (2011) manipulated the viewing perspective of the action (0, 90 and 180 degrees) being observed whilst simultaneously recording from area F5. In addition, they investigated actual vs filmed actions. They showed that the percentage of neurons responding to filmed actions was less than for actual actions, this challenges previous findings suggesting that mirror neurons are not active for filmed movements (Ferrari et al., 2005). A small proportion of mirror neurons (17/224) actually had a preference for filmed actions over actual actions.

The authors showed that mirror neurons can having different firing rates for the angle of viewing depending on whether the neuron is tuned for 90 degrees, 180 degrees or for 0 degrees. Interestingly, they did not investigate a 270 degree angle. One might expect them to have a similar discharge as for 90 degrees if they are achieving an understanding of the position to the monkey.

Although the authors provide evidence of some view based encoding within area F5, many of their neurons had overlapping view preferences suggesting that they do not have a view-based preference. Many of the neurons were also selective for 0 degrees (first person perspective), which might imply that mirror neuron activity represents the first person perspective and therefore such neurons might have more of a role in motor learning rather than in action understanding (Caggiano et al., 2011). One possible reason for finding more neurons tuned to 0 degrees is that the object appeared to be larger on the screen for the 0 degree view (as displayed by their figure), although F5 is not known to have retinotopic representations which might somehow represent the size of the image. Another possible explanation might be that the monkeys were participating in mental imagery or rehearsal, and this perhaps highlights a problem of using filmed actions.

On a side note, it is important to note that these authors achieved the view based experiment by using video images, which means that all the actions were, in effect, carried out in the extrapersonal space (as they are within the screen) and the monkey was therefore unable to interact with any of the objects. However, their other results on actual vs filmed actions make it unlikely that this factor could explain their findings on view-based variation in mirror neuron discharge.

The implications of this experiment is that only ~14% of F5 mirror neurons reported by Caggiano et al. (2011) responded to filmed actions alone, meaning that if videos are not used for observation of actions, it is unlikely that many mirror neurons will go undetected. In agreement with the findings of Kraskov et al. (2009), the data show that some mirror neurons exhibited suppressed discharge during action observation, since the average activity of some mirror neurons, after subtraction of the baseline firing rate, was below zero.

Goal coding mirror neurons in parietal cortex

Mirror neurons have also been discovered in PFG (Fogassi et al., 2005). The authors trained monkeys to reach and grasp a piece of food to eat or to reach and grasp a piece of good to place in a container (the container placed near the monkey's mouth). The authors found a substantial number of mirror neurons in parietal cortex and showed that mirror neurons in this area fired either for grasping to eat or grasping to place, i.e. they reflected the goal of the action, despite the kinematics of both these actions being similar (Fogassi et al., 2005). Mirror neurons in parietal cortex that responded differentially to observation of identical actions when they were embedded in different contexts were reported by Yamazaki et al., (2010). These authors also found another type of mirror neuron that showed similar responses to different actions but with a common 'motor goal': for example, when the monkey was handed a food reward sealed within a container. The neuron discharged when either the experimenter or the monkey opened the container, but it also responded when the experimenter closed the container. The neuron had a similar firing rate for

both opening and closing the container even though the kinematics of the actions was completely opposite.

The authors argue that mirror neurons in parietal cortex might be responding to an arbitrary categorisation of actions based on context. In fact, the experimenter pulled the lid off the container with her fingers, whilst the monkey pushed a button on the device to open the lid, and so the actions are quite dissimilar (Yamazaki et al., 2010). The authors go on to propose that the concept of generalisation of action understanding and semantic systems might be more developed in humans (Yamazaki et al., 2010).

These results fit quite well with previous fMRI literature where the parietal lobe was shown to be more involved when a specific goal was involved compared when there was no goal (Buccino et al., 2001). In this experiment the blood-oxygen-level-dependent (BOLD) activity of inferior parietal region was modulated whether the performed actions had goals (e.g. grasping an apple) or just mimicking the action (without the apple). Thus the neurons in this region have functional relevance to the motor act (Buccino et al., 2001). In contrast, studies by Fogassi's group have suggested that some parietal neurons respond differently according to the planned action (e.g. 'grasp to eat' vs 'grasp to place'; Fogassi et al., 2005).

Taken together, the current literature suggests that there are different classes of mirror neurons in parietal cortex compared with area F5. It might be that a combination of these types of mirror neurons provides the necessary information to fully formulate the goal and motor representation of the observed movements.

1.3.5 Mirror neurons and action suppression?

Recently it has been suggested that mirror neurons could be involved in the observer's capacity to withhold their own motor response while they watch another's actions (Kraskov et al., 2009). This capacity is obviously essential to prevent the activation of mirror neurons leading to automatic imitation by the observer. These authors showed that half of identified pyramidal tract neurons (identified though antidromic stimulation and collision tests) tested in F5 showed a significant decrease in modulation of their activity when the monkey observed a precision grip task. 17/64 of mirror neurons showed complete suppression during observation even though they were active during the period where the monkey itself was performing a precision grip to obtain a small food reward. The disfacilitation of PTNs, which themselves might provide excitatory inputs to spinal circuits controlling active grasp, could be a means of inhibition of movement during action-observation stages and, if this is the case, provides a new additional function of mirror neurons (Kraskov et al., 2009).

1.3.6 Other types of single unit activity not associated with overt movement

Mental rehearsal and motor imagery can also lead to modulation of firing rates during observation of an action but are not associated with action understanding. Single neuron studies in PMd (Cisek and Kalaska, 2004) and M1 (Tkach et al., 2007, Dushanova and Donoghue, 2010) have suggested that neurons active in action observation may reflect mental rehearsal of a learned motor action. Tkach et al., (2007) found neurons that responded to viewing a cursor move on a screen and also

when the monkey itself moved the cursor. Dushanova and Donoghue (2010) found cells that fired in response to viewing a point to point arm reaching task (mentioned above in more detail). Typically, these neurons fired in anticipation of an action instead of responding to it, i.e. they fired at an earlier time point compared with when the neuron was active during execution of the task. Thus timing of activation/suppression of a neuron during action observation vs mental rehearsal is very important in distinguishing these two types of activity. Apart from this temporal difference, the firing characteristics are the same action execution and observation. In addition, mental rehearsal neurons modulate their firing rates depending on whether the trial is rewarded (Dushanova and Donoghue, 2010).

1.3.7 Function of mirror neurons: social importance of hand function

The studies described above have broadly been taken to establish that the main function of mirror neurons is for understanding the actions of other individuals. In particular, the fact that they can be triggered when an individual is given enough mental clues to assimilate the motor goal outcome and is independent of kinematics strongly supports this argument. 'Action understanding' means that the observer of the motor action is able to 'understand' the intention of the movement. Note that the term 'understanding' may imply a cognitive process, but in fact the mirror neuron system is a fast, relatively low-level system that is primed by the action and does not require cognitive processing. The 'Direct Matching Hypothesis' suggests that actions made by others are understood when the corresponding mirror neuron is activated (Rizzolatti and Luppino, 2001), although it does not imply that it is the only way in which we understand the actions of others. This view is further strengthened by

monitoring the BOLD activity of patients without limbs during action observation. In these instances the individuals recruit the appropriate motor repertoire to carry out the goal (Gazzola et al., 2007).

Of possible relevance here is the finding that some patients with ASD (autism spectrum disorder) show an inability to understand the actions of others that they observe. It might be explained by a defect in mirror neuron activity. Cattaneo et al. (2007) showed that when normal children observe the execution of grasping to eat there was anticipatory activation of the mylohyoid (tongue) muscle (detected by surface EMG electrodes), a muscle that would normally be used during execution of the eating task. In contrast, children with ASD showed very little activation of mylohyoid.

Others have suggested that mirror neurons have a function in the internal representation of the movement so that it can assist in motor learning (Jeannerod et al., 1995). In this way it is similar to corollary discharge (Sommer and Wurtz, 2008). However, this does not preclude a function in motor preparation; as neurons do not continue to fire in the period between action and action observation in a task involving the monkey carrying out the action immediately after watching the experimenter (Gallese et al., 1996).

Another possible role of mirror neurons is that they facilitate imitation. In macaque imitation studies it is known that macaques tended to fixate on the imitator more often compared with the non-imitator. Ferrari et al. (2009) suggested that a 'direct' and an 'indirect' mechanism is present for imitative behaviours and thus underpins the function of the mirror system. The 'direct' pathway of parieto-premotor areas

with ventro-lateral prefrontal cortex may influence motor output during action-observation whilst the 'indirect' pathway could use the mirror system for complex behaviours in delayed imitative behaviour. One study shows that in infant macaques, those infants that showed greater imitative behaviours such as tongue protrusion and lip smacking (evoked by the experimenter carrying out these behaviours) resulted in better reaching-grasping abilities later on (Ferrari et al., 2009). But otherwise, it is generally thought that the capacity to imitate is not well-developed in macaques.

1.3.8 Controversy surrounding the function of mirror neurons

There has been much debate over mirror neurons having a role in action understanding. Hickok and Hauser (2010) claim that sensorimotor learning is a more appropriate idea for the function of mirror neurons. They claim that observed actions are inputs for action selection and argue that this can be experimentally tested by showing that action understanding and the motor system functionally dissociate, i.e. that animals can understand actions that they cannot execute.

This hypothesis is extremely hard to test. Firstly, the term 'understanding' must be better defined. It appears that Hickok and colleagues interpret understanding as a high level cognitive process, whilst others might instead interpret it as a low level priming system.

1.3.9 Investigating the mirror neuron system in humans

Non-invasive imaging techniques have been used in humans to assess the action observation circuit. Their use has provided ideas that there might be homologous areas in human cortex to monkey area F5. The human inferior frontal gyrus (IFG) has been the main focus for studies aimed at identifying a mirror neuron system in the human brain (Decety et al., 1997, Buccino et al., 2004, Iacoboni et al., 2005, Kilner et al., 2009). There is one published account of an invasive study in humans, in which single units with mirror neuron properties were found, but in brain areas that are not really considered to be part of the mirror neuron system in the monkey (SMA and hippocampus) (Mukamel et al., 2010).

TMS is a common technique used to evaluate the corticospinal excitability. Fadiga and colleagues were the first to show an increase or facilitation of MEPs during observation of an action using this technique (Fadiga et al., 1995). They showed that MEPs elicited from flexor digitorum superficialis and the first dorsal interosseous muscles were facilitated, compared with rest, when observing the fingers of an actor closing on an object. They used two control conditions to show that these effects were not due to motor preparation or unspecific factors such as arousal or attention.

Similarly, Catmur et al. (2010) carried out TMS experiments during observation of actions but instead applied the TMS to areas PMv and PMd to elucidate whether these brain areas might contribute to the facilitation effects seen by Fadiga and colleagues. They report that premotor-M1 connections modulate M1 corticospinal excitability at 300 ms after onset of the observed movement. Interestingly, they showed that the mirror effects they see could be 'reversed' with 'counter mirror

training' (in which you are trained to perform one movement while observing a movement involving a different muscle) and that the counter mirror effects were modulated by stimulation to premotor cortices.

Their research highlights several points. Firstly, that PMd might have mirror activity, supporting other work in the monkey (Cisek and Kalaska, 2004, Dushanova and Donoghue, 2010). Secondly, that counter-mirror training modifies the same brain areas involved in the original mirror effects. This implies that there is a degree of flexibility through which mirror properties can be forged and supports the idea proposed by C. Heyes on the associative learning theory (Press et al., 2011). This theory suggests that any given motor area that has access to sensory information has the potential to develop mirror effects given enough experience (Heyes, 2010).

Thus far, there has been only a very limited analysis on the latency of mirror neuron activation with respect to the observed movement; however the mirror effects seem to be present much later in premotor cortex (300 ms) than for action selection and grasping (~150 ms; see Prabhu et al., 2007). More research on the temporal activation of mirror neurons is clearly required to further elucidate their role.

Cortico-cortical interactions during an observed movement have been first investigated by Strafella & Paus (2000). Using a paired pulse TMS regime, they showed that action observation resulted in a reduced intracortical inhibition at 2 ms interstimulus interval (ISI; short-interval cortical inhibition 'SICI') and reduced facilitation as well at 12 ms ISI (SICF; short interval cortical facilitation) (Strafella and

Paus, 2000). They again found a muscle-specific effect in the observed action. They used three different conditions to make their observations: rest, observation of hand-writing and observation of arm movements. Whilst the test stimulus alone induced a facilitation specific to the muscle involved much like the earlier work described above (Fadiga et al., 1995, Catmur et al., 2010), they found that in the hand-writing condition there was reduced cortical inhibition and reduced facilitation, these being the driving factors for the excitatory drive onto the corticospinal neurons generating the signals for movement that can be influenced by TMS (Floeter and Rothwell, 1999). It is interesting that they found a conflict in the cortical processes, in that a reduced inhibition and reduced facilitation might lead to no overall change in the corticospinal excitability. This might be because there are inhibitory and facilitatory effects at play during observation of movement.

Functional MRI techniques have also been used to determine possible mirror areas (Kilner et al., 2009). The problem is that although there is a large spatial overlap between areas involved during execution and observation of movements in the IFG (Rizzolatti et al., 1996, Buccino et al., 2001), and many of the neurons in the monkey homolog of these areas, such as the 'canonical ' visuomotor neurons are known not to have mirror properties (Rizzolatti and Craighero, 2004). Kilner et al. (2009) found that the average peak execution area was 6.7 mm more lateral than the action observation area, suggesting that the locations of peak effects are in different areas.

Thus there is an on-going debate as to whether this approach can confirm the existence of mirror neurons in the human brain, as opposed to two overlapping

populations of neurons located within the IFG (see Kilner et al. 2009). In order to overcome this difficulty, Kilner and colleagues used a 'repetition suppression' approach to reveal genuine mirror neuron activity in the human IFG. This technique is believed to select mirror neuron activity because repeated stimuli bring about activation of the same neuronal population, leading to the amplitude of the response adapting or reducing (Grill-Spector et al., 2006, Dinstein et al., 2007, Dinstein et al., 2008). This phenomenon should only be present if the same population of neurons are activated by both action observation and execution. The authors found that there was a small area within human IFG that suppressed when an action was followed by observation of an action and it also suppressed for the reverse situation when observation was followed by execution, and thus provided evidence of mirror activity in human IFG (the homolog of monkey area F5 where mirror neurons have been found).

In the only study to date of direct recording of human mirror neurons Mukamel et al. (2010) recorded from 1177 cells in human medial frontal cortex and temporal cortex whilst patients grasped or observed grasp as well as facial expressions. The authors found that many of the cells were active for both the conditions, thereby satisfying the main mirror neuron criterion. They even found cells that were active during execution but suppressed during action observation much like the neurons found in a recent paper outlining a new class of mirror neuron (Kraskov et al., 2009).

The spinal circuitry during action observation has been investigated through measurements of the H-reflex. Initially it was proposed that the modulation of the H-reflex during observation of hand action contradicted the findings of Fadiga

(Baldissera et al., 2001), as motoneurons of the FDS (flexor digitorum superficialis) muscle had a decrease in H-reflex facilitation during hand closing. However, in a subsequent experiment using a detailed temporal EMG study it was shown that in all subjects the FDS muscle showed peak activation during the hand-opening phase. This finding highlighted that the decrease of FDS facilitation during hand closing is not inverted mirror behaviour but directly reflects its activation pattern during action execution (Montagna et al., 2005).

Ideas about the organisational principles of mirror activity in humans have been investigated using experiments involving different effectors (Jastorff et al., 2010). In a recent study, video clips contained images of different effectors (foot, hand, and mouth) used to carry out a series of motor acts. They found that premotor cortices had activity that grouped according to the effector whilst activity in the IPL clustered according to the type of motor act. Movements bringing the object toward the agent (grasping and dragging) activated more ventral areas compared with the opposite type of movements. These results suggest that the representations of hand motor acts in human AIP are used as templates for coding motor acts executed with other effectors. This study indicates that mirror neurons in different areas might have different functions and extract different sensory information depending on anatomical connections with other brain areas.

1.4 INHIBITION OF MOVEMENT

Classically, inhibition of movement has been thought to originate from frontal areas of the cerebral cortex, more specifically dorsolateral prefrontal cortex, inferior frontal cortex and orbitofrontal cortex (Aron et al., 2004b). These ideas have been formed using data from lesion studies; patients with frontal lobe damage typically are unable to inhibit their movements or impulses. In addition the greater the damage to the pre-frontal cortex the worse the response inhibition as measured using the stop signal reaction time (the reaction time for inhibiting a response that has already been cued) (Aron et al., 2003). Similarly, in monkey lesion experiments, performance during a No-go task is impaired (Iversen and Mishkin, 1970).

Patients with focal hand dystonia (FHD) have been reported to have behavioural abnormalities during voluntary inhibition tasks, in that they have a higher threshold for eliciting intracortical inhibition within M1 (Stinear and Byblow, 2004). The reasoning behind this is thought to be due to basal ganglia dysfunction (Hallett, 1998). In addition, patients with ADHD (attention deficit hyperactivity disorder) have higher thresholds for intracortical inhibition and suggest that both groups of patients might have abnormal inhibitory function within motor cortex (Moll et al., 2000). FMRI analysis has also consistently shown that inhibition activates right-lateralised IFC regions (Garavan et al., 1999, Rubia et al., 2003).

Inhibiting a response is thought to be a cognitive process (Verbruggen and Logan, 2008, Verbruggen and Logan, 2009). This has been classically tested using Go/No-go tasks and stop-signal tasks. Several principles for inhibition are thought to be at play. One example is task-set switching. The idea is that there is a “switch-cost” when

halting an action. This is calculated by subtracting the average reaction time of trials where a switch in behaviour did not have to be made from the average reaction time of switch trials. This is because switching a response requires a different attentional modulation and subsequent preparation of a different response.

Inhibition during one trial is known to have an after effect on the subsequent trial. That is, if you suppress a response to an object then responding to that object in the next trial has been shown to be slower in comparison to another object in that position. This is known as negative priming. The importance of these findings is that suppressing movements will have after effects, maybe even at the single neuronal level, thus the order of object presentation is also important.

Importantly, inhibition in the cognitive sense is not the same as inhibition in the neurophysiological sense. It is likely that the prefrontal cortex suppresses basal-ganglia output possibly via the subthalamic nucleus (STN) as patients with deep brain stimulation to the STN have an improved response inhibition (Aron et al., 2004b). Memory can also have an important role in an inhibitory response, in that areas of DLPFC (dorsolateral prefrontal cortex) have been associated with inhibition of unwanted memories through connections with the medial temporal lobe, a central area for memory (Anderson et al., 2004).

There is a strong likelihood that there is a common circuitry to all inhibitory responses. This current circuit might involve the right prefrontal cortex, basal ganglia, motor cortex and memory related MTL (medial temporal lobe) (Aron et al., 2004b). The current thinking is that the left prefrontal cortex might maintain the typical goal related behaviour (MacDonald et al., 2000, Garavan et al., 2002, Aron et al., 2004a),

the ACC (anterior cingulate cortex) detects conflicts when the stimulus does not match the goal (Gehring and Knight, 2000), the right prefrontal cortex suppresses the inappropriate response subcortically via STN (Burman and Bruce, 1997) or through the motor or premotor cortex (Sasaki and Gemba, 1986, Kraskov et al., 2009).

TMS has also been used to try and elucidate the cortical processes during inhibition of a movement. Corticospinal excitability has been shown to be suppressed during volitional inhibitory tasks (Hoshiyama et al., 1996). This suppression is thought to be taking place in antagonist as well as agonist muscles, and is therefore non-specific. At the same time, SICI is thought to be increased (Waldvogel et al., 2000) whilst long intracortical inhibition (LICI) has been shown to be reduced, indicating that SICI and not LICI might be mediating volitional inhibition during No-go trials.

Sohn (2002) showed that volitional inhibition in motor cortex was observed 100-500 ms after the No-go cue was presented. The TMS was triggered to the average reaction time of the Go trials as this was thought to be when volitional inhibition is at maximum. Subjects were asked to extend their right index finger only after Go but to remain relaxed after No-go (Sohn et al., 2002). However, this means that the true state was not tested, as this is merely relaxation of a muscle on command, and not a true volitional inhibition (Coxon et al., 2006). The suppressed response in muscles is most likely non-specific (between agonist and antagonist) (Hoshiyama et al., 1996).

Go/No-go paradigms (Miller et al., 1992, Kalaska and Crammond, 1995, Port et al., 2001) and stop signal reaction tasks (Scangos and Stuphorn, 2010, Mirabella et al., 2011) have also been used to investigate inhibitory responses in monkeys. These have been in areas M1, PMd, SMA and pre-SMA. LFP analysis in area SMA suggests

that SMA displays changes in activity to suggest causality in inhibition of movements (Chen et al., 2010), but this finding does not tie in with the single neuron study that only found 8/335 neurons exhibiting this behaviour (Scangos and Stuphorn, 2010). The LFP however, might be reflecting the inputs from other brain regions rather than the local cortical activity (Logothetis, 2003).

1.4.1 Inhibition and areas PMv and M1

In recent times it has been suggested that PMv might also play a role in action reprogramming by inhibiting M1 corticospinal activity associated with undesired movements when motor plans change (Buch et al., 2010).

Initial studies from this laboratory have shown that PMv can facilitate rather than inhibit M1 (Cerri et al., 2003, Shimazu et al., 2004). However, in the awake monkey, both facilitatory and inhibitory effects on M1 were observed (Kraskov et al., 2011) and the motor output from M1 was affected in a grasp-specific manner (Prabhu et al., 2009). There is also evidence that PMv's activity is dynamic and based on context and that PMv inhibits M1 activity when a new action must be selected. Using a paired pulse (8 ms ISI) approach it has been shown that within 75 ms of the task change, activity in PMv modulates M1. The relatively short ISI used to measure this suggests that the connection with M1 is likely to be more direct and not via a multi-synaptic route (Buch et al., 2010).

The last cortical site before a motor command descends to the spinal cord and leads to excitation of muscles and movement is area M1. This is therefore likely to be a key

area to stop a motor response when all other up-stream mechanisms of inhibition might have failed (Aron, 2009). It is therefore highly interesting to investigate pyramidal tract neurons (neurons that have axons that pass through the medullary pyramid) because, although they are highly collateralised to reach other parts of the motor system, their final output reaches the spinal cord.

1.4.2 A spinal substrate for suppressing actions during action observation

Evidence for the involvement of the spinal cord in action observation is controversial (Baldissera et al., 2001, Montagna et al., 2005). The activity is of real importance because motor-evoked potentials provide the entire corticospinal activity, rather than the modulation of the activity within the spinal cord itself.

However, a detailed metabolic study using 8 monkeys has been performed to try and elucidate how action observation affects the spinal cord (Stamos et al., 2010). The activity of the final output of the cortex for movement (mostly through the pyramidal tract) in addition to the activity in the spinal cord might help to answer questions about the overall activity of the circuit during action observation. Ultimately, although facilitation of the cortico-spinal tract is seen during action observation, there is no overt movement, meaning that movement has to be suppressed or inhibited at some point along the pathway. The corticospinal tract can exert both facilitation (via monosynaptic and oligosynaptic pathways) and inhibition (via oligosynaptic pathways) at the spinal level (see Porter and Lemon, 1993).

In the experiment the monkeys were trained to either perform reach-to-grasp movements or to observe the experimenter performing the same movements. The

authors found that the metabolic activity (measured glucose utilisation – ^{14}C-deoxyglucose method) in the cervical enlargement of the spinal cord was suppressed bilaterally during observation whilst the ipsilateral cord was active during execution. This might help explain why we do not produce any overt movement (in the form of EMG activation) and imitate everything that we observe. One problem with this experiment was that they had to separate execution and observation parts of the experiment, thereby no monkey carried out both action execution and observation. This was due to the fact that they required a spinal cord that was only exposed to either the execution or observation condition.

The authors suggest that this inhibition might be brought about through descending control from premotor cortices as these areas have been shown to facilitate the motor cortex (Shimazu et al., 2004, Schmidlin et al., 2008, Prabhu et al., 2009), whilst inhibiting the spinal cord (Moll and Kuypers, 1977, Sawaguchi et al., 1996). This may suggest a dual mechanism; one mechanism facilitates the motor cortex outputs that lead to activation of grasp-related circuits at the spinal level, whilst another suppresses the overt movement by inhibition non-specifically at the level of the spinal cord via the premotor cortex. It is tempting to speculate that the corticospinal output from the PMv, whose function has never been fully explained, might serve to mediate movement suppression.

The question arises as to how you can have activity in the output cells of the cortex (PTNs) without the generation of overt movement. Although PTNs in area F5 have been shown to have mirror properties, it is important to assess whether the primary

motor cortex (M1) also contains mirror neurons, since this area has classically been shown to be much more closely involved in the generation of movement and contains many more PTNs. If there are mirror neurons in M1 and some of them can be identified as PTNs, a further question is whether their level of activity during execution and observation is similar.

1.5 NEUROPHYSIOLOGY OF CORTICAL CELL TYPES AND CELL CLASSIFICATION

1.5.1 Cell identification for better understanding of cell types

For knowledge to progress about the nature of the cortical activity associated with a wide range of different brain functions it is becoming increasingly important to identify the cortical neurons involved. The neocortex is comprised of a range of different pyramidal cells and interneurons, and distinguishing between these two groups of neurons in recordings made from awake, behaving animals is a key issue. Early investigators first suggested that interneurons, with high spontaneous firing rates, had 'thin' action potentials of short duration and could be distinguished from pyramidal cells with longer action potentials and lower, regular spiking pattern of discharge (Mountcastle et al., 1969). These differences were subsequently confirmed by detailed intracellular studies in brain slices from rodents (Connors et al., 1982, McCormick et al., 1985, Contreras, 2004). Given that the duration of the intracellularly recorded action potential at half-amplitude is directly related to the time between the negative trough and the subsequent positive peak of the

extracellular spike waveform (Henze et al., 2000, Gold et al., 2006), it was argued that the spike duration should provide a means of distinguishing cortical interneurons from pyramidal cells in extracellular recordings.

In vivo studies using high density recordings from rat neocortex (Bartho et al., 2004) further suggested that the duration of the unfiltered spikes provided the most reliable indicator of recordings from putative inhibitory interneurons vs pyramidal neurons. A number of recent reports in the awake, behaving monkey have applied this criterion as a means of identifying different cell types in cortical recordings, allowing better definition of local cortical circuitry underlying a variety of brain mechanisms involved e.g. in motor planning (Kaufman et al., 2010), control of arm direction (Merchant et al., 2008) and attention (Mitchell et al., 2007).

To verify the hypothesis that spike durations of extracellular action potentials can be used as a reliable classifier of cell type one would need to record from identified interneurons and pyramidal cells. One class of pyramidal neuron that can be unambiguously identified in the motor areas of the cortex is the pyramidal tract neuron (PTN). These are the layer V neurons whose axons pass through the medullary pyramidal tract, and which, for the most part, project to the spinal cord as the corticospinal tract (Humphrey and Corrie, 1978). PTNs can be identified by their antidromic discharge in response to stimulation of the ipsilateral pyramidal tract (Evarts, 1964, Lemon, 1984). The antidromic nature of the response can be verified by the collision test (Baker et al., 1999b, Kraskov et al., 2009, Lemon, 1984). The antidromic latency (ADL) of a given PTN is a reflection of axonal conduction velocity, and previous studies have shown that the ADL is also related to cell size (Deschenes

et al., 1979, Sakai and Woody, 1988), with the shortest ADL (fastest axons) being recorded from the large pyramidal neurons or Betz cells, which are a characteristic feature of the primary motor cortex.

A number of intracellular studies in the cat have shown that there is a clear relationship between the duration of the action potential and the axonal conduction velocity (Baranyi et al., 1993, Calvin and Sypert, 1976, Sakai and Woody, 1988); with 'slow' PTNs having longer spikes than 'fast' PTNs. There is, however, a paucity of comparable data on extracellular PTN spike duration from the awake, behaving monkey, in which the conduction velocity and organisation of the corticospinal tract is different to that in the cat (Lemon, 2008). As a result, whether or not spike duration could be reliably used to distinguish between interneurons and all types of pyramidal neurons in extracellular recordings in awake monkeys remains unclear.

1.6 THESIS OUTLINE

This thesis encompasses experiments in both monkeys and humans. Multi-electrode recording techniques have been utilised in areas M1 and F5 of the awake, behaving macaque monkey to attempt to elucidate some of the mechanisms that allow us to suppress our movement even though we can have a profound modulation of neurons that directly affects downstream spinal targets. After Chapter 2, where I describe the common methods to the experiments, Chapter 3 will examine whether mirror activity is present in primary motor cortex (M1) and in particular in pyramidal tract neurons (PTNs). Thereafter, a comparison of activity between action execution and action observation will be presented. In addition, the neuronal activity of mirror

neurons will be examined in relation to a No-go paradigm to examine whether suppression during action observation shares a similar mechanism with self-inhibition of movement.

Chapter 4 primarily focuses on a comparison of F5 mirror PTNs activity across execution and observation conditions, together with some preliminary findings that give us some insight into the differences of mirror activity between areas M1 and F5.

Chapter 5 utilises the same task apparatus used in the monkey experiments. However, in this case, cortical activity is measured indirectly using TMS in human volunteers. Single pulse and paired pulse techniques will show that indirect measures of cortical activity are more variable and make it difficult to make any solid conclusions.

In a separate analysis, Chapter 6 looks at whether spike duration is a reliable indicator of cell type and suggests that current techniques employed to distinguish cell types in primary motor cortex are unlikely to be sufficient.

Finally Chapter 7 will attempt to unify the results from these experiments and addresses the original question of how we are able to suppress movement during action observation.

CHAPTER 2: General Methods

2.1 BEHAVIOURAL TASK

2.1.1 Monkeys

All experimental procedures were approved by the Local Ethical Procedures committee and carried out in accordance with the UK Animals (Scientific Procedures) Act. Experiments involved three adult purpose-bred Rhesus (*M. mulatta*) monkeys, (M43, female 5.5 kg, M44, male 7.1kg and M47, male 5.0 kg). The care and housing of these animals was in accordance with guidelines for non-human primates issued by the UK National Centre for the 3Rs. Additional recorded data from two other purpose-bred rhesus monkeys (M, female 6.0 kg and L, female 5.3 kg) were kindly provided by Prof. Stuart Baker's laboratory at Newcastle University (Witham and Baker, 2007). For Chapters 3 and 4 we used data collected from M43 and M47. For Chapter 6, data were used from M43, M44, M and L.

2.1.2 Training

Initial training comprised training the monkeys to enter a training cage. The cage was used to transport the monkey into the laboratory from the housing area. Positive reinforcement techniques were used at all stages of the training and recording phases. After the monkey got accustomed and comfortable with the laboratory setting, the monkeys were trained to pull on an object (on a spring loaded shuttle) to obtain a small food reward in order to familiarise them with the concept of working to receive small food rewards. The monkeys were then trained to accept a neck restraint (a smooth metal collar). This allowed us to transfer the monkey from the

training cage into the experimental primate chair. A loose-fitting seat plate was then placed above the hips.

After surgical implantation of a tissue-friendly (Tekapeek) headpiece, monkeys were then trained to accept head restraint using a metal disc to fix the head to the recording rig. This allowed for stable recordings of extracellular single cell activity.

2.1.3 Mirror task

Monkeys were trained to perform the mirror task which involved both an action execution and action observation component. In addition, one of the monkeys (M47) was trained to perform a Go/No-go task embedded in the mirror task. The monkeys were trained until they were proficient at the task with minimal errors (in M43 it only took a few sessions since the task was very simple (see below)) and M47 – 7 months)

Monkey M43:

In this experiment, a precision grip was used by either experimenter or monkey to grasp a small food reward, placed on a table in between them. For **action execution**, the experimenter took a small piece of food and placed her hand on a homepad on her side of the table. After a short delay (1.5s) she released the homepad and placed the food reward on the table to the monkey's left where it could easily reach and grasp with its left hand (contralateral to the cortical recordings). The experimenter's release of the homepad cued the monkey's reach-to-grasp movement. Execution trials were carried out in blocks of ten trials, interleaved with those for **action observation**. In the latter, the monkey sat quietly resting its hands on the table placed in front of it. A small piece of food was placed above a central sensor on the

table, in the monkey's midline but beyond its reach (around 42 cm from the monkey's edge of the table). Each trial began with the experimenter's right hand resting on a homepad. About 1.5 s later a tone sounded which cued the experimenter to release the homepad (HPR) and slowly approach the food and grasp it in a precision grip between thumb and index finger, but not move it. The experimenter wore a glove on the right hand and this glove contained a small magnet at the tip of the index finger. As the experimenter approached the food reward, a magnetic sensor embedded in the table beneath it was activated and generated a sensor pulse. Trials were repeated once every 4-5s in a block of 10, and on average, the monkey was rewarded after every fifth trial.

Monkey M47:

The monkey sat facing a human experimenter with the carousel device between them (**Fig.2.1**); this could present a graspable object (small trapezoid, ring and sphere affording precision grip, hook grip and whole-hand grasp, respectively, see **Fig. 2.2**) to either. During **execution trials,** the monkey sat quietly, resting its hands on two homepads (HP) placed at waist level (**Fig. 2.1A**, HP-M: monkey).The experimenter placed their right hand on another homepad on their side of the carousel (**Fig.2.1A**, HP-H: human). The timeline for each trial is indicated by the coloured markers in **Fig. 2.1E and F**. Each trial began with the monkey resting both hands on their respective homepads. After a short delay (~ 0.8 s), an object (any one of the objects shown in **Fig 2.2**, e.g. small trapezoid; 9 mm x 11 mm x 26 mm; see **Fig. 2.1C**), mounted on the monkey's side of the carousel (**Fig. 2.1A**, OBJ-M), became visible when an opaque screen (**Fig. 2.1A**, S-E: screen- execution), placed in the monkey's line of sight with

the object (see **Fig. 2.2**), was electronically switched to become transparent. After a variable time period (0.8-1.5 s), a green LED came on, changing the illumination around the object and acting as a GO signal (**Fig. 2.1E**) for the monkey to release its right hand from the homepad (**Fig. 2.1E**, HPR), reach out and grasp the presented object (**Fig. 2.1D**). The object was mounted on a low-friction, spring-loaded shuttle (**Fig. 2.1C**), and the monkey was required to displace it by around 5-8 mm (**Fig. 2.1G**), applying a load force of around 0.6 N and pulling the object upwards, towards the monkey. The correct extent of displacement was monitored by a Hall effect sensor on the shaft of the shuttle, and fed back as an audible tone to the monkey. Displacement onset (DO, **Fig. 2.1E**) was determined from the Hall effect signal. The monkey held the object steadily in its displaced position for 1 s and then released it (H_{ON} to H_{OFF}), and placed his hand back to the right homepad. Around 1 s after the trial was completed, the monkey received a small piece of fruit as a reward at the end of each execution trial; this was delivered directly to the monkey's mouth.

During **observation trials**, which were interleaved with execution trials using a pseudorandom process, the roles were simply reversed. The carousel turned so that the object was now on the experimenter's side. After all the homepads were depressed, the trial began, and the same objects became visible, to the experimenter and to the monkey who viewed in through a second switched screen (**Fig. 2.1B**, S-O: screen-observation). In these trials, the green LED cued to the experimenter to GO, releasing their right hand from the homepad (**Fig. 2.1B**, HP-H), reaching and grasping the object, displacing it and holding it for 1s, then releasing it (**see Fig. 2.1H**). The monkey also received a small fruit reward at the end of each observation trial.

The carousel device allowed us to determine the precise timing of each event making up the whole action. While the human and monkey grasps were very similar, the kinematics of the monkey's action was faster than for the experimenter: HPR to DO was 0.31 s for the monkey and 0.45 s for the experimenter. GO to HOLD-OFF was typically 1.9 s for the monkey and 2.1 s for the human.

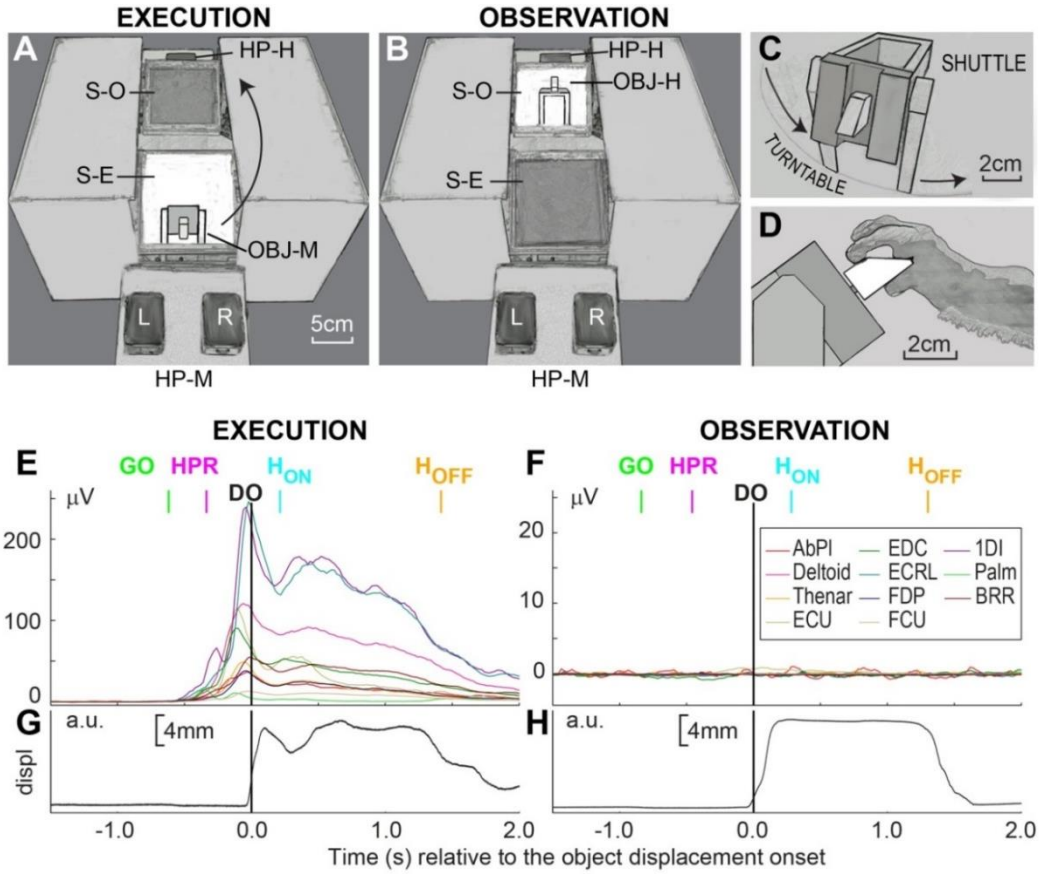


Figure 2.1 Experimental apparatus

The diagram shows the monkey's perspective of a carousel device used to present an object during execution **(A)** or observation trials **(B)**. HP-M: homepads-monkey, left and right. HP-H: homepad experimenter. S-E & S-O: screens which could be electronically switched from opaque to transparent during execution (S-E) or observation trials (S-O), allowing monkey direct view of the object (OBJ-M) when the monkey grasped it (A) and of the same object (OBJ-H) when experimenter grasped it (B).

C Close up of trapezoid object (affords precision grip) mounted on a spring-loaded shuttle.

D Side-view of monkey grasping the trapezoid object using precision grip.

E-H Average EMG traces from 11 hand or arm muscles from one session in M47 for execution **(E)** and observation trials **(F)**. During execution all muscles were active, but there was no modulation during observation. Note that a 10 times higher gain was used for observation trials to emphasise absence of EMG activity (note different y-scale). Averages aligned to the onset of the object displacement (DO) by the monkey **(E)** or human **(F).** Average displacement of object shown for execution and observation trials in **G** and **H,** respectively. The median time of other recorded events relative to DO are shown as vertical lines above; GO: go cue, HPR: homepad release, H_{ON}: stable hold-onset, H_{OFF}: stable hold-offset. Muscles colour-coded as follows AbPl: abductor pollicis longus, deltoid, thenar, ECU: extensor carpi ulnaris, EDC: extensor digitorum communis, ECR-L: extensor carpi radialis longus, FDP: flexor digitorum profundus, FCU: flexor carpi ulnaris, FDI: first dorsal interosseous, Palm: palmaris, BRR: brachioradialis.

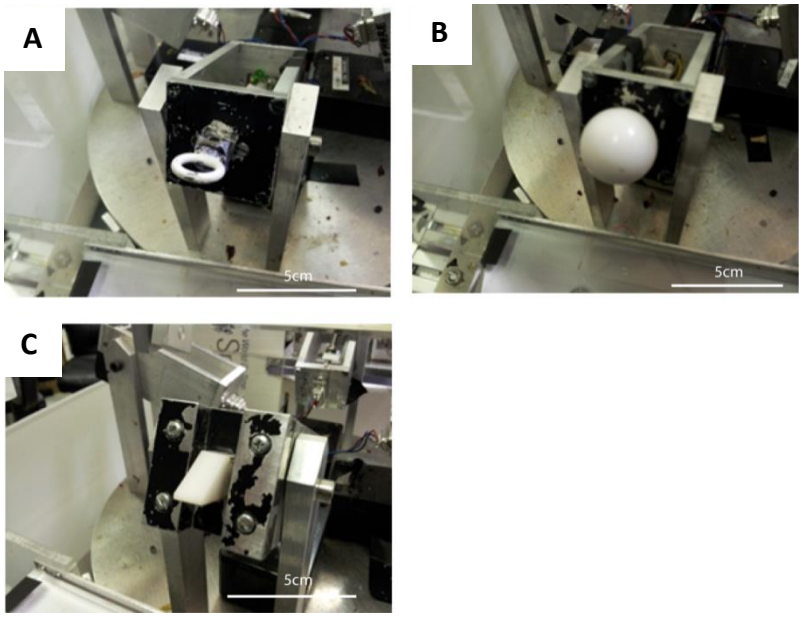


Figure 2.2 Objects

The photos show three objects presented on the carousel to both the monkey and the experimenter. These were the ring **(A)**, which is grasped with the index finger in a hook grasp, the sphere **(B)**, which affords whole-hand grasp and the small trapezoid **(C)**, affording precision grip. On any given trial, one of these objects would be presented to either the monkey or the experimenter.

2.1.4 Go/No-go task

In addition to the mirror task described for **M47**, embedded in the task design we implemented a Go/No-go paradigm. This involved training the monkey to withhold its movement following presentation of a cue. Instead of a green LED indicating that the monkey or the experimenter should grasp the object, on some trials (20% of all trials and pseudo-randomised), a red LED would illuminate the object and indicated the monkey or the experimenter not to move or attempt to grasp the object. The low proportion of No-go trials was to ensure that the monkey would be preparing for a movement.

2.2 SURGICAL PROCEDURES

2.2.1 Structural MRI

Structural MRI scans were carried out for each monkey at the early stage of training. Using images acquired on a 3T Siemens Trio MRI scanner (voxel size: 0.5 x 0.5 x 0.5 mm) allowed design of a custom-fitted Tekapeek headpiece for head restraint of the monkey (for experimental recording sessions) and to plan the craniotomy for optimising the chamber location using the sulci (central and arcuate) and anatomical landmarks for future recording (see **Fig. 2.3**). Monkeys were scanned under full anaesthesia (ketamine 0.08 mg/kg i.m. and domitor 0.11 mg/kg i.m. and repeated approximately every 45 minutes), whilst being placed in a plastic stereotaxic head holder. The whole procedure took around 2-2.5 hours, whilst each MRI scan took approximately 45 mins.

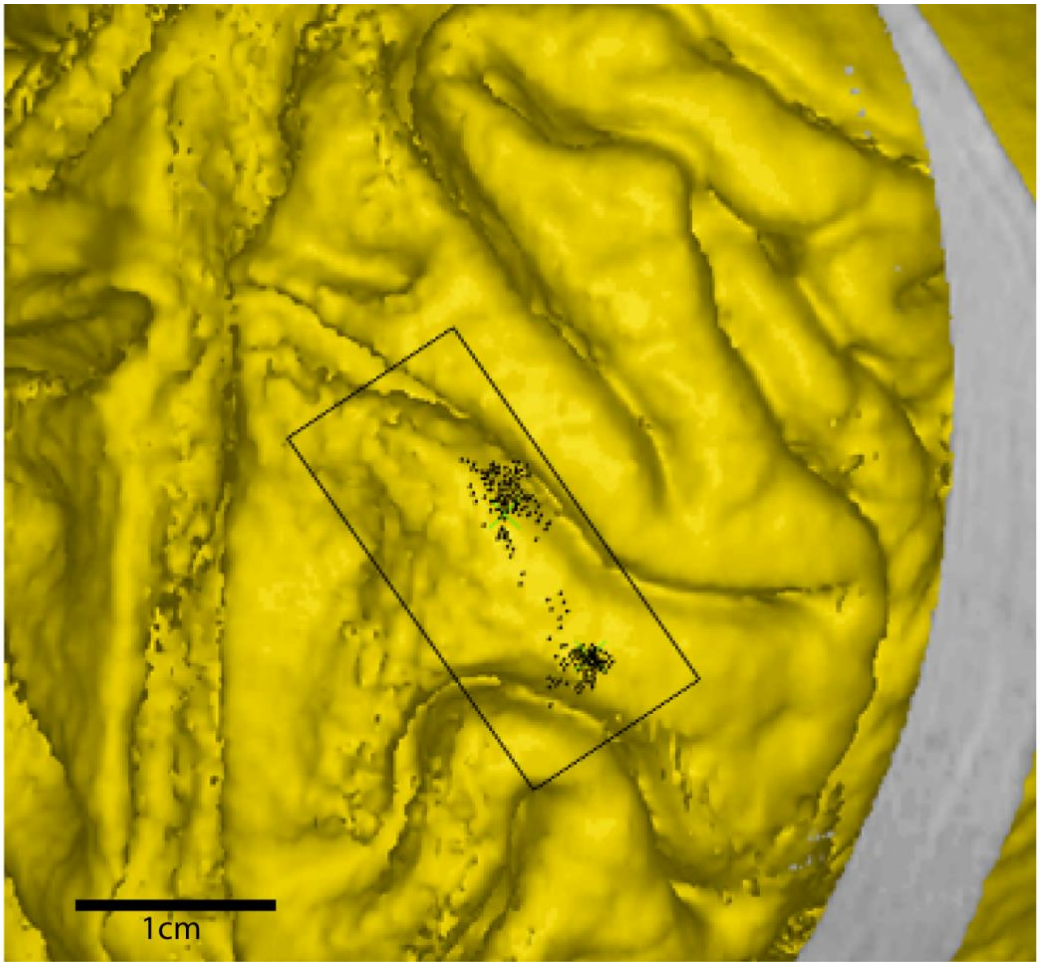


Figure 2.3 Structural MRI with Chamber location and penetrations

The diagram shows the structural MRI obtained from M47. In addition the chamber location and penetrations have been superimposed to show the recording penetrations made close to the central and arcuate sulci. Each dot represents a single penetration. Note that several electrodes were used at each penetration site.

2.2.2 Surgical implantation

Three different surgical procedures were carried out on each monkey, each under deep general anaesthesia (induced with ketamine (10 mg/kg i.m) and maintained with 1.5-2.5% isoflurane in O_2) and under aseptic conditions. In the first, a custom-fitted Tekapeek (high strength biocompatible thermoplastic) headpiece was surgically implanted to allow head restraint. The headpiece was secured to the skull

with four special bolt assemblies in which a titanium disc was placed epidurally using a small (9 mm diameter) hole in the skull, and subsequently manoeuvred beneath the skull to align with a 4 mm burr hole. A M2.5 titanium bolt was then passed through the hole, screwed into the disc and locked in position (Lemon, 1984).

In the second surgery, chronic electromyogram (EMG) patch electrodes were implanted in up to 11 arm, hand and digit muscles (Brochier et al., 2004) and run subcutaneously to a multipin connector externalised in the monkey's back. In the third surgery, a recording chamber was mounted over M1 and F5. The stereotaxic locations of the arcuate and central sulci, visible through the dura were measured, as were a number of fiducial markers on the lid of the recording chamber. Stimulating electrodes were chronically implanted in the medullary pyramid for subsequent antidromic identification of pyramidal tract neurons. A pair of fine tungsten electrodes (240 µm shank diameter with an electrode tip impedance of 20-30 kΩ) were implanted stereotaxically at AP +2 mm, lateral -4.5 mm and height (range: -3.4 to -7 mm) below the intraural line for the anterior electrode and AP -3 mm, lateral -5 mm and height (range: -9.2 to -12 mm) for the posterior electrode. The final depth of the implanted electrodes was determined by stimulating with pulses of up to 300 µA whilst lowering the electrode, looking for motor responses as the tip passed through various brainstem motor nuclei or nerves (abducens (detected by monitoring eye movements), facial (mouth movements) and hypoglossal (tongue movements)), and then searching for the lowest threshold for activation of a short-latency (1.0 ms) antidromic volley recorded from the dura over the ipsilateral motor cortex. The threshold was 20-22 µA (range). After the surgery, we tested the

response to PT stimulation in the awake monkey. Delivering shocks of 150-200 µA evoked short-latency (6-10 ms) EMG responses in hand and forearm muscles.

2.2.3 Chamber maintenance

The recording chamber was regularly cleaned to prevent infection. After every second recording session the dura was exposed and covered with 5-Flurouracil (5-FU) for 5 minutes, and then washed through with plenty of saline (Spinks et al., 2003). This anti-mitotic agent was used to help prevent fibroblast proliferation and angiogenesis. In addition, 5–FU has been shown to have both bacteriocidal and bacteriostatic effects, helping to maintain the health of the dura by preventing infection.

After breaks in recordings, it was sometimes necessary to perform a dura strip. Over time the dura becomes thick and fibrous and makes it hard to penetrate with electrodes. These short surgical sessions were carried out under general anaesthesia (Ketamine/Domitor i.m.) and involved using a corneal hook, small dura scissors and low pressure suction under a microscope to carefully remove excess tissue away from the recording areas (Spinks et al., 2003).

2.3 EXPERIMENTAL PROCEDURES

2.3.1 Recordings

We used two Thomas recording drives (16 and 7 channels, see **Fig. 2.4**) to record simultaneously from the hand regions of M1 and ventral premotor cortex (area F5). During initial mapping sessions, both drives were fitted with a linear array head (see **Fig. 2.4**). The head allowed for an inter-electrode distance of 0.5 mm. This broad spacing allowed us to quickly map the activity of the area so that we were able to locate the hand areas of M1 and F5 within a few sessions. Once we had a better understanding of the location we changed the linear array head to a 4x4 rectangular array for the 16 drive and a circular array for the 7 drive (see **Fig. 2.4**). These heads had a smaller inter-electrode distance (300 µm) and allowed for a targeted penetration in the hand area of M1 and F5. Typically >4 glass insulated platinum electrodes (diameter, 80 µm) were loaded into each drive. The impedance of the tip of these electrodes was measured before each use and documented (1-2 MΩ). We either carried out single area recordings (M1 or F5) or dual recordings in M1 and F5. During dual recording sessions, the 16 drive would be positioned for penetration in the hand area of M1 whilst the 7 drive would be used for recording from area F5. After the monkey had been head restrained, the drives were positioned above the monkey's head on a sturdy metal plate and directed at an angle best suited for successful transdural penetration. The drive's stereotaxic position was calculated by triangulation using the co-ordinates of 4-5 fiducial markers (present on the chamber lid) before each recording session. The points measured were used to calculate the position of the drive within a chamber map using custom-written Matlab software

(**see Fig. 2.7**). Previous penetrations and their ICMS effects were saved onto this chamber map, this allowed us to make an estimate of the location for the penetration for the current recording session.

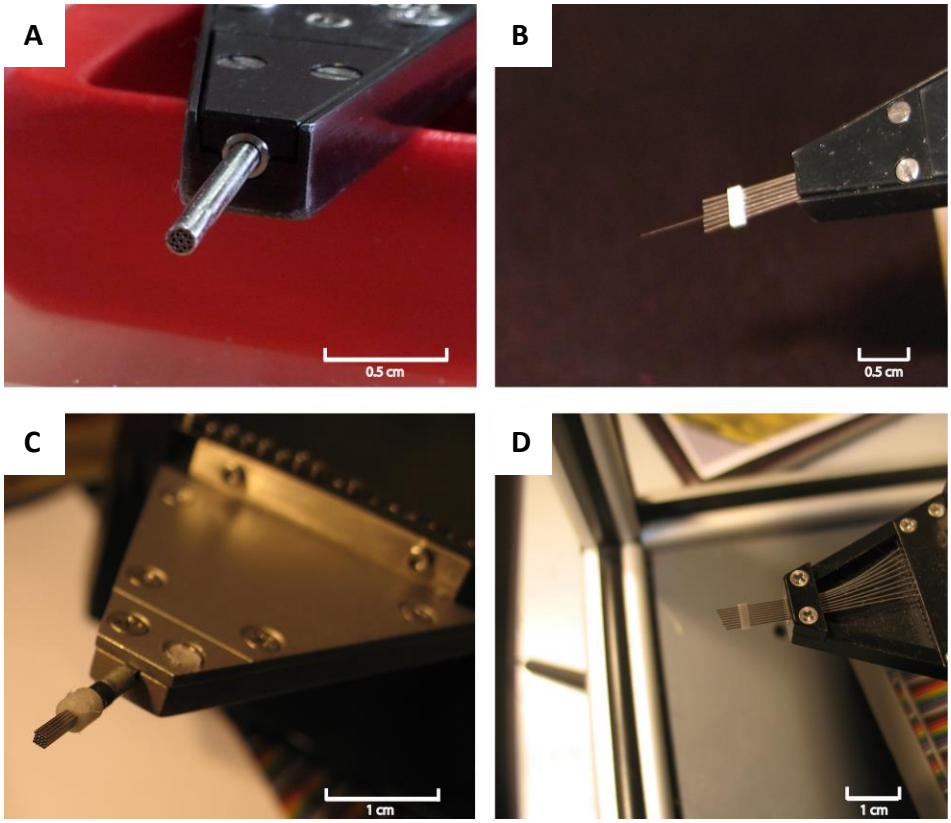


Figure 2.4 Recording drives and heads

The figures shows the 7 channel drive with circular array **(A)** and linear array **(B)**, used for recordings in hand area of F5. We used the 16 channel drive with rectangular array **(C)** and linear array **(D)** to record from area M1. The linear array head allowed us to map the area (large inter-electrode distance), whilst the pointed array allowed us to make more focused recordings from more interesting areas.

Once the location of the penetration had been determined, we slowly lowered each electrode whilst listening to the recording and watching the electrode at the dura surface with a binocular microscope. Once we heard activity or saw the electrode penetrate the dura, we stopped moving the electrode and then penetrated with

another electrode. After all the electrodes had penetrated we raised each of them slowly whilst listening and carefully monitoring the oscilloscope until we could not see any further evidence of neuronal activity. This allowed us to calibrate the depth of the penetration with the dural surface. To allow for any cortical depression that might have been caused by the transdural penetration we waited at least 10 minutes before re-advancing the electrodes into the cortex.

2.3.2 PTN identification

Since we were mainly interested in recording from the output neurons of the motor cortex we were primarily interested in recording from identified PTNs (pyramidal tract neurons). During the recording session, a search stimulus of 250-300 μA (biphasic pulse, each phase 0.2 ms) was applied to the pyramidal tract electrodes and responses from well-isolated neurons were confirmed as PTNs by their invariant response latency (jitter <0.1 ms) and by applying a collision test (Lemon, 1984); we noted the antidromic latency, collision interval and threshold for each PTN. The PTN response had an invariant latency because it was antidromic and not synaptic; any latency jitter was generally taken to indicate a synaptic rather than an antidromic effect (Lemon, 1984).

A successful collision occurred when a spontaneous spike was used to trigger the pyramidal tract stimulation at a desired delay after the spontaneous discharge of a discriminated neuron. Spikes were discriminated on-line using a software-based discriminator with two voltage-time windows. Triggering the stimulator evoked an antidromic spike that travelled towards the cortex. If the cell we recorded from had an axon in the pyramidal tract and the timing was within the collision period, then

the antidromic spike would collide with the spontaneous spike. This means that the antidromic volley never reached the cortex and could not be detected at the recording electrode, indicating that the cell that was being recorded had its axon within the pyramidal tract. Please see **Fig. 2.5** for further details. Note that the collision interval, which reflects the refractory period of the stimulated axon should be brief and characteristic for each PTN (Lemon, 1984).

The sample of PTNs was unbiased in terms of their task-related activity, which was not tested until antidromic identification and stable recordings had been achieved, although it might be biased in terms of recording from the biggest cells with the fastest conduction velocities (see Chapter 6).

At the end of the recording session, ICMS was delivered at each electrode at the same depth to characterise the motor output of the area we recorded from, we noted the depth and threshold if we found a response. An isolated stimulator (custom made, optically isolated stimulator) was used to deliver trains of 13 pulses at 333 Hz, intensity typically up to 50-60 µA , duty cycle 0.5 Hz.

2.3.3 Technical recording parameters

Pre-amplification (x20, Thomas Recording headstage amplifier), the signals from each electrode were further amplified (typically x150) and broadly band-pass filtered (1.5 Hz–10 kHz). Data were acquired using a PCI-6071E, National Instruments card at 25 kHz sampling rate and were recorded together with electromyographic activity (5 kHz), eye movement signals, and times of all task events and the home pad, object displacement and sensor signals (1 kHz).

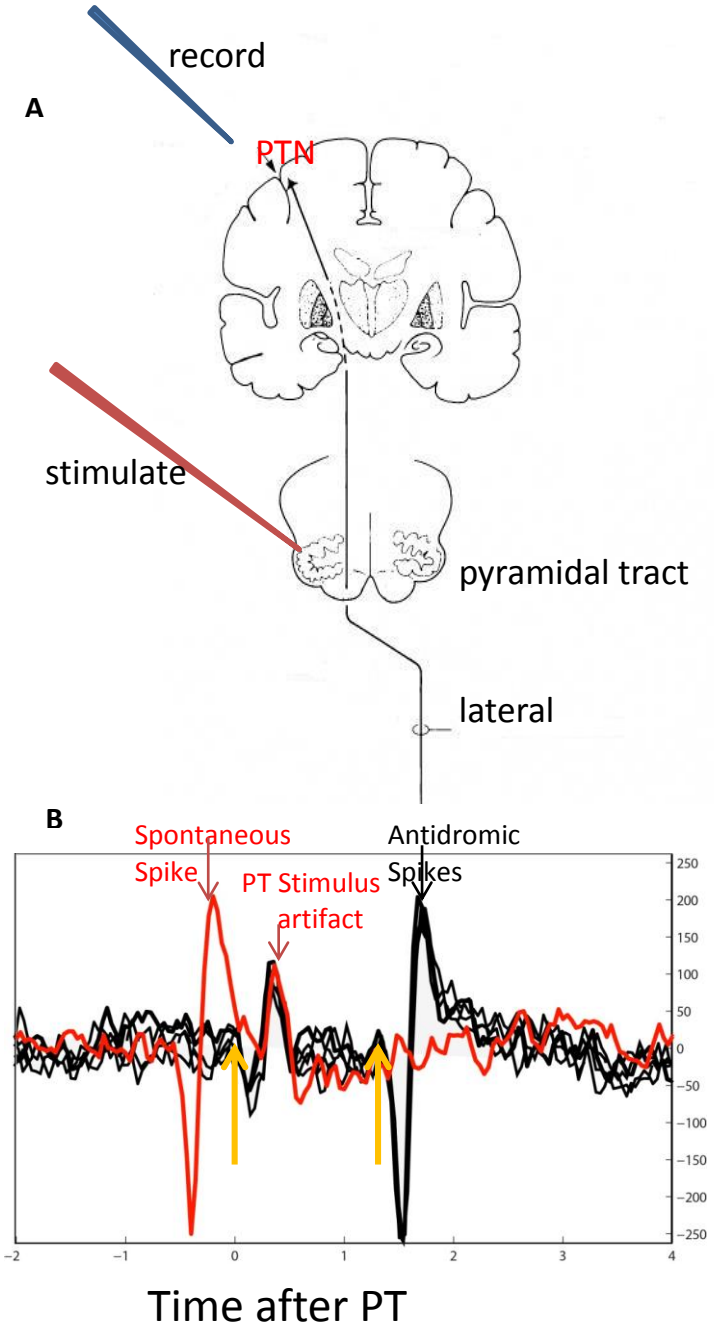


Figure 2.5 PTN identification

(A) Diagram showing how we identify PTNs. We record from the cortex whilst simultaneously stimulating the pyramidal tract at the level of the medulla (shown by red electrode). **(B)** Sweeps of responses of a PTN to stimulation of the pyramidal tract. The black traces show several sweeps following stimulation of the pyramidal tract at time zero. Each sweep shows the presence of an antidromic spike at around 1.2 ms after the PT shock. The lack of jitter (<0.1 ms) identifies the spike as antidromic. The antidromic latency (ADL) is measured from the first orange arrow to the next. This is a measure of the conduction velocity. The red trace is a single sweep in which there has been a collision between the spontaneous spike (generated in the cortex) and the antidromic volley (ascending towards the cortex), hence the antidromic spike is not seen at the recording electrode.

2.3.4 Recording locations

All our recordings were taken from the primary motor cortex (M1) and premotor cortex (F5). M1 units were recorded from locations rostral to the central sulcus (anterior bank). F5 units were recorded in the rostral division of PMv (see **Figs. 2.3 & 2.7**).

2.3.5 Histology

At the end of the experiment in M43, the monkey was killed by an overdose of pentobarbitone (50 mg kg^{-1} i.p. Euthanal; Rhone Merieux) and perfused through the heart. The cortex and brain stem were photographed and removed for histological analysis. Frozen sections of the brainstem were cut at 30 µm and stained with a Nissl stain and Luxol fast blue so that the implanted electrode tips were confirmed to be in the pyramidal tract. M47 is still alive.

2.4 DATA ANALYSIS

2.4.1 Spike discrimination

To detect spikes we used simple thresholding applied to software filtered data (acausal 4^{th} order, elliptic, 300Hz-3 kHz). Single neurons were clustered using modified *Wave_clus* software (Quiroga et al., 2004). We used an extended set of features which included not only wavelet coefficients but also the first three principal components. Spike shapes of PTNs obtained after clustering were checked against shapes of spontaneous spikes which collided antidromic spikes during PT stimulation (see **Fig. 2.5**). This was confirmed for data recorded both before and after recording

of activity during task performance (Kraskov et al., 2009). During spike discrimination, a very short (200 μs) 'dead' time between two consecutive spike events was used which allowed detection of different units which fired close together in time. For bursting units, clusters with minimum 1 ms interspike interval were accepted; for other units a minimum interspike interval of 2 ms was set. **Fig. 2.6** shows an example of clustered units from one recording sessions. The different coloured spikes are sorted into 3 clusters (blue, red, green) based on their spike shapes as described above.

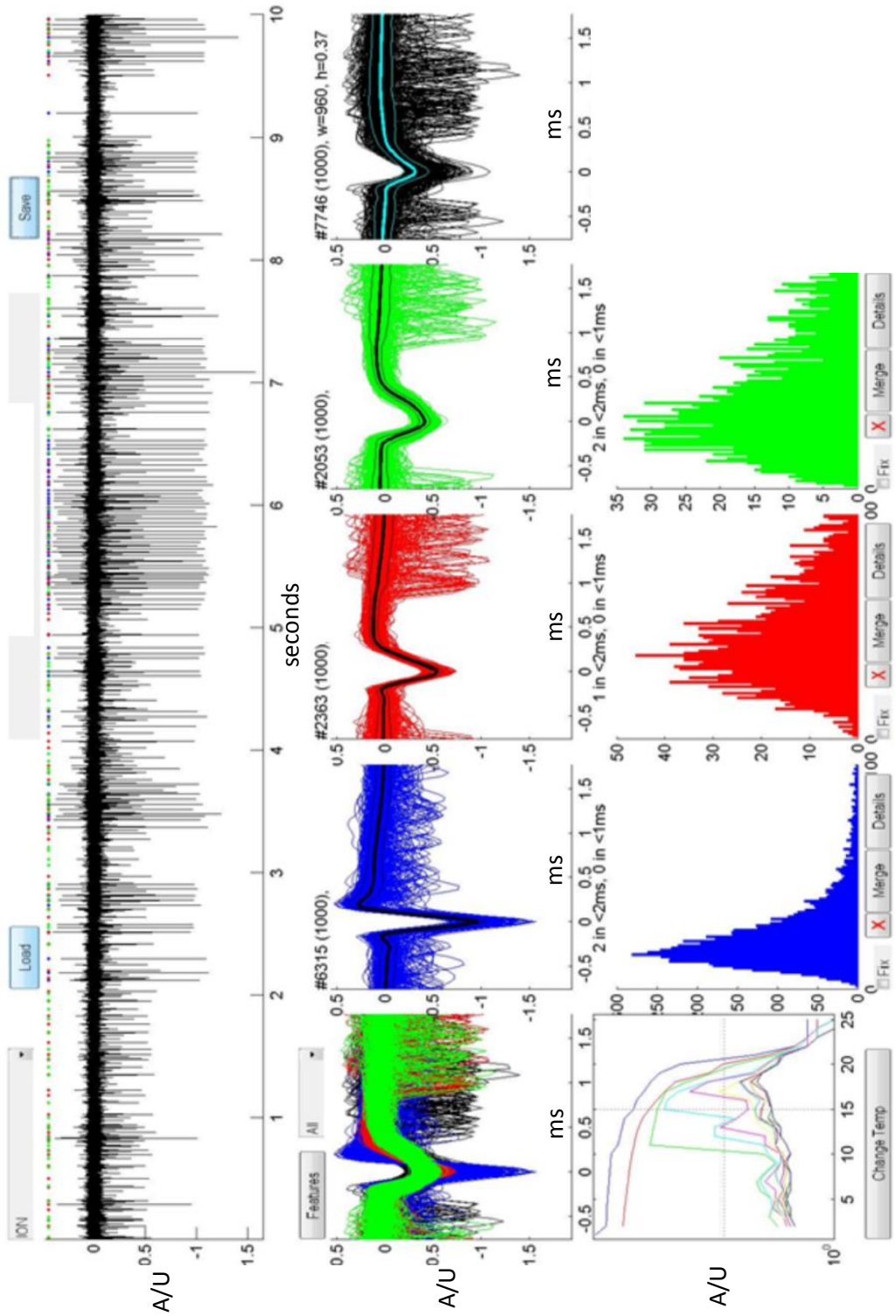


Figure 2.6 Discrimination of Spikes and Clustering

A screen shot of *Wave-clus* from one recording. The first 10 seconds of the recording is shown at the top, with the corresponding spikes and clusters (shown as blue, red and green dots). The selected temperature (principle component parameters) is shown by the crosshair on the bottom left plot. Three clusters are shown, 2 of which are PTNs (blue and red). 1000 of the spikes are shown in the plots of each cluster with a corresponding inter-spike interval histogram below.

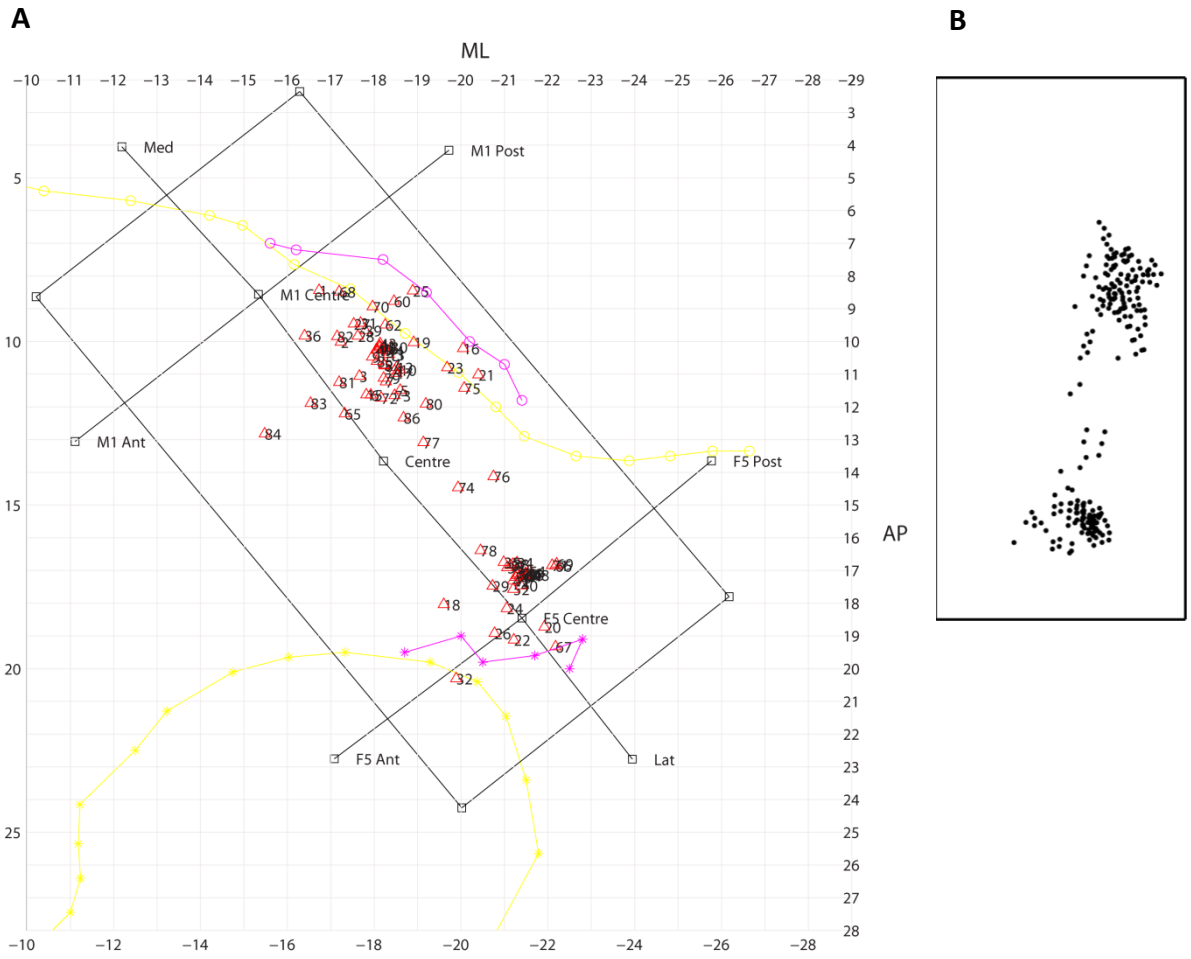


Figure 2.7 Chamber map and penetrations

(A) Penetration locations are shown in M47 in M1 and F5. The central and arcuate sulci measured at the time of the surgery are shown in magenta, whilst that measured from the MRI are in yellow. **(B)** Flat view of penetrations in M1 and F5

2.4.2 EMG analysis

Recordings were made from the muscles listed in the legend to **Fig. 2.1 E-F**. Data were bandpass filtered between 30 and 500 Hz (4th order Butterworth), rectified, averaged over trials and then smoothed using a 100 ms moving window.

2.4.3 Eye movements

For monkey M47 we simultaneously recorded the eye with a non-invasive ISCAN camera system (ETL-200, 120 Hz). We were able to calibrate the position of the object (for execution and observation locations) so that we were able to identify when the object was being fixated during trials. We designed a plate holding 7 orange LEDS (see **Fig. 2.8**) that could be attached to the carousel at the execution and observation positions. Before recording the activity during the mirror task we placed the plate in the 'observation' position and turned each LED one at a time (in darkness). The monkey would then saccade to the position of the illuminated LED. The last LED was positioned on top of the object. We would then repeat this whilst placing the plate in the 'execution' position. We were then able to analyse and calibrate the eye position data off-line.

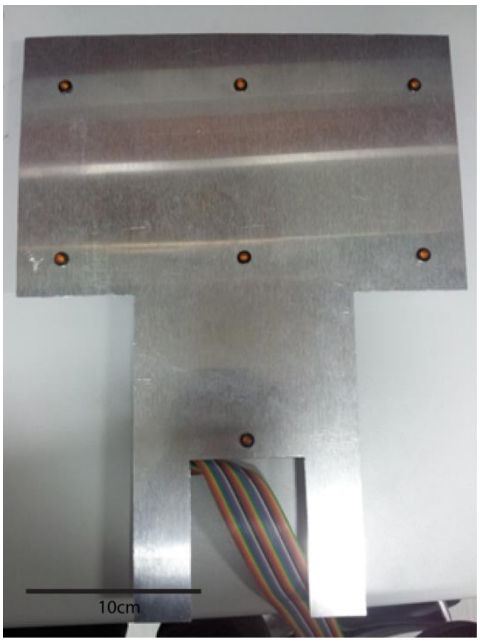


Figure 2.8 Eye movements calibration equipment
Plate housing 7 LEDs used for calibration of eye position. The plate was placed in the execution position (near the monkey's object) and each LED would be activated in isolation whilst simultaneously recording the eye position using an external infra-red camera. This procedure would be repeated at the location of the experimenter's object. In this way we were able to calibrate the eye position data during the task offline.

CHAPTER 3:
M1 corticospinal mirror neurons and their role in movement suppression during action observation

3.1 ABSTRACT

Evidence is accumulating that neurons in primary motor cortex (M1) respond during action observation (Tkach et al., 2007, Dushanova and Donoghue, 2010) a property first shown for mirror neurons in monkey premotor cortex (Gallese et al., 1996). We now show for the first time that the discharge of a major class of M1 output neuron, the pyramidal tract neuron, is modulated during observation of precision grip by a human experimenter. We recorded 132 pyramidal tract neurons in the hand area of two adult macaques, of which 65 (49%) showed mirror-like activity. Many (38/65) increased their discharge during observation (facilitation-type mirror neuron), but a substantial number (27/65) exhibited reduced discharge or stopped firing (suppression-type). Simultaneous recordings from arm, hand and digit muscles confirmed the complete absence of detectable muscle activity during observation. We compared the discharge of the same population of neurons during active grasp by the monkeys. We found that facilitation neurons were only half as active for action observation as for action execution, and that suppression neurons reversed their activity pattern and were actually facilitated during execution. Thus although many M1 output neurons are active during action observation, M1 direct input to spinal circuitry is either reduced or abolished and may not be sufficient to produce overt muscle activity.

In a set of further experimental studies, we analysed data collected from monkey M47 that had been trained on the more complex task design and found evidence of M1 PTNs that modulated their firing rate after a No-go cue. These experiments suggest that one way in which we inhibit movement during action observation is by reducing the firing of PTNs in motor cortex.

3.2 INTRODUCTION

Mirror neurons are particularly fascinating in that they are activated not only by one's own actions but also by the actions of others. Mirror neurons in macaque area F5 were originally shown to respond during both the monkey's own grasping action and during observation of grasp carried out by a human experimenter (Gallese et al., 1996, Rizzolatti et al., 1996). Recordings made in adjacent primary motor cortex (M1) were reported as lacking mirror-like activity, and this was taken as indirect evidence that the monkey was not making covert movements while it observed actions. This conclusion was very much based on the idea that M1, unlike premotor cortex, is an 'executive' structure, whose activity has many 'muscle-like' features, which can be reliably linked to the production of movement (Kakei et al., 1999, Todorov, 2000, Lemon, 2008, Scott, 2008).

However, since 1996, evidence has since been steadily accumulating for the presence of mirror-like activity in M1, both in monkeys (Tkach et al., 2007, Dushanova and Donoghue, 2010) and humans (Fadiga et al., 1995, Hari et al., 1998, Montagna et al., 2005, Press et al., 2011, Szameitat et al., 2012). This activity has been open to a number of interpretations, including a role for M1 as part of a frontal network

involved in mental rehearsal or simulation of the observed action (Cisek and Kalaska, 2004). In monkey studies, it has been shown that a considerable proportion of M1 neurons (46-70%) can be activated during observation of a familiar directional reaching task (Tkach et al., 2007; Dushanova and Donoghue, 2010).

The executive role of M1 in the brain's motor network is strongly supported by the architecture of its outputs to the spinal cord (Dum and Strick, 1991, Lemon, 2008, Porter and Lemon, 1993, Rizzolatti and Luppino, 2001). M1 outputs project to all the brainstem pathways giving rise to descending motor pathways, as well as projecting, as the corticospinal tract, to influence both medial and lateral motor groups, controlling axial and distal muscles. The latter include the direct cortico-motoneuronal projections to alpha motoneurons innervating arm and hand muscles. Given this architecture, it is a challenge to explain why the presence of extensive mirror-like activity within M1 does not lead to movement. To understand this we recorded from identified corticospinal neurons in M1 and showed that although many of these neurons exhibit mirror-like activity, there were major differences in their pattern and extent of discharge during action execution versus action observation.

3.3 METHODS

Please see section 2.1.3 for a detailed description of the task.

3.3.1 Firing rate analysis

For M47, to test whether a cell showed any modulation of firing rate during action observation or action execution, we used a one-way ANOVA for three phases of the task: baseline (500 ms before the GO cue), reach (HPR to DO) and hold (H_{ON} to H_{OFF}). We performed a Bonferonni corrected posthoc test in order to compare the neuronal activity relating to the movements (reach and hold) with the static presentation of the object (baseline). Similarly, we carried out an ANOVA using the same factors on execution data.

For M43, we compared modulation of firing rate during the 500 ms before the onset of the experimenter's movement (HPR) with the 1000 ms period centred on the time of grasp (sensor signal). For execution, we confirmed that PTNs modulated their firing rate during the monkey's grasp.

For graphical display in **Figs. 3.2-4, 3.7 and 3.8**, we smoothed the average time course of each PTN's discharge over a 400 ms moving window (20ms bins with 20ms steps) and normalised it by subtracting baseline activity and then dividing by its absolute maximum, defined using execution and observation trials (this was either the absolute maximum during execution or observation). For graphical display in **Fig. 3.10**, we smoothed the averaged time course in a similar way; however, the absolute maximum/minimum was defined using execution No-go and Go trials.

3.3.2 Spike-triggered averaging of EMG

For M47, averages were made for each PTN from all discriminated PTN spikes and EMG recorded during the task. The identification of CM cells used the criteria employed in earlier studies from this laboratory (Quallo et al., 2012). EMG from each muscle recorded simultaneously with the PTN, was full-wave rectified and averaged with respect to spike discharge over a period -20 ms before and 40 ms after spike discharge. Averages were compiled with a minimum of 2000 spikes. This procedure was not carried out in M43 because there were a smaller number of observation trials and therefore there were too few spikes were available for compiling averages.

3.4 RESULTS

3.4.1 Database

PTNs were recorded in 27 and 40 sessions in M43 and M47, respectively, and over periods of 25 and 10 weeks, respectively. PTNs were recorded for a minimum of 10 observation and 10 execution trials. Most recordings were from large, fast PTNs: antidromic latencies ranged from 0.51 to 5.35 ms (median 1.05 ms) (Vigneswaran et al., 2011). Most PTNs were recorded from tracks in the M1 hand region close to the central sulcus and at sites from which digit movements could be evoked with low-threshold intracortical microstimulation (< 20 μA, 79% of PTNs; < 10 μA, 55%).

A total of 132 PTNs were recorded from M1 in the two monkeys (M43, 79 PTNs; M47, 53 PTNs).

Please note that the analysis up to section **3.4.7** is based on one object (small trapezoid that affords precision grip) and data from monkeys M43 and M47. The rest of the analysis is on data from all three objects and based on data collected from monkey M47.

3.4.2 EMG activity during execution and observation

In both monkeys, we recorded from up to 11 different arm, hand or digit muscles to confirm that the monkey did not make covert movements as it watched the experimenter (Kraskov et al., 2009). Electromyogram recordings during execution all showed marked activity, but were silent during observation (cf. Chapter 2 **Fig. 2.1F**; note difference in gain, EMG activity is plotted at 10x the gain for observation to reveal even small levels of activity).

3.4.3 Types of mirror PTN

Mirror neurons are neurons that modulate their firing rate during observation of a grasp and are facilitated during execution. In total 77/132 PTNs (58%) showed significant modulation during action observation. **Fig. 3.1** shows examples of mirror neurons. These can be classified either as 'facilitation' type mirror neurons, which increased discharge during observation trials (cf. Gallese et al., 1996; **Fig. 3.1A,C**); or as 'suppression' type, in which discharge was reduced or abolished during observation (cf. Kraskov et al., 2009; **Fig. 3.1B,D**). The key events in each trial are

shown by coloured symbols superimposed on the rasters of unit activity. For M47 (**Fig. 3.1A, B**), the rasters are aligned to displacement onset (DO) for both conditions (cf. Chapter 2 **Fig. 2.1**). The facilitation PTN shown in **Fig. 3.1A** became active soon after homepad release (HPR), but the activation was much more pronounced for execution (dashed line in averaged spike activity) than for observation (solid lines).

The suppression mirror neuron shown in **Fig. 3.1B** had a steady baseline discharge of around 30-35 spikes/s which decreased to around 20 spikes/s soon after the GO signal in the observation condition. In striking contrast, it showed a marked increase in discharge during execution up to a peak of 90 spikes/s: it reversed its pattern of activity as the task changed from observation to execution.

In M43, the task was more naturalistic. For observation trials, a contact sensor signal allowed us to align rasters with the moment the experimenter first grasped the piece of fruit. The facilitation PTN shown in **Fig. 3.1C** increased its discharge shortly before the experimenter's grasp, and peaked around 500 ms after it. For execution trials, rasters were aligned with the onset of the monkey's muscle activity (see Methods); this PTN showed a complex pattern of early suppression followed by later activation, which was again much greater than the peak rate during observation (95 vs 45 spikes/s). The PTN shown in **Fig. 3.1D** had a baseline firing rate of around 10 spikes/s which was completely suppressed during observation, while it showed pronounced activity (peak of 75 spikes/s) late in the monkey's own reach-to-grasp.

There were some differences in the kinematics, with the monkey moving more rapidly than the human (cf. Chapter 2, **Fig. 2.1G vs H**); however this is unlikely to

explain the difference in firing rate since we could not find any consistent correlation between firing rate and movement time across execution and observation trials. It is also worth noting that the reversal of pattern in suppression mirror neurons could not be explained by any differences in the kinematics of human vs monkey action.

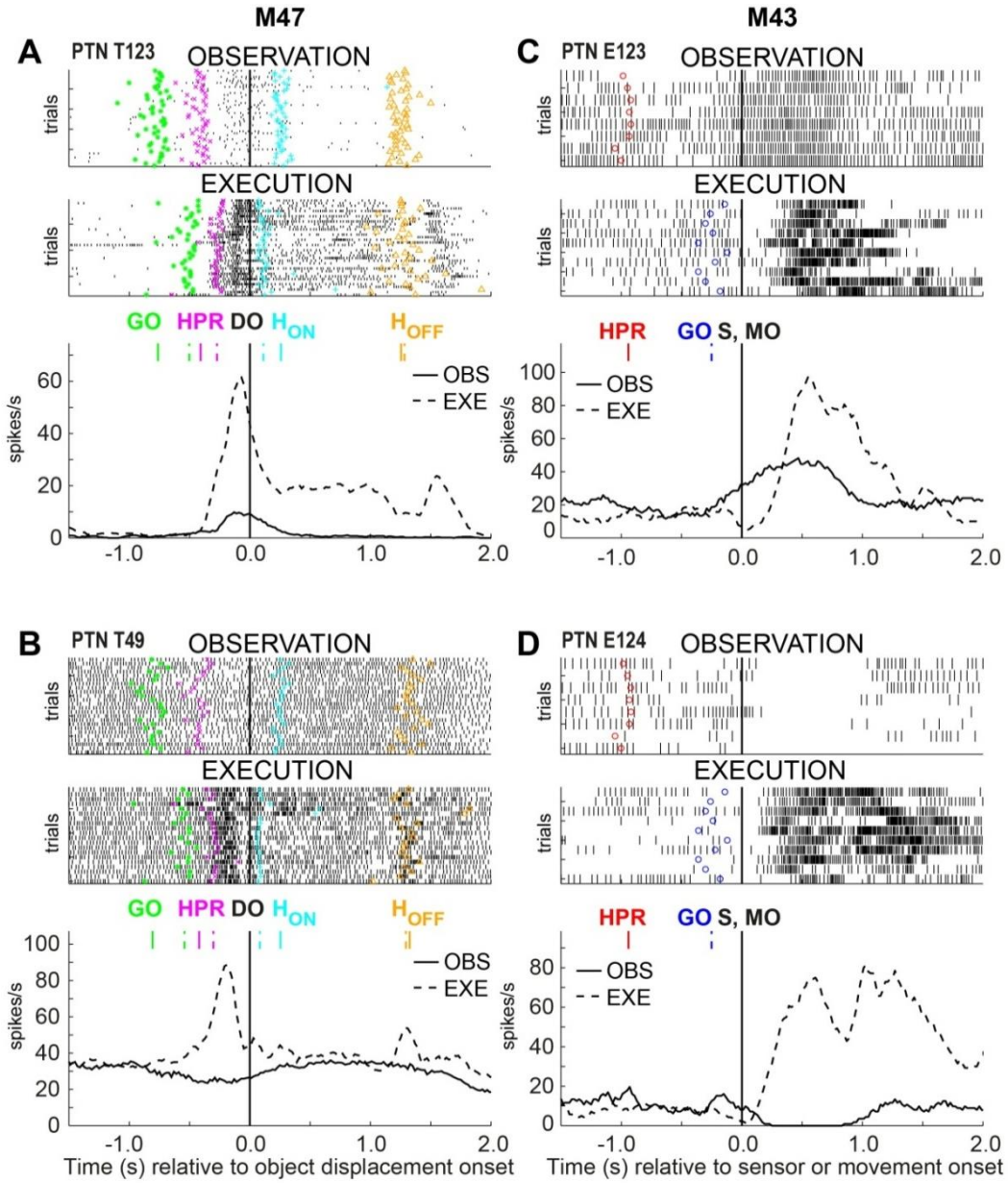


Figure 3.1 Mirror PTNs in M1

Examples of M1 facilitation **(A and C)** and suppression **(B and D)** mirror PTNs in M47 **(A and B)** and M43 **(C and D)**. Each panel consists of raster plots for observation and execution trials and corresponding histograms (solid and dashed lines, respectively). Histograms were compiled in 20 ms bins and then smoothed using a 140 ms sliding window. In **(A)** and **(B)**, all data were aligned to onset of the object displacement (DO); other behavioural events are indicated by coloured markers for each trial on raster plots and with vertical lines on histograms (cf. Figure 2.1). In **(C)** and **(D)**, all execution trial data were aligned to movement onset (MO), defined using onset of the monkey's biceps EMG activity. All observation trial data were aligned to a sensor signal (S), which detected first contact of the experimenter with the object. HPR indicates beginning of the experimenter's movement in observation trials. GO markers indicate the cue for the monkey to grasp the reward in execution trials.

3.4.4 Population activity during observation and execution

Fig. 3.2 shows the population analysis of M1 PTNs modulated during observation (n=77). In M47 we recorded 35 PTNs (**Fig. 3.2A**) of which the majority (24/35, 68.6%) were facilitated during observation (Obs, F), and most of these (20/35, 57.1%) were also facilitated during execution (Exec, F-F type, light red). A few PTNs showed either suppression (F-S, 3 PTNs (8.6%) dark red) or were non-significant (ns) (1 PTN, 2.9%) during execution. The remaining 11/35 PTNs (31.4%) showed suppression during observation; 7/35 (20%) were facilitated during execution (S-F, light blue), with a few also suppressed (S-S, 3 PTNs, 8.6%) or ns (1 PTN, 2.9%) during execution.

Rather similar results were found in M43 (**Fig. 3.2B**): again many PTNs (21/42, 50%) showed facilitation during observation, and most were also facilitated during execution (18/42, 42.9%). Almost all PTNs exhibiting suppression during observation (21/42, 50%) reversed their activity and were facilitated during execution (20/42, 47.6%). Note that of the 77 PTNs shown in **Fig. 3.2A and B**, only 65 would be strictly classified as mirror neurons, i.e. PTNs which were either facilitated or suppressed during observation *and* facilitated during execution.

Fig. 3.2C compares the time-resolved normalised firing rates of mirror neurons during observation and execution (M47). We selected the two main sub-groups of PTNs: facilitation mirror neurons that were also facilitated during execution (n=20 F-F type PTNs, red traces in **Fig. 3.2C**), and suppression mirror neurons, which reversed their firing pattern and were also facilitated during execution (n=7 S-F PTNs, blue

traces). During observation (shown on left) both sub-groups modulated their background firing rate shortly after the experimenter's HPR, with peak modulation at DO. During execution (shown on right) facilitation PTNs were around three times as active compared with observation; discharge increased to 64% of the maximum modulation above baseline (see section **3.3.1**) vs only 17% during observation. The sub-group of suppression PTNs reversed their pattern of discharge from 19% of the maximum modulation *below* baseline for observation to 47% *above* it for execution. Changes in firing rate were sustained at lower levels during the hold period.

Similar patterns were found in M43. **Fig. 3.3A-B** shows the time resolved population analysis. For facilitation mirror neurons (F-F type, n= 18), discharge during execution (B) was 60% of the maximum modulation above baseline vs 44% for observation (A). Suppression mirror neurons (S-F type, n=20) discharged at 31% *below* baseline during observation but reversed to 63% *above* it for execution. Clearly, there are some differences between the population data obtained from the two monkeys (cf. **Fig. 3.2C**). Some of the differences might be due to the fewer behavioural events to align the data. For example, we did not have a true initiation of movement signal (such as homepad release) for M43, and had to infer this time point from the onset of muscle EMG activity (biceps muscle, corresponding to the monkey lifting its hand off the homepad). However, the same conclusions with respect to the overall level of activity could be made within each monkey.

In **Fig. 3.2D** we estimate changes in maximum firing rates (non-normalised) when the task switched from observation to execution. Pooling data from both monkeys, we

calculated the mean firing rate for 38 F-F type mirror neurons (red bars), i.e. those facilitated during execution (E) but strongly attenuated during observation (O). The blue bars represent 27 S-F type PTNs, which were suppressed for observation but facilitated for execution. The difference in mean firing rate of facilitation vs suppression PTNs in observation was around 5 spikes/s/PTN. The next, green bar, combines results from these two sets of mirror neurons and shows that compared with the execution condition, the population mean firing rate during observation represented a mean disfacilitation of around 45 spikes/s/PTN. On the right of **Fig. 3.2D**, we estimated the same change for a group of 34 'non-mirror' PTNs recorded in the same monkeys. By definition, these PTNs showed no significant modulation during observation, so they were also effectively disfacilitated during observation.

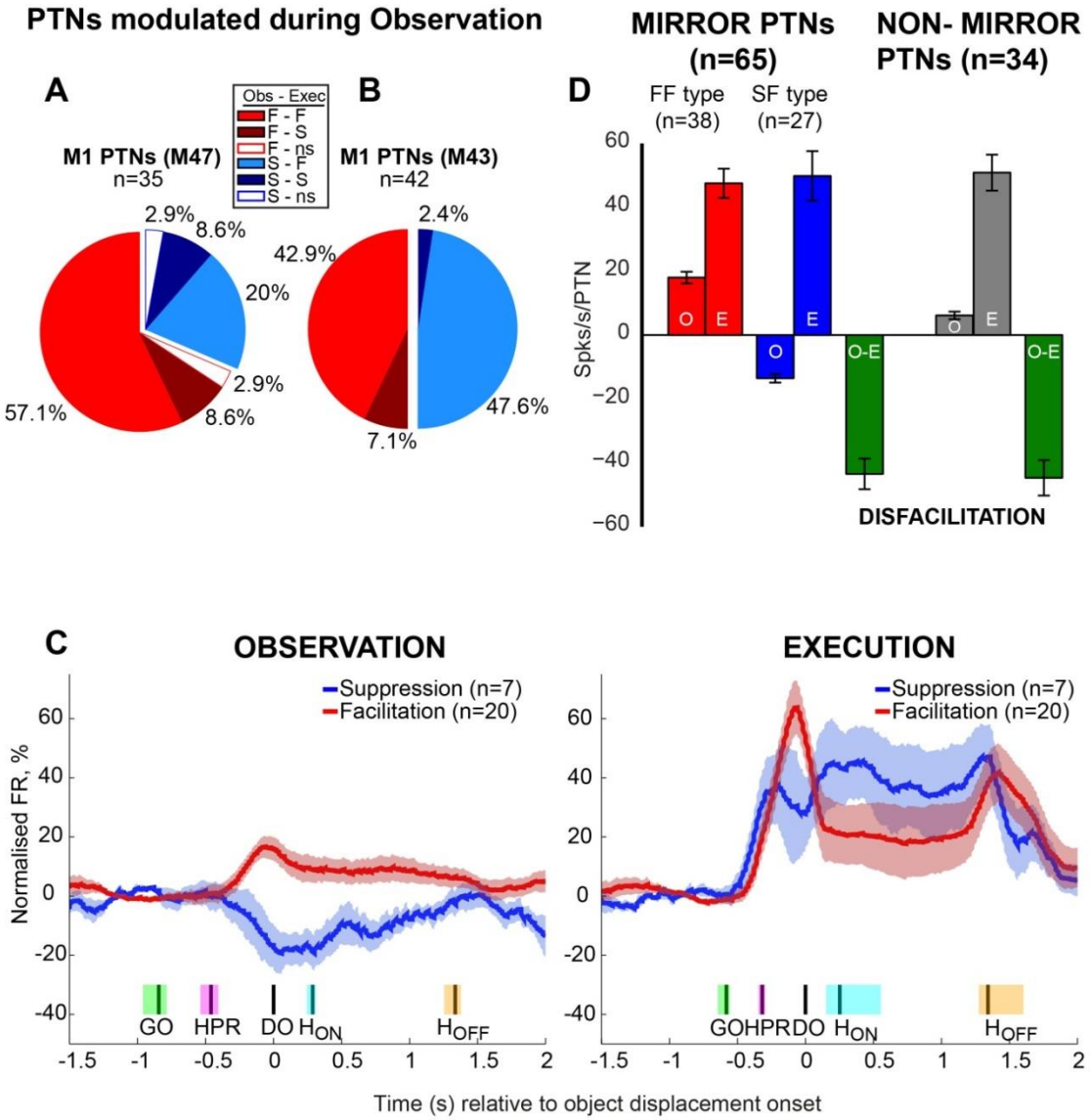


Figure 3.2 Population Activity of M1 Mirror Neurons (M47)

(A and B) Pie charts showing different types of facilitation (red, F) and suppression (blue, S) PTNs recorded during action observation (Obs in inset box) in M47 **(A)** and M43 **(B)**. Lighter shades of both colours indicate proportions of these neurons whose discharge was facilitated during execution (Exec in inset box); darker shades indicate proportions showing suppression during execution (a relatively small proportion). ns, no significant change in modulation during execution.

(C) Left: population averages during observation for corticospinal mirror neurons (M47) that were activated during execution and whose discharge was significantly suppressed (blue) or facilitated (red) during observation (together with SEM, shaded areas). Firing rates were normalised to the absolute maximum of the smoothed averaged firing rate of individual neurons defined during execution and observation trials, and baseline firing rate was subtracted. Data aligned to DO, the median (black line), and the 25th to 75th percentile times of other events recorded are shown as shaded areas: GO (green), HPR (magenta), hold HON (cyan), and HOFF (yellow). Firing rates were smoothed using a 400 ms sliding window in 20 ms steps. Right: population

average for the same groups of mirror neurons during execution. Facilitation-type PTNs showed higher discharge rates during execution compared with observation trials, and suppression type PTNs changed pattern to facilitation during execution.
(D) Maximum firing rate of PTNs during observation and execution trials, expressed as raw firing rates (with SEM). Results from both monkeys were pooled. Red bars show average rates for 38 M1 PTNs facilitated during both observation (O) and execution (E) (F-F type). Note the much lower rate during observation. Blue bars show rates for 27 M1 PTNs suppressed during observation (O) and facilitated during execution (E) (S-F type). The left green bar shows the mean firing rate for all these mirror PTNs in observation minus that in execution, to capture the total amount of disfacilitation in the output from these neurons that occurred during observation. On the right are similar results for PTNs that did not show any mirror activity.

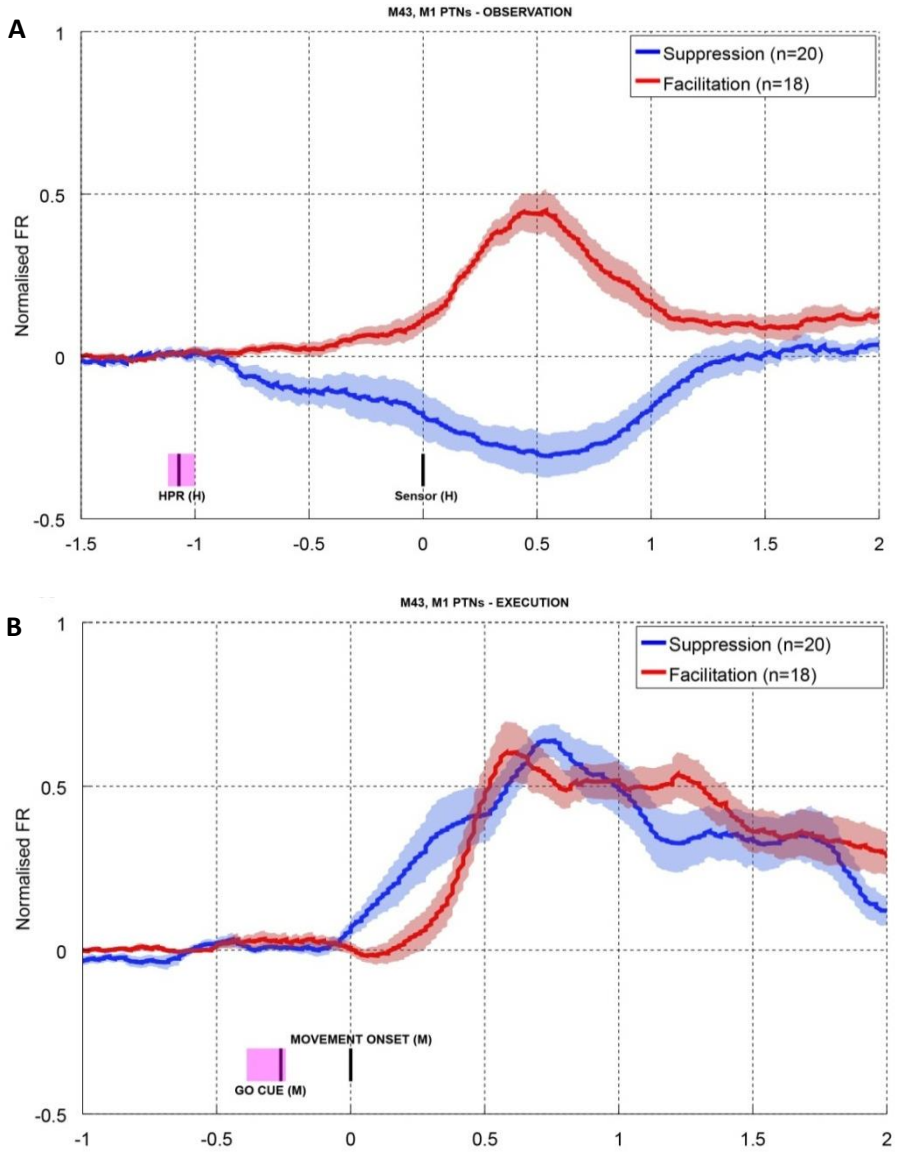


Figure 3.3 Population Activity of M1 Mirror Neurons (M43)

(A) Population averages during observation for corticospinal mirror neurons (M43) that were activated during execution and whose discharge was significantly suppressed (blue) or facilitated (red) during observation (together with SEM, shaded areas). Firing rates were normalised to the absolute maximum of the smoothed averaged firing rate of individual neurons defined during execution and observation trials, and baseline firing rate was subtracted. Data aligned to sensor, the median (black line), and the 25th to 75th percentile times of other events recorded are shown as shaded areas: HPR (magenta). Firing rates were smoothed using a 400 ms sliding window in 20 ms steps. **(B)** Population average for the same groups of mirror neurons during execution. Facilitation-type PTNs showed higher discharge rates during execution compared with observation trials, and suppression type PTNs changed pattern to facilitation during execution.

3.4.5 Different firing patterns during observation

For the main analysis we were only concerned with pure facilitative or suppressive effects during observation (F or S types, the vast majority of neurons showed this activity); however, it is important to note that these were not the only patterns of firing. Many neurons actually showed differential activity during the reach/grasp and hold phases of the observed action rather than just a pure facilitation or suppression effect. In monkey M47, there were two main components to the observed task: reach/grasp and hold. We were able to classify neurons based on their firing rate of these epochs. The following analysis comprises data taken from trials in which precision grip was the grasp performed by the experimenter and from monkey M47. This is used as an example to illustrate the patterns of firing during observation.

Fig. 3.4 shows four groups of neuron that we classified based on the firing rate during either the reach and grasp or hold phase. The four groups were ~,- (the classification was based on suppression only (-) during the hold phase; '~' signifies that these neurons were not classified on the basis of their reach/grasp activity);

-,~ (classification based on suppression of activity below baseline only during the reach/grasp phase), ~,+ (facilitation above baseline only during the hold phase) and +,~ (facilitation above baseline only during the reach/grasp phase).

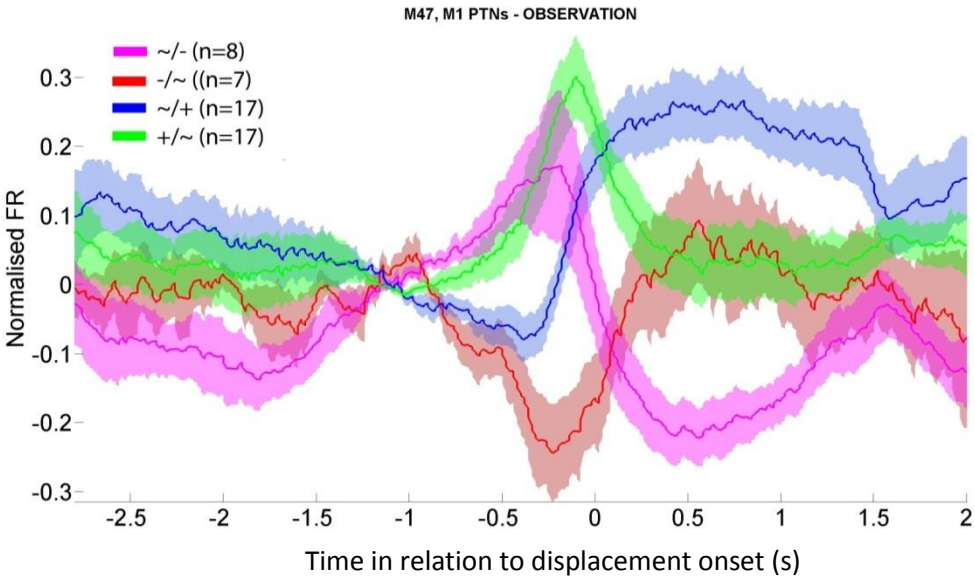


Figure 3.4 Firing patterns during Observation

Neurons have been categorised according to activity in either the reach and grasp or hold phase, (~,- ; -,~ ; ~,+ ; +,~) .+/- signifies that the neuron is classified based on the activity in either the first or second phase (first symbol denotes activity in the reach and grasp phase, second symbol applies to the hold phase), whilst '~' signifies that the neurons were not classified on the basis of their activity during that particular phase. Data are aligned to object displacement. Firing rates were smoothed using a 400 ms sliding window in 20 ms steps.

The (~,-, magenta trace, n=8) group showed a clear facilitation (~18% of the maximum modulation) during the reach and grasp phase even though neurons with suppressed activity in the hold phase were included (~20% below baseline). This type of mixed activation during observation was common but it is unclear why these neurons' discharge was both facilitated and suppressed during the same overall grasping action. We speculate that this might be due to the differences in the level of engagement of the mirror system between the dynamic phase (reach and grasp) and isometric phase (hold), but this is yet to be tested experimentally.

The red trace shows PTNs whose discharge showed suppression during the reach and grasp phase but which were not selected based on activity of the hold phase (n=7). These neurons suppressed their activity to 23% below baseline just before displacement onset (time zero on plot), before returning to baseline firing throughout the hold period.

Neurons that were facilitated during the hold period (23% of maximum modulation above the baseline, blue trace, n=17) tended to have a small level of suppression during the reach/grasp period (~5%). PTNs that were facilitated during the reach and grasp phase (green trace, n=17), modulated their activity to around 30% of the maximum modulation just prior to displacement onset but their activity decreased back to baseline during the hold period.

It is clear that M1 PTNs show different patterns of activity during observation of grasp, and although half of each of these curves are arbitrarily defined, we still find remarkably similar activity in the period that is not required for definition of the subtype ('~'). Note the SEMs are quite small in comparison to the overall modulation of the means. It might be that these neurons are mirroring different parts of the action; however, this requires further experimental testing.

3.4.6 CM cells as mirror neurons

In M47 we carried out spike-triggered averaging to determine whether PTNs, whose discharge was modulated during action observation, also exerted post-spike facilitation of hand muscles, identifying them as cortico-motoneuronal cells (Maier et al., 1993, Porter and Lemon, 1993). Of the 34 mirror PTNs tested, five (15%) had clear post-spike effects; three were facilitation and two were suppression mirror neurons. **Fig. 3.5** shows an example of a CM cell that was also a mirror neuron. The neuron was a facilitation mirror neuron that was strongly facilitated to around 80 spikes/s and 10 spikes/s in execution and observation trials, respectively. Spike – triggered averaging of the FDI EMG revealed post-spike facilitation of this muscle (see peak at ~10ms).

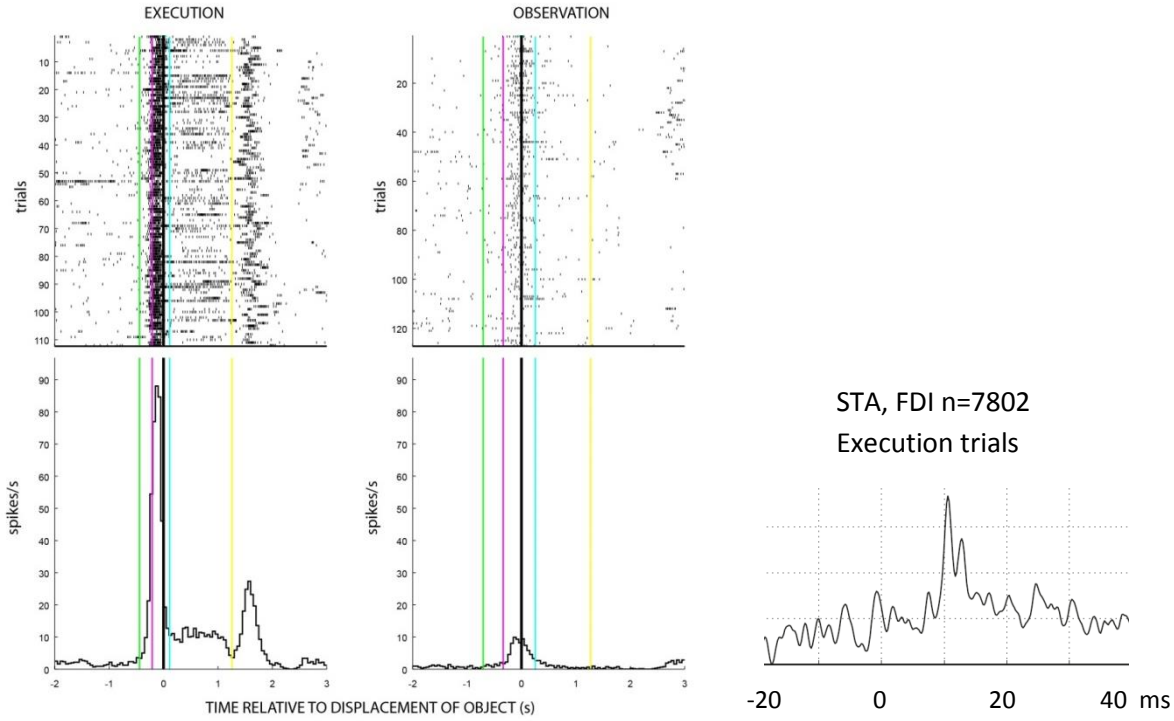


Figure 3.5 CM Mirror cell

An example of a classical mirror neuron that was also a CM cell.
Left: Rasters and histograms of one CM cell. Data are aligned to DO (black line), and the median times of other events recorded are shown as vertical lines: GO (green), HPR (magenta), HON (cyan), HOFF (yellow). Data have been binned in 50 ms bins.
Right: Spike triggered average of the FDI EMG using 7802 spikes; a clear post-spike facilitation of EMG is present at ~ 10ms.

3.4.7 Analysis of mirror neuron PTNs during different types of grasp

In addition to performing the experiment with the trapezoid object we also trained monkey M47 to grasp two other objects: a sphere and a ring. These objects afforded different grips (whole-hand-grasp and hook grasp respectively, see **Fig. 2.2** for description and illustration of grasps). This allowed us to compare the activity during execution and observation with several different grasps. Note that the results described are only based on data from monkey M47.

3.4.8 Grasp selectivity in execution

Fig. 3.6A-B shows raster plots and histograms of the firing of example PTNs which modulated their activity differently dependent on the object that was being grasped by the monkey. Although the grasps were carried out pseudo-randomly, the rasters have been sorted so that the trials involving the same object are adjacent and are aligned to the displacement of the object (start of the movement). Trials involving the hook grip of the ring are in red, whole-hand grasp of the sphere in green and precision grip in blue. Task related events for each trial are superimposed on top of the rasters with the median time of these events drawn as a vertical line and projected onto the histograms.

In general, for execution trials, we found that grasp selectivity sometimes manifested as a graded response, that is, a similar temporal firing pattern but different amplitude, dependent on the grasp. **Fig. 3.6A** shows an example of a PTN with a graded response; the neuron actually suppressed its activity after the GO signal irrespective of the object being grasped. Subsequently, after the homepad was released the neuron increased its firing rate and reached a maximum of ~180, 60 or 25 spikes/s depending on the object that was grasped at the time of displacement onset (ring, sphere, trapezoid, respectively). Note that for execution of precision grip there was actually a double peak of activation, not seen for the other grasps.

However in other neurons, grasp selectivity could also manifest as different temporal patterns dependent on the grasp e.g. **Fig. 3.6B**: The PTN fired only during the release

phase in trials in which the ring was grasped (~ 100 spikes/s), but suppressed its activity below baseline at the time of displacement onset. The same neuron increased its firing during the hold phase in trials in which the sphere or trapezoid was grasped.

We carried out a two-way ANOVA, using epoch and grip type as factors for M1 PTNs in M47. 52/53 (98%) had significantly modulated firing rates during execution trials. We found that 45 of the 52 (86.5%) cells that had significantly modulated firing rates during execution trials also had different firing rates for the different grasps or grasp selectivity.

3.4.9 Lack of grasp selectivity during observation

In contrast to the selectivity during execution, during observation we found much less grasp selectivity in M1; although many neurons did show some subtle differences, the difference in firing rates between the objects was much less compared with execution. **Fig. 3.6C** shows an example of the subtle differences seen during observation of grasp. **Fig. 3.6C** shows the rasters and histogram for one mirror PTN aligned to displacement of the object during observation. It is clear that the activity was rather similar during observation of a precision grip or whole-hand grasp; the neuron increased its firing rate around 0.6 s before displacement of the object and reached a maximum firing rate of just under 60 spikes/s. In contrast, trials in which a hook grasp was being observed the neuron started to fire 0.5s before displacement onset and reached a higher maximum of around 65 spikes/s. It is clear

that although there are subtle statistical differences between the curves shown here, there is much less of an overall difference during observation compared with execution.

We carried out a two-way ANOVA (as described above) for observation trials. 48/53 (90%) M1 PTNs were significantly modulated during observation. We found that 19/48 (40%) M1 PTNs that were modulated during observation also had significantly different firing rates for the different grasps. However, all these cells had very subtle, but statistically significant, differences in the firing rates, similar to that shown in **Fig. 3.6C**.

Mirror neurons could show either facilitated or suppressed discharge for the different grasps; not all neurons that mirrored one grasp necessarily mirrored another type of grasp. As I described earlier, we found 27 mirror neurons using trials in which the small trapezoid was being grasped in a precision grip. Interestingly, when we used trials in which the ring was being grasped, we found that a smaller number of neurons mirrored (n=20), and many of these mirror neurons overlapped with the neurons that mirrored precision grip. However, two of the neurons were unique, that is, they did not show mirror activity during observation of either precision grip or whole hand grasp, only the hook grasp of the ring. Using trials in which the sphere was grasped, we found 25 mirror PTNs, three of which were unique to the whole hand grasp of the sphere.

For the hook grasp we found (75%, n=15) neurons of the F-F or facilitation type, the remaining (25%, n=5) were the S-F or suppression type. We also found a similar

proportion of facilitation (74%, n=20) and suppression (26%, n=7) mirror neurons for precision grip. For whole-hand grasp, we found a larger number of facilitation mirror neurons (84%, n=21) compared with suppression mirror neurons (16%, n=4).

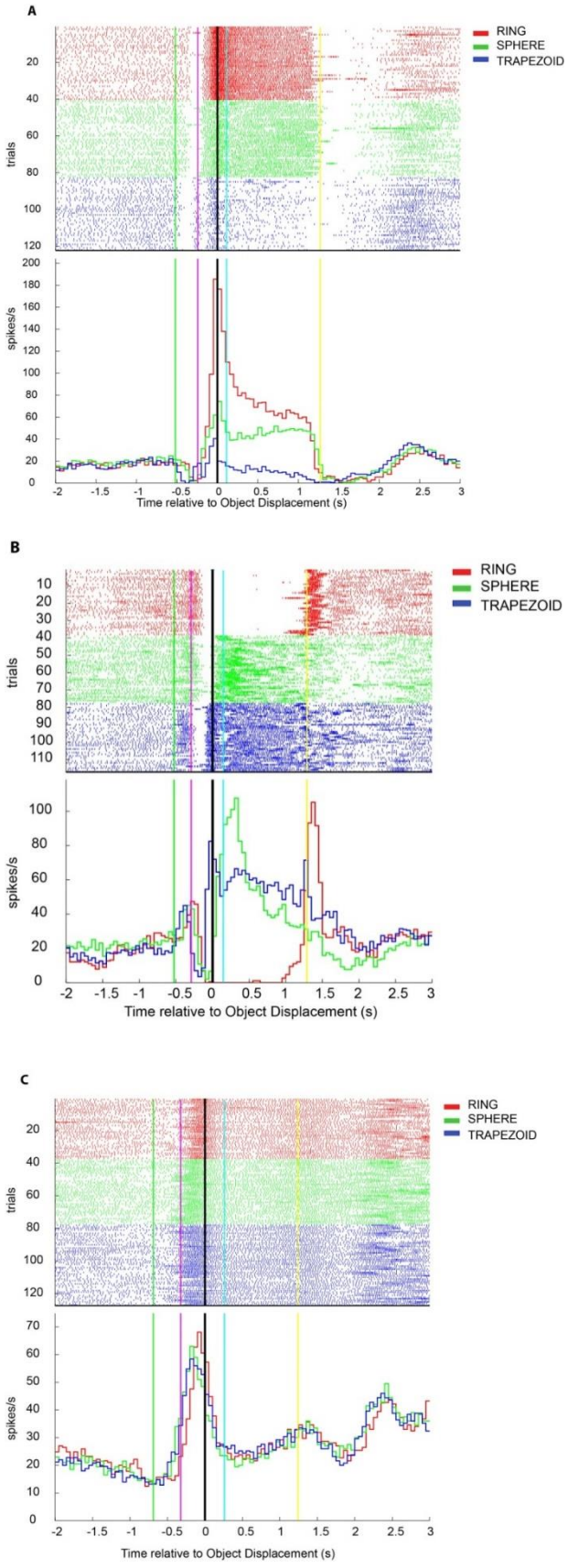


Figure 3.6 Examples of Grasp Selectivity

(A) Raster and histogram plots of one PTN showing different firing rates for different grasps during execution. Data is aligned to displacement of the object (black line), and the median times of other events recorded are shown as vertical lines: GO (green), HPR (magenta), HON (cyan), HOFF (yellow). Rasters have been grouped in relation to the object being grasped, ring (red), sphere (green) and trapezoid (blue). Although the presentation of all objects was randomised during the recording, they are grouped together on the plot for easier visual inspection. **(B)** As above. **(C)** Raster and histogram plots of one PTN showing statistically different firing rates for different grasps during **observation**.

3.4.10 Population activity for other grasps

Fig. 3.7 shows the population average of mirror neurons (F-F, S-F types) for the hook grip. **Fig. 3.7A** shows the average neuronal activity for facilitation (red trace, n=15) and suppression (blue trace, n=5) during observation. **Fig. 3.7B** shows the activity of these same neurons during execution. During observation (**Fig. 3.7A**) both facilitation and suppression neurons modulated their activity at around HPR, with peak modulation at displacement onset. During execution (**Fig. 3.7B**) facilitation PTNs discharged at around 50% of the maximum modulation above the baseline vs 23% during observation. For suppression mirror PTNs the activity during observation was 17% below baseline compared with 50% above it for execution. Interestingly, the suppression neurons sub-group were on average slightly facilitated/ back to baseline during observation by hold onset (HON).

Fig. 3.8 shows the population plots for whole-hand grasp. During observation facilitation neurons (red traces, n=21) discharged at around 18% above baseline compared with ~70% above baseline during execution. Suppression PTNs (blue traces, n=4) once again reversed their activity from 25% below baseline during observation to ~55% above baseline during execution. Notably, there was a double peak of activation similar to that seen for the precision grip (cf. **Fig. 3.2C**). Many of the mirror neurons that showed suppression of discharge during precision grip also had a double peak of activation during execution trials (see **Fig. 3.6B** for example). These cells typically increased their firing rate before being suppressed and then fired again at a higher rate. It is not clear why these cells displayed this activity, but the

suppression seen during observation might be correlated with the suppression during execution trials.

There are two additional differences of note when comparing the population plots for the three different grips (cf. **Figs. 3.2C, 3.7 and 3.8**): suppression mirror neurons for whole-hand grasp also have suppressed activity during the hold phase (~15% below baseline, see blue trace on **Fig. 3.8B**). This is in contrast to suppression PTNs for hook and precision grips, which are facilitated during execution (~40%, cf. **Figs. 3.2C & 3.7B**). It is also noteworthy that the suppression mirror neurons for the hook grip started and reached their maximum modulation later on average compared with facilitation neurons during execution. This is in contrast to the population plots for precision and whole-hand grips shown in **Fig. 3.2C & 3.8A** (suppression neurons were modulated earlier compared with facilitation neurons).

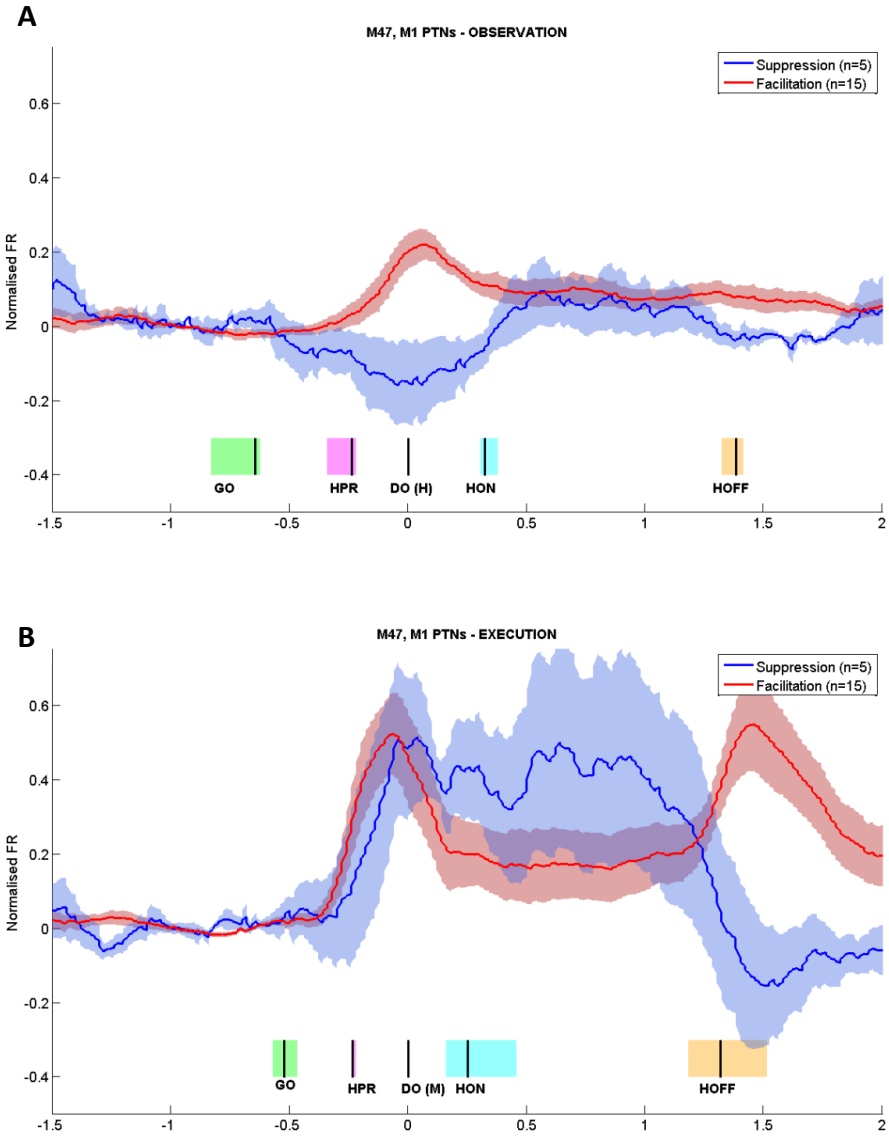


Figure 3.7 Population average (Hook Grip)

(A) Population averages during observation of a hook grip for corticospinal mirror neurons (M47) that were activated during execution and whose discharge was significantly suppressed (blue) or facilitated (red) during observation (together with SEM, shaded areas). Firing rates were normalized to the absolute maximum of the smoothed averaged firing rate of individual neurons defined during execution and observation trials, and baseline firing rate was subtracted. Data aligned to DO, the median (black line), and the 25th to 75th percentile times of other events recorded are shown as shaded areas: GO (green), HPR (magenta), hold HON (cyan), and HOFF (yellow). Firing rates were smoothed using a 400 ms sliding window in 20 ms steps. Right: population average for the same groups of mirror neurons during execution of hook grip. Facilitation-type PTNs showed higher discharge rates during execution compared with observation trials, and suppression type PTNs changed pattern to facilitation during execution. **(B)** Population average for the same groups of mirror neurons during execution of hook grasp.

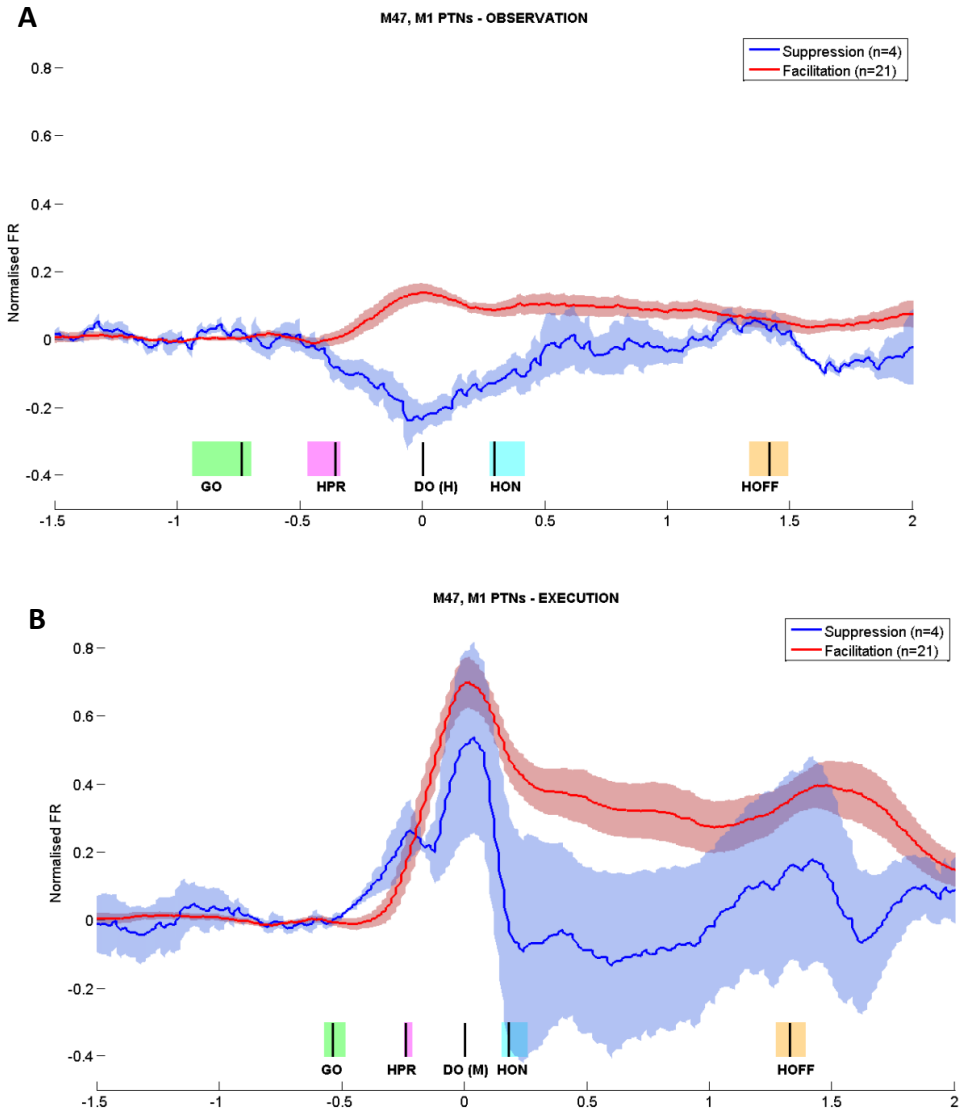


Figure 3.8 Population average (Whole-hand Grip)

(A) Population averages during observation of a whole-hand grasp for corticospinal mirror neurons (M47) that were activated during execution and whose discharge was significantly suppressed (blue) or facilitated (red) during observation (together with SEM, shaded areas). Firing rates were normalized to the absolute maximum of the smoothed averaged firing rate of individual neurons defined during execution and observation trials, and baseline firing rate was subtracted. Data aligned to DO, the median (black line), and the 25th to 75th percentile times of other events recorded are shown as shaded areas: GO (green), HPR (magenta), hold HON (cyan), and HOFF (yellow). Firing rates were smoothed using a 400 ms sliding window in 20 ms steps. **(B)** Population average for the same groups of mirror neurons during execution of whole-hand grasp.

3.4.11 Go/No-go response

In addition to the mirror experiment, as outlined in the methods, we also recorded spiking activity of single neurons during a Go/No-go paradigm.

Fig. 3.9 shows examples of the No-go effects we found in primary motor cortex. **Fig. 3.9A** shows the rasters and histogram of a PTN during No-go trials (this neuron was actually a facilitation mirror neuron).

Fig. 3.9A shows the activity of the neuron during the execution No-go phase (left panel) and observation No-go phase (right panel). The rasters and histograms have been aligned to the No-go signal (red led). During execution No-go trials it is clear that the neuron increased (from 20 to 50 spikes/s) and decreased its firing rate over a short duration (approx. 100-150ms). This occurred after the No-go cue. This effect is clearly seen on the raster plots.

This brief burst of activity or 'blip' is not present (no significant change from baseline) on trials in which the monkey watched the experimenter perform the same task (see right panel). In this part of the experiment, the monkey had to remain still whilst observing the experimenter react to a No-go cue. The firing rate of this neuron did not significantly change after the experimenter received the cue.

Fig. 3.9B shows another example of a neuron with a No-go effect. On No-go execution trials, the PTN slowly increased its firing rate from <25 to 40 spikes/s just before the cue and then showed a brief burst of activity to 70 spikes/s. It then sharply decreased its firing rate to baseline (~20 spikes/s). Once again, this is only seen on execution trials (this burst was not present on observation trials (right panel)). It is

important to remember that the trials were completely interleaved with execution and observation Go trials and so the effects are not caused by any predictability in trial type.

To quantify the activity of PTNs that showed this effect we carried out a one-way ANOVA. We compared the neuronal activity in the 500 ms before the cue onset to the activity in the first 150 ms after the cue. We chose these timings because on visual inspection most of the responses were seen very early after the cue. We found that discharge of 14/53 (26%) PTNs were significantly modulated after the No-go cue.

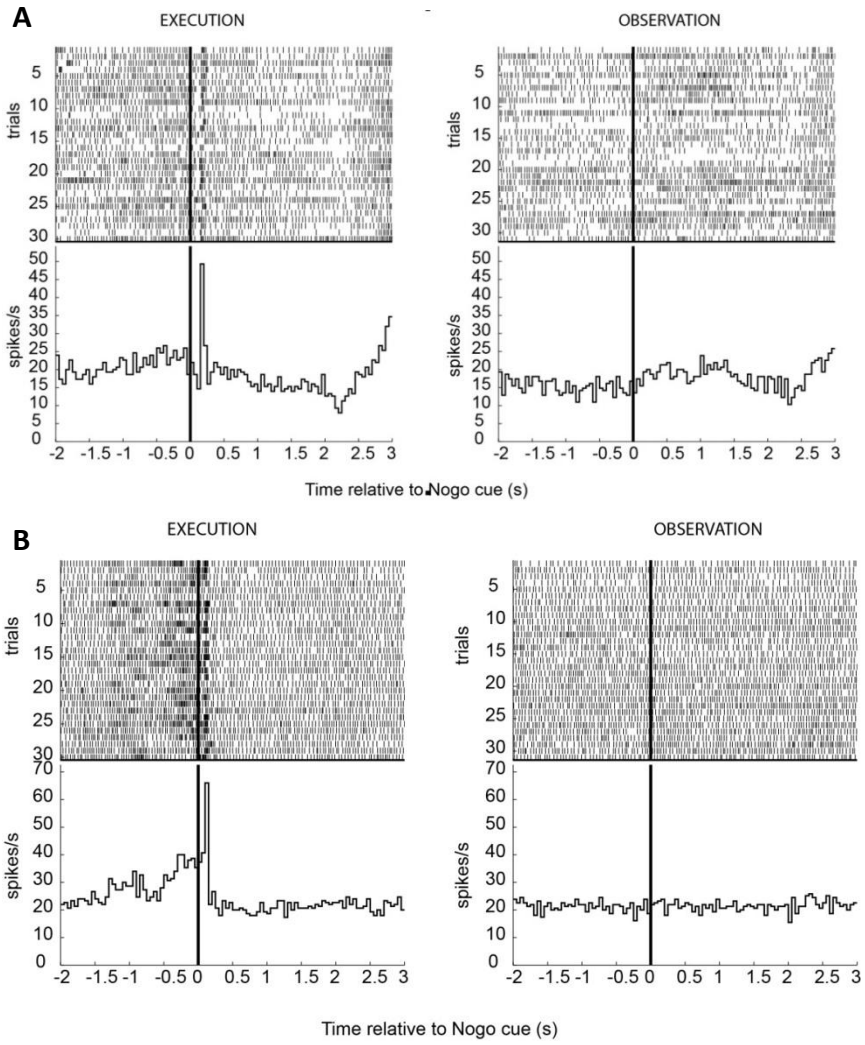


Figure 3.9 Examples of No-go effect

(A-B) Raster and histograms of PTNs following a No-go cue. Data are aligned to the No-go cue (black line). Left: Execution of a No-go. Right: Observation of a No-go (performed by the experimenter).

We categorised the activity based on whether this initial component was facilitated or suppressed in relation to the baseline. We found 10 neurons that were significantly facilitated and 4 neurons that were suppressed after the cue onset (although these cells became facilitated later in the trial).

Fig. 3.10A shows the population averages of these sets of neurons during No-go trials, data have been normalised across Go and No-go trials so that the depth of the

modulation can be compared across conditions. We also plot the activity of the same neurons during Go trials (**Fig. 3.10B**). **Fig. 3.10C** shows the population activity superimposed. It is clear that the No-go responses follow a similar pattern to the Go responses and only differ after the presentation of the cue. For the No-go facilitation neurons (n=10), the neurons start ramping their activity before the Go/No-go cue and continue to increase their activity after the onset of the cue, however, on No-go trials the response is similar but clearly smaller. The maximum activity for these 10 neurons is around 21% of the maximum modulation. During Go trials the average modulation was much higher at around 55% of the maximum modulation.

Some neurons significantly suppressed their activity shortly after the Go/No-go cue (n=4, blue traces). During No-go trials, these neurons suppressed their activity to around 9% below baseline; shortly after, they increased their activity above baseline (~9%). When we examined their activity during Go trials, it is clear that these same neurons also suppress their activity to a similar extent (compare light blue and dark blue traces on **Fig. 3.10C**). However, after the initial suppression they are more strongly modulated above baseline (~79% maximum modulation).

We also carried out a statistical analysis comparing the Go with the No-go responses within the same neuron. For the facilitation type responses, the No-go and Go responses were significantly different from each other for a period of 100ms (light red trace compared with the dark red trace) to on average 140ms after the onset of the cue. For the suppression type neurons, they become significantly different from each other somewhat later, at 200ms after the cue.

We did not find that this ‘No-go’ effect was specifically restricted to mirror neurons; out of the 14 neurons that were significantly modulated after the No-go cue, seven neurons were facilitation type mirror neurons, one was a suppression mirror neuron and six were non mirror neurons.

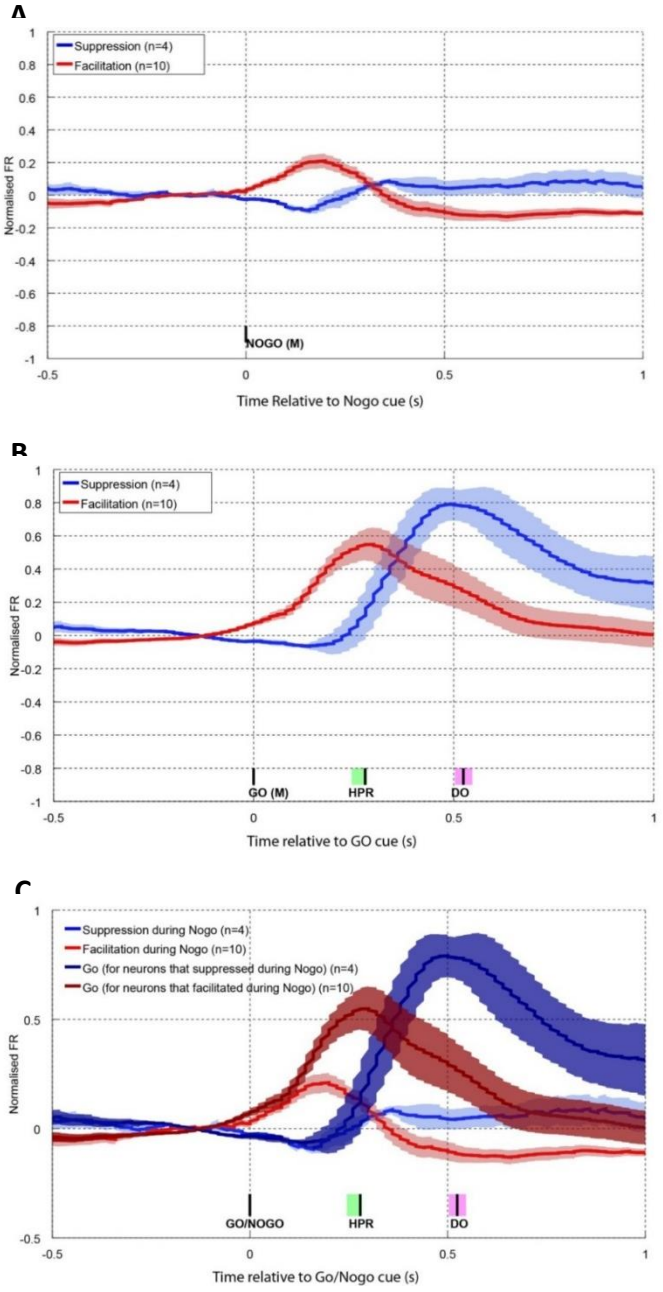


Figure 3.10 Population average of No-go responses

(A) Population averages during execution of a No-go for corticospinal neurons (M47) discharge was significantly suppressed (blue) or facilitated (red) during the initial 150 ms following the onset of the No-go cue (together with SEM, shaded areas). Firing rates were normalised to the absolute maximum of the smoothed averaged firing rate of individual neurons defined during execution Go and No-go trials, and baseline firing rate was subtracted. Data aligned to the No-go cue, the median (black line). **(B)** Population average for the same groups of neurons during execution Go trials. PTNs showed higher discharge rates during execution compared with execution No-go trials. Again data are aligned to the GO cue and the 25th to 75th percentile times of other events recorded are shown as shaded areas: HPR (magenta). Firing rates were smoothed using a 400 ms sliding window in 20 ms steps. **(C)** Traces from A and B superimposed onto the same plot. Lighter shades represent activity during No-go trials, whilst darker shades represent the activity during GO trials.

3.4.12 Eye movements

For a given PTN there did not appear to be any correlation between the firing rate and eye movements. For example, the monkey routinely made a saccade to the object when it was first made visible, but we did not see any modulation of PTN discharge at this time. However, 19 PTNs showed a significant correlation between the time the monkey spent looking at the object and the neuronal firing rate. The monkey spent less time looking at the object during observation than during execution. However, the object fixation pattern between both conditions was highly correlated (0.92, $p<0.05$) emphasising that the monkey paid attention to the experimenter's actions during observation trials although this was not explicitly required in the task design.

3.5 DISCUSSION

3.5.1 Mirror Neurons in Primary motor cortex

The primary finding of this study reveals that there is widespread mirror activity amongst PTNs in the hand area of macaque primary motor cortex. Using data from two monkeys, we have shown that there is significant modulation of firing rate in over half of recorded corticospinal neurons during observation of a precision grip carried out by a human experimenter. Most of these PTNs (38/65, 58.5%) were categorised as 'facilitation ' mirror neurons, similar to those originally described by Gallese et al. (1996), increasing their discharge during both observation and execution. However, these neurons were far less active for observation than execution (**Figs. 3.2C-D & 3.3**), with the overall normalised firing rate down to less than half that when the monkey performed the grip. This comparison is valid in that

both human and monkey performed a similar set of actions on the same trapezoid object, and both used a precision grip. Just as had previously been demonstrated in area F5 of premotor cortex (Kraskov et al., 2009), we also found a significant proportion of 'suppression' mirror neurons in M1 (27/65, 41.5%). During action observation, these neurons either decreased their firing rate (solid line in **Fig. 3.1B**) or stopped firing altogether (**Fig. 3.1D**). Nearly all of these 'suppression' PTNs reversed their pattern of activity during execution, and increased their firing rate.

The significance of these findings is that M1 contributes 50% of the descending corticospinal projection from the frontal lobe (Dum and Strick, 1991), which terminates heavily in lower cervical cord (Maier et al., 1993) and includes direct cortico-motoneuronal projections directly influencing activation of digit and other muscles (Lemon, 2008). Thus, during observation, there is modest modulation of descending pathways that might influence downstream spinal targets involved in control of digit and other muscles.

During observation, discharge in M1 facilitation mirror PTNs was attenuated (compared with activity during execution) and was even reversed in suppression mirror PTNs. Taken together, this would mean that that M1 output to spinal interneurons and motoneurons involved in generating movements in hand and digit muscles could be strongly disfacilitated during observation (green bars in **Fig. 3.2D**), but nonetheless still be above baseline. Metabolic activity in the monkey spinal cord has been reported to be depressed during action observation (Stamos et al., 2010). A reduction in activity during action observation might arise from two possible scenarios that are not mutually exclusive, while this could reflect active inhibition; it

could presumably also have resulted from a disfacilitation of descending excitation as described here.

We do not know whether the effects at spinal level of our sample of mirror PTNs were excitatory, inhibitory, or mixed (Olivier et al., 2001, Porter and Lemon, 1993). There is one notable exception to this, namely the mirror PTNs identified as cortico-motoneuronal cells (see **Fig 3.5**) (Lemon, 2008). These neurons within M1 are connected monosynaptically to α-motoneurons. Since the synaptic terminals of these cells on spinal motoneurons are not subject to presynaptic inhibition (Jackson et al., 2006), there is no obvious mechanism to prevent discharge in these cells facilitating their target motoneurons. So it is interesting that two of the five cortico-motoneuronal cells that we identified showed suppression of activity during observation. Such a mechanism might help to prevent this input contributing to unwanted discharge of motoneurons and movement. Suppression of discharge was also seen for a small population of PTNs during execution trials (dark colours in **Fig. 3.2A, B**); PTN disfacilitation has been reported before for tasks requiring skilled movements of the digits (Maier et al., 1993) including tool-use (Quallo et al., 2012).

Why are M1 output neurons modulated during action observation? If M1 is considered to be part of a larger 'action observation network' (Fadiga et al., 1995, Hari et al., 1998), then it is not surprising that the output neurons, which are strongly embedded in the intrinsic cortical circuitry (Jackson et al., 2002, Weiler et al., 2008) are also modulated. However, because of the functional proximity of M1 corticospinal neurons to the spinal apparatus, to avoid overflow of their activity into unintended, overt movements during processes which involve action observation, it

may be important to attenuate or block that activity. This may involve inhibitory systems operating at both cortical (Aron et al., 2007, Duque et al., 2012) and subcortical levels (Gilbertson et al., 2005). Viewed in this way, action observation is yet another manifestation of the dissociability of motor cortex and muscle activity, such as that seen in BMIs (Carmena et al., 2003, Davidson et al., 2007, Fetz and Cheney, 1987, Fetz and Finocchio, 1971), recently reviewed by (Schieber, 2013), and provides further reasoning for re-examining the concept that PTNs act as "upper motor neurons" (Schieber, 2011). Clearly, there are mechanisms present that can attenuate or reverse cortical activity, to stop an overflow of activity reaching the final target muscles. The activity itself reaching the cord might have a role in learning motor acts (see Chapter 7).

These findings show for the first time that PTNs in primary motor cortex exhibit mirror activity when monkeys watch humans grasping. The presence of this activity in the corticospinal output must have consequences for spinal networks supporting voluntary movements. The striking differences between M1 PTN activity for observation vs execution may help us understand more about the patterns of PTN discharge that lead to movement, as well as those that don't. They may also help to explain why we don't imitate every action that we observe.

3.5.2 Grasp selectivity during execution and observation

We also looked at grasp selectivity using data from one monkey (M47) that had been trained to grasp and observe three objects (ring, sphere, small trapezoid).

Although there were some neurons with grasp related selectivity (n=19), on the whole the differences in the firing rates for the different grasps were much smaller during observation of a grasp compared with execution (86.5% vs 40% of modulated units). This might mean, at least for primary motor cortex, that during observation of grasp there is more generalisation of the grasping action (very similar to the original "broadly congruent" type neurons described in the original mirror neuron studies e.g. di Pellegrino et al., 1992, Gallese et al., 1996). The neurons seem to respond to the overall grasping action with some subtle differences for the different types of grasp. This is in direct contrast to execution trials, where we find much more varied activity for the different grasps (86.5% of the units modulated during execution also had significantly different firing rates for the different grasps).

Interestingly, the monkey did not have to extract any grasp related information in order to obtain a food reward. He merely had to sit and keep its homepads depressed. One interesting question for further experimentation might be if the monkey had to use the information about the experimenter's type of grasp, would these same mirror neurons show a greater difference in firing patterns across the objects. This certainly cannot be ruled out.

We confirmed our previous findings that the firing rate of PTNs in motor cortex during observation is much lower compared with execution (all objects showed a much lower firing rate during observation compared with execution; **Fig. 3.2C, 7 and 8**). Interestingly, we show that there is considerable variation in the pattern of firing during execution for mirror PTNs that is dependent on the object (ring vs whole-hand

grasp) being grasped and the mirror neuron type (F-F vs F-S). Notably, mirror neurons whose activity was suppressed during hook grasp of the ring showed a higher firing rate during the hold period of execution trials compared with suppression mirror neurons for grasp of the sphere. It is still unclear what these differences might mean or reveal about the pattern of firing seen during observation, but one hypothesis is that there might be a correlation between the pattern firing rate during execution and observation. That is to say, if a neuron shows suppression during execution it might be more likely to be a suppression mirror neuron. These hypotheses are untested and require further data and analysis.

We also show that there is a much more complex firing pattern of mirror PTNs during observation of a grasp than a mere facilitation or suppression of activity. Although, much of the data on mirror neurons has previously described a pure facilitative or suppressive effect (Gallese et al., 1996, Rizzolatti et al., 1996, Kraskov et al., 2009) the pattern can be mixed (i.e. facilitation combined with suppression). For example, **Fig. 3.4** shows that when we look for those mirror neurons that had suppressed activity during the hold phase, tended to show facilitation during the reach and grasp phase, and thereby exhibit a mixed effect over the whole reach-grasp-hold action. In M47 we were able to accurately define this activity due to the additional behavioural markers recorded simultaneously, which was not possible in M43.

3.5.3 No-go response in primary motor cortex

We also report the presence of a No-go response within primary motor cortex, namely, a sharp increase and decrease in the firing rate of neurons on trials in which the monkey had to inhibit or suppress its movement. These were typically short duration (100-150 ms). One possible hypothesis to explain these findings might be that an external signal reaching primary motor cortex could be inhibiting the output cells of motor cortex. If the neuron continued to fire it might lead to strong activation of downstream spinal targets, possibly leading to unwanted overt movement. We found that the discharge of 26% of PTNs in M1 was significantly modulated after the No-go cue. It appears that the responses to the No-go cue are shorter and smaller versions of the Go response (compare light traces with dark traces in **Fig. 3.10C**). During Go trials the facilitation type neurons seem to have peak activity around the time of HPR, whilst the suppression time neurons have peak activity after HPR and nearer to displacement onset. This might suggest that the facilitation type neurons are more involved in the reach component of the grasp, whilst the suppression types are more closely linked to the grasp (displacement of the object); this would fit with the finding that in the wider literature, M1 neurons exhibiting suppression of activity during movement has mostly been reported for grasp-related actions (Hepp-Reymond et al., 1978, Quallo et al., 2012).

For the facilitation type, the Go and No-go traces become significantly different from each other around 140ms after the cue, whilst the suppression type become significantly different at around 200ms after the cue. The key difference between

the No-go and Go condition is that there is no movement during the No-go. However, as we have shown, there can be concurrent neuronal activity.

This might suggest that the level of activity during the No-go condition is not sufficient to elicit overt movement. As in the action observation condition, modulation of PTNs does not necessarily lead to movement.

Interestingly, we found that the No-go effect was not restricted to mirror neurons or even suppression mirror neurons. We had previously hypothesised that since suppression mirror neurons might play a role is suppressing activity during action observation by reducing their firing rate to below the baseline that they might also be suppressed when the monkey had to inhibit its own movements. Although we did find suppression mirror neurons with this activity it was not restricted to these particular types of mirror neurons. This might be the case because there is a fundamental difference in terms of inhibition of movement in execution vs observation. In the action-observation scenario, there is clear emphasis on the action being observed, compared with execution No-go, when you are primed to make a movement but have to withhold the response. Execution vs observation might be a more low-level computation compared with more higher level cognitive models of inhibition of movement that might be mediated by the prefrontal cortex (Aron et al., 2004b)

However, one common finding for suppression of movement during action observation and suppression during self-inhibition of movement is that when there is absence of movement, the amplitude of the responses of PTNs in M1 is lower.

During action observation, there is a reduction of activity; during No-go trials, there is also a reduction of activity (facilitation type neurons are at 21% of the maximum modulation during No-go trials compared with 55% during Go trials, and suppression type neurons are at a maximum of 9% compared with 79% during Go trials.

From these observations, it is clear that the long held beliefs of PTN activation leading to activation of downstream spinal targets and thereby causing movement is not as simple as it first seemed. We have shown that there can be widespread cortical activation and spiking in PTNs in a mirror task (where there is mere observation of a movement with no concomitant EMG activation) or even a scenario where suppression of movement is required (No-go).

CHAPTER 4:
F5 Corticospinal Mirror Neurons

4.1 INTRODUCTION

Mirror neurons were first discovered in the premotor cortex (area F5) of the macaque monkey (di Pellegrino et al., 1992, Gallese et al., 1996, Rizzolatti et al., 1996). More recently, it has been shown that corticospinal neurons in this area can have mirror properties and thereby can directly affect downstream spinal targets (Kraskov et al., 2009). This means that the 'mirror neuron system' must include projections to the spinal cord.

Since PTNs terminate in the spinal cord and can directly affect the spinal circuitry and motor output, it is of interest to directly compare the depth of modulation of neural activity during execution of a grasp with observation of the grasping action. Although the role of F5 corticospinal projections in movement is not well known (see Schmidlin et al., 2008, Borra et al., 2010), given that PTNs fire during both observation and execution of a grasp, it is a challenge to explain why in one scenario there is no overt movement, whilst there is movement in the other. Comparing the modulation and profile of activity between execution and observation might help us better understand the differences in activity that results in movement vs no movement.

Whilst there has been much research on the presence of mirror neurons in area F5, as yet, no systematic comparison between execution and observation has been carried out. This is important, when considering the functional role of area F5 may have in movement generation. It is also of interest to make a comparison of the activity between areas M1 and F5.

4.2 METHODS

The design of the experiment is identical to that described in Chapter 3, except that the data described here, is in relation to recordings made in the premotor cortex (area F5). In addition to the experiment outlined in the Chapter 3, we carried out some additional tests in monkey M47, which was trained to perform a more complicated version of the task. These additional tests involved manipulating the visual information that the monkey had during the observation of grasp. These are described as follows:

4.2.1 Screen Covered

The Screen Covered condition involved the screen being covered by a small opaque wooden cover during observation trials, and thereby not allowing vision of the experimenter's action. This meant that the monkey did not have vision of the grasp but only had vision of the reach and audio feedback that the experimenter was holding the object in the electronically defined window. During execution trials, the monkey performed the task under normal vision (i.e. the screen allowing vision of the monkey's action was operating as in the standard trials).

4.2.2 No movement

We also carried out a 'No-movement' condition. In this condition, after a set of normal trials had been completed, in which the experimenter would carry out the action observation experiment by correctly grasping and holding the object after the GO cue, we instructed the experimenter not make a reach to grasp action (although the homepad was released, the hand remained on the homepad) after the GO cue (importantly, the monkey probably expected the experimenter to move). These trials

were pseudo-randomised with the execution trials where the monkey had to grasp the object normally.

4.2.3 Decoding using observation data

For M47 we carried out a decoding analysis on all cells collected from M1 and F5. We trained a linear classifier to decode grip type for execution trials using spike data from observation trials. We used 14 time points around the displacement of the object as inputs to the linear classifier (-0.3s to +0.35s). We conducted a 10 nested, 10 fold cross validation analysis of single unit firing rate to test whether we could decode grasp during **execution trials** using a classifier trained on **observation trials**. Some neurons were excluded from the analysis because they contained trials which did not contain sufficient spikes (at least one spike), in addition we also only included cells that were significantly modulated during both execution and observation, and this left 135 cells for this analysis. The chance level was 33% (since there were three objects). The significance level (~40%) was estimated using the cumulative binomial test with $p<0.05$.

4.3 RESULTS

4.3.1 Recordings

PTNs were recorded in 24 and 10 sessions in M43 and M47, respectively, and over a period of 32 and 11 weeks, respectively. PTNs were recorded for a minimum of 10 observation and 10 execution trials. Most PTNs were recorded from tracks in the F5 hand region close to the arcuate sulcus (see Chapter 2, **Fig. 2.3**) at sites from which

activity was related to hand movements or evoked hand or digit movements from ICMS.

We analysed recordings from area F5 from monkey M43 to carry out a depth of modulation analysis similar to the analysis of M1 PTNs completed in Chapter 3. Whilst we were able to record 19 PTNs under the new experimental design in monkey M47, only 3 were mirror neurons and so PTNs from M47 have been left out of the population analyses, instead they have been used to confirm the presence of mirror neurons under the new task setup and to describe some preliminary findings in the 'screen covered' and 'No-movement' conditions described previously in the methods.

A total of 76 PTNs were recorded in area F5 from two monkeys (M43, 57 PTNS; M47, 19 PTNS). Once again, both monkeys had EMG recordings to confirm the absence of muscle activity during observation trials. We found evidence for both mirror neuron subtypes in both monkeys (facilitation and suppression). **Fig. 4.1** shows single neuron examples of these types of mirror neurons. For M43 (**Fig. 4.1 A-B**), the data shown in observation trials are aligned to the sensor or experimenter's grasp (see Chapter 2). The neuron shown in **Fig. 4.1A** is an example of a facilitation mirror PTN. During observation trials, the activity of this neuron reached 25 spikes/s around 600 ms after the experimenter's hand made contact with the sensor. During execution trials, which have been aligned to the movement onset (determined from EMG onset of the monkey's biceps muscle, corresponding to lifting of the hand to release the homepad), the neuron showed a similar increase in firing rate to around 30spikes/s,

at around 600 ms after movement onset. **Fig. 4.1B** shows an example of a suppression mirror neuron. This example neuron had a baseline firing of approximately 10 spikes/s. In observation trials (right), there was a complete suppression of activity soon after the experimenter contacted the sensor, and in many trials (see raster plots) the cell did not fire at all. In direct contrast, during execution trials (left), there was a double peak of activation (reaching ~65 spikes/s) just after movement onset and 550 ms after movement onset. **Fig. 4.2A-B** show data obtained from M47 on the new task. All data are aligned to the displacement of the object (monkey or experimenter depending on execution or observation trials, respectively). **Fig. 4.2A** shows another example of a classical mirror neuron. During observation trials, there was a sharp increase in the firing rate shortly after the experimenter released her homepad (magenta vertical line) to reach a maximum of ~33 spikes/s which was mostly sustained for much of the hold period. During execution trials there was a pause shortly after the GO cue (~30 spikes/s) followed by a large peak of activity around displacement onset (~55 spikes/s). **Fig. 4.2B** shows an example of a suppression mirror neuron recorded in the new task setup, during observation trials (right), the background firing rate was ~12 spikes/s and shortly after homepad released (magenta line) there was a suppression of firing to ~5 spikes/s with a minimum at around displacement onset of the experimenter's object. In contrast, during execution trials, this neuron decreased its firing rate shortly after homepad release (magenta line) but then was strongly facilitated to around 45 spikes/s shortly after displacement onset.

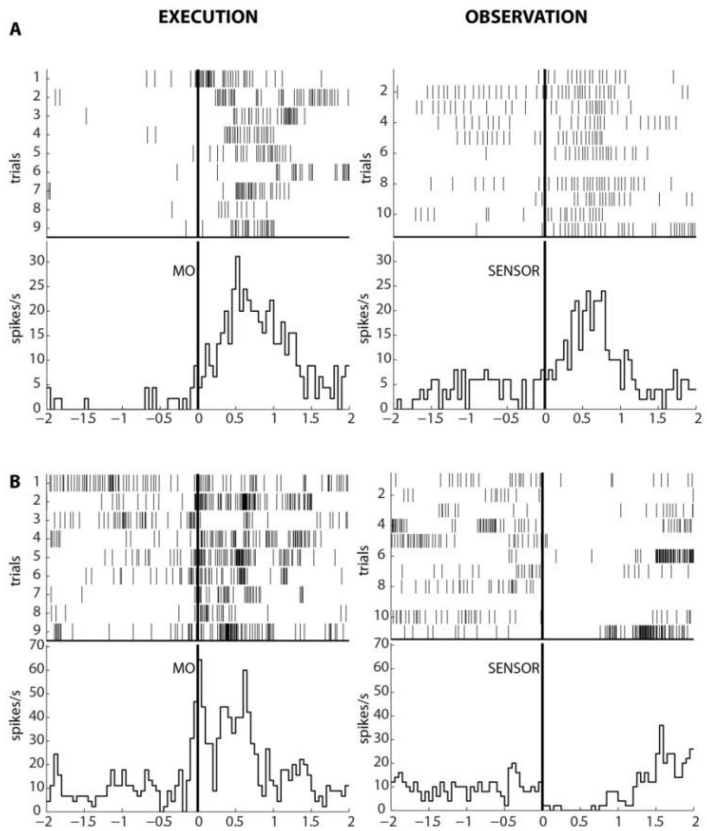


Figure 4.1 Examples of F5 Mirror PTNs (M43)

Examples of F5 facilitation **(A)** and suppression **(B)** mirror PTNs in M43. Execution and observation data is plotted in the first and second column, respectively. Histograms were compiled in 20 ms bins. All execution trial data were aligned to movement onset (MO), defined using onset of biceps EMG activity. All observation trial data were aligned to a sensor signal, which detected first contact of the experimenter's hand with the object.

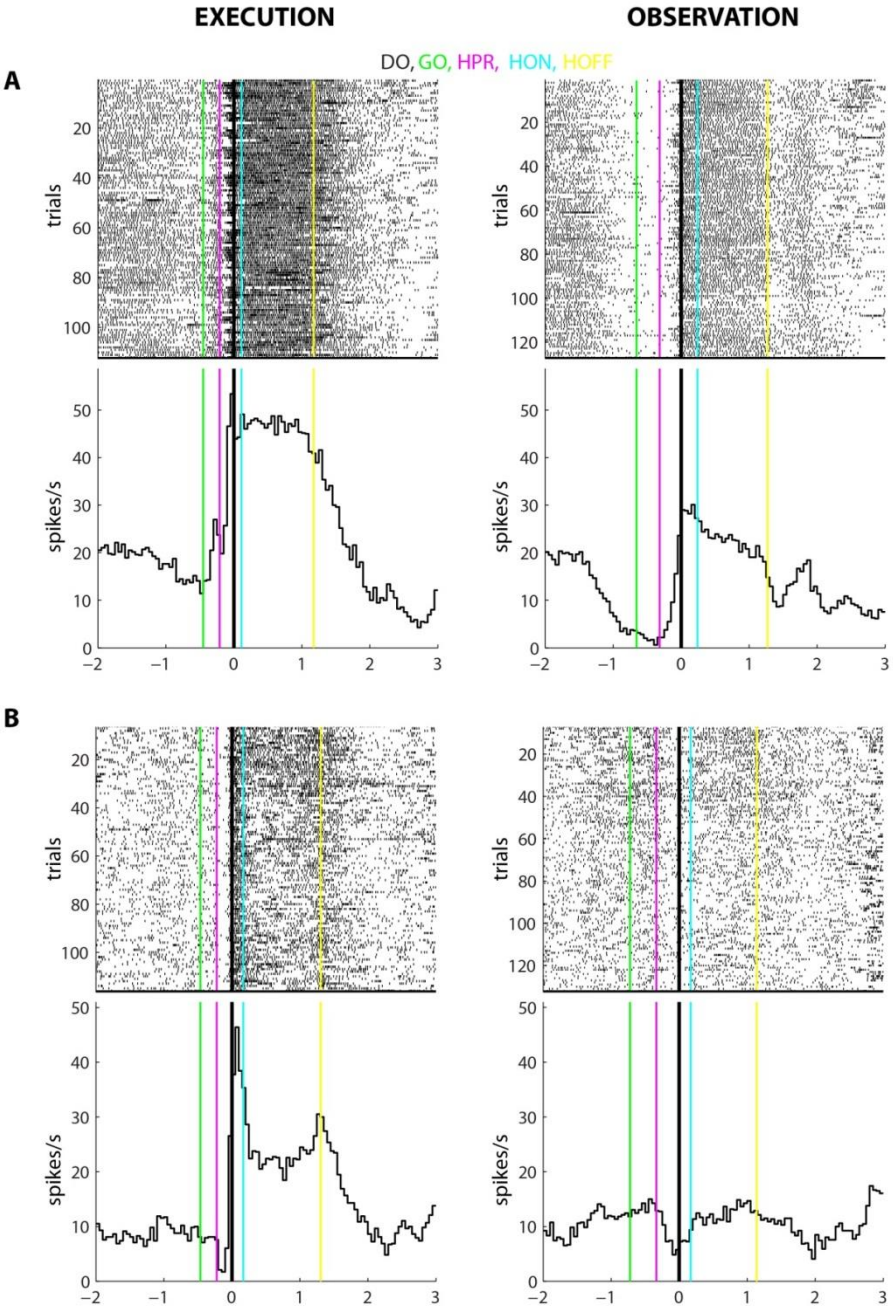


Figure 4.2 Examples of F5 Mirror PTNs (M47)

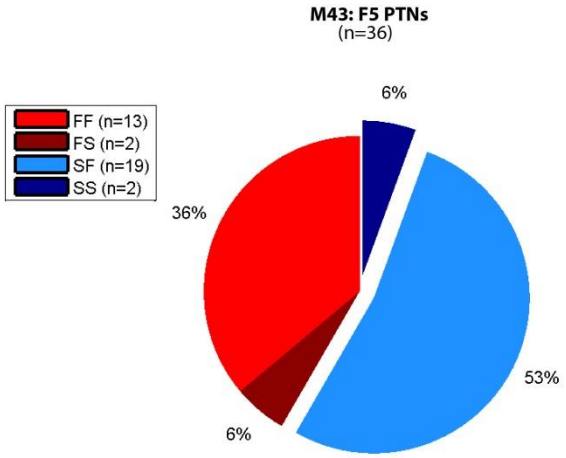
Examples of F5 facilitation **(A)** and suppression **(B)** mirror PTNs in M47. Execution and observation data is plotted in the first and second column, respectively. Histograms were compiled in 20 ms bins. All data were aligned to onset of the object displacement (DO); other behavioural events are indicated by coloured markers for each trial on raster plots and with vertical lines on histograms (cf. Chapter 2, Fig. 2.1).

4.3.2 Population analysis

In M43 we found that the discharge of 36/57 (63%) neurons was significantly modulated during observation of the task. **Fig. 4.3** shows a breakdown of all neurons whose activity was modulated during observation. PTNs have been classified based on their activity during observation and execution; the four classes of neurons are F-F (facilitated during observation and execution), F-S (facilitated during observation and suppressed during execution), S-F (suppressed during observation and facilitated during execution), S-S (suppressed during observation and execution).

Furthermore, 32/57 (56%) PTNs could be classified as mirror neurons (neurons that modulated their activity during observation, whilst in addition, being *facilitated* during execution). 13 neurons (36%) were of the type F-F (i.e. these neurons would be classified as "classical" mirror neurons, light blue) and 19 (53%) of the type S-F ("suppression" mirror neurons, light red). We also found a small number of neurons that were either of the F-S (n=2, dark red) or S-S (n=2, dark blue) type.

Figure 4.3 Neurons modulated during action observation (M43)



Pie chart showing different types of facilitation (red, F) and suppression (blue, S) F5 PTNs recorded during action observation in M43. FF denotes facilitation during observation and execution, FS: facilitation during observation and suppression during execution; SF: suppression during observation and facilitation during execution; SS: suppression during observation and execution.

Fig. 4.4 shows the population averages for monkey M43; we plot the average normalised firing rate (normalised across observation and execution, so that the depth of modulation can be compared) of all PTN mirror neurons +/- SEM (lighter shades). For the facilitation type (F-F type, n=13, red trace), discharge during execution reached a maximum of 45% of the maximum modulation above baseline vs 51% during observation of grasp. Suppression mirror neurons (n=19, S-F type, blue trace) discharged at 46% *below* the baseline during observation vs 50% *above* the baseline during execution. Note the temporal differences in the population plots for observation. The maximal suppression (at time 0, sensor signal indicating the onset of the experimenter's grasp) occurred earlier compared with the maximal facilitation, which occurred after the grasp was completed.

In a similar analysis to that carried out in Chapter 3 (see Chapter 3, **Fig 3.2D**), we estimated the maximum firing rates (non-normalised) during execution and observation of the task, and calculated the change from execution to observation (see **Fig. 4.5**- green bars). This was to calculate the actual amount of activity in terms of PTN spikes per second reaching the spinal circuitry.

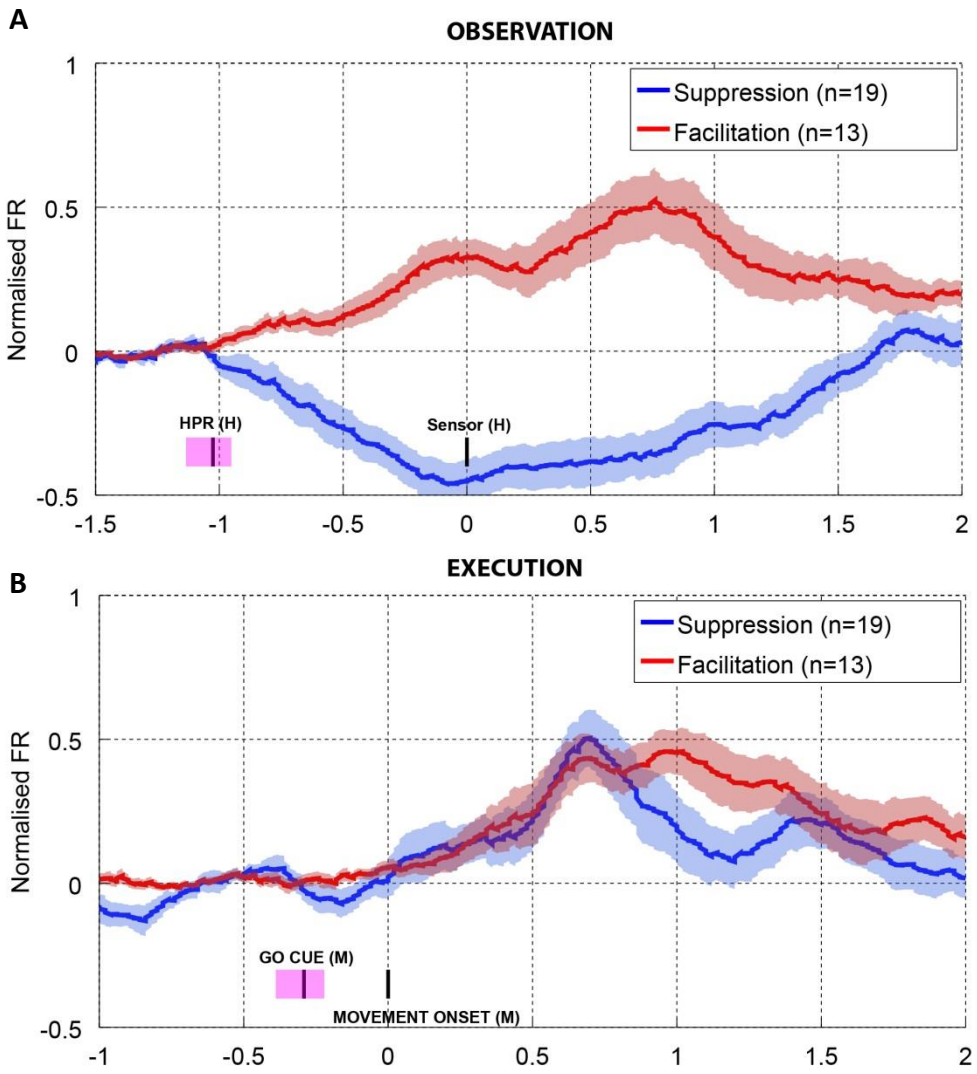


Figure 4.4 Population averages of F5 Mirror PTNs (M43)

(A) Population averages during observation for corticospinal mirror neurons (M43) that were activated during execution and whose discharge was significantly suppressed (blue) or facilitated (red) during observation (together with SEM, shaded areas). Firing rates were normalised to the absolute maximum of the smoothed averaged firing rate of individual neurons defined during execution and observation trials, and baseline firing rate was subtracted. Data aligned to sensor, the median (black vertical line), and the 25th to 75th percentile times of other events recorded are shown as shaded areas: HPR (magenta). Firing rates were smoothed using a 400 ms sliding window in 20 ms steps. **(B)** Population average for the same groups of mirror neurons during execution. Facilitation-type PTNs showed higher discharge rates during execution compared with observation trials, and suppression type PTNs changed pattern to facilitation during execution.

We found that for the F-F type mirror neurons in area F5 (red bars), there was a similar level of activity across observation and execution with the mean firing rate being ~25 spikes/s/PTN during the observation condition and ~28 spikes/s/PTN during execution. For the S-F type neurons, there was a decrease in the mean firing rate from baseline (~10 spikes/s/PTN) during observation, whilst during execution there was a reversal of the activity, with activity actually being facilitated above the baseline (~22 spikes/s/PTN). This means that overall there was a disfacilitation of total PTN activity from execution to observation of about (~20 spikes/s/PTN), or in other words, during execution, there are on average ~20 spikes per PTN more than compared with observation. Note, that the activity of the F-F type is quite similar across execution and observation. Non-mirror neurons (neurons that are significantly facilitated during execution but show no significant change during observation) also contribute to an overall disfacilitation of the spinal targets, as these neurons fired at ~45 spikes/s during execution whilst barely firing above baseline during observation. The disfacilitation attributed to the non-mirror population (~40 spikes/s/PTN) is actually greater than the mirror population (~20 spikes/s/PTN) in F5.

Unfortunately, the sample of mirror neurons found in monkey M47 was too small (n=3) to make comments on the population activity.

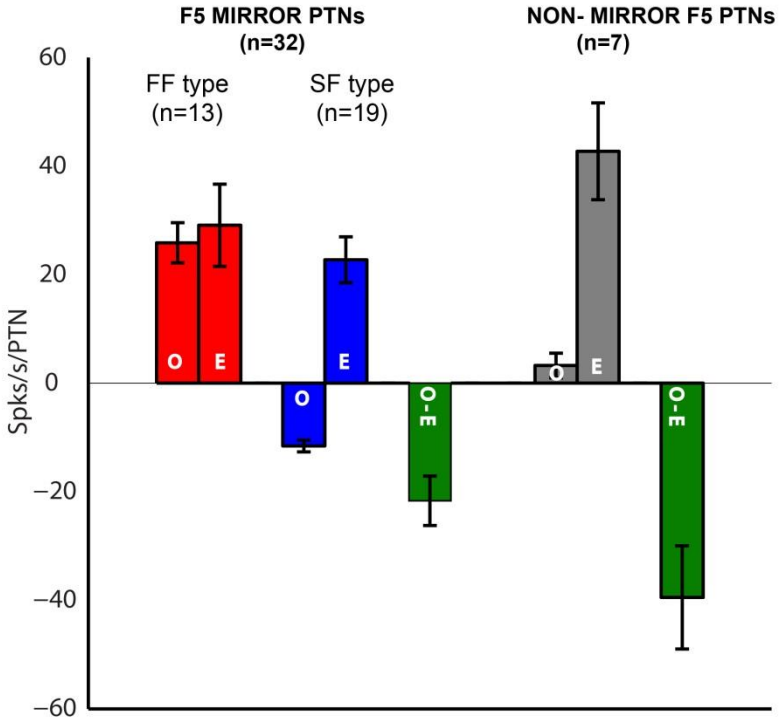


Figure 4.5 Firing rates of F5 PTNs

Maximum firing rate of F5 PTNs during observation and execution trials, expressed as raw firing rates (with SEM). Results from **M43** only. Red bars show average rates for 13 F5 PTNs facilitated during both observation (O) and execution (E) (F-F type). Note the similar rate during observation. Blue bars show rates for 19 M1 PTNs suppressed during observation (O) and facilitated during execution (E) (S-F type). The left green bar shows the mean firing rate for all these mirror PTNs in observation minus that in execution, to capture the total amount of disfacilitation in the output from these neurons that occurred during observation. On the right are similar results for F5 PTNs that did not show any mirror activity.

4.3.3 Additional properties of mirror neurons in F5

Although the sample in M47 was small, we were able to perform two additional tests which involving manipulating the visual information that the monkey received during the task. These provided us with some preliminary data for further investigation.

In the first condition, we covered the screen during observation trials whilst the experimenter continued grasping the objects as normal. In this way the last part of

the action or grasp was hidden, but the reach was visible. **Fig. 4.6B** (screen covered condition, green traces) shows the histogram and raster plot for one PTN we recorded in area F5 that continued to fire on observation trials (**Fig. 4.6B**) even though the monkey had no clear view of the grasping action, the neuron started to fire with the release of the experimenter's homepad and peaked at around 45 spikes/s before the displacement of the object (which the monkey was presumably only able to infer from the sound of experimenter displacing the object into the electronically defined window). Note that although the PTN still fired, the depth of modulation was less (peak, ~45 spikes/s) compared with under full vision of the grasping action (see **Fig. 4.6B**, red trace, peak ~110 spikes/s). There was also a delay in the initiation of firing of this PTN in the screen-covered condition (post-cue in the normal scenario to just before HPR in the screen covered condition, see shift in red vs green traces in **Fig. 4.6B**). Importantly, the activity of this neuron was unchanged during execution trials (see **Fig. 4.6A**, compare red and green traces).

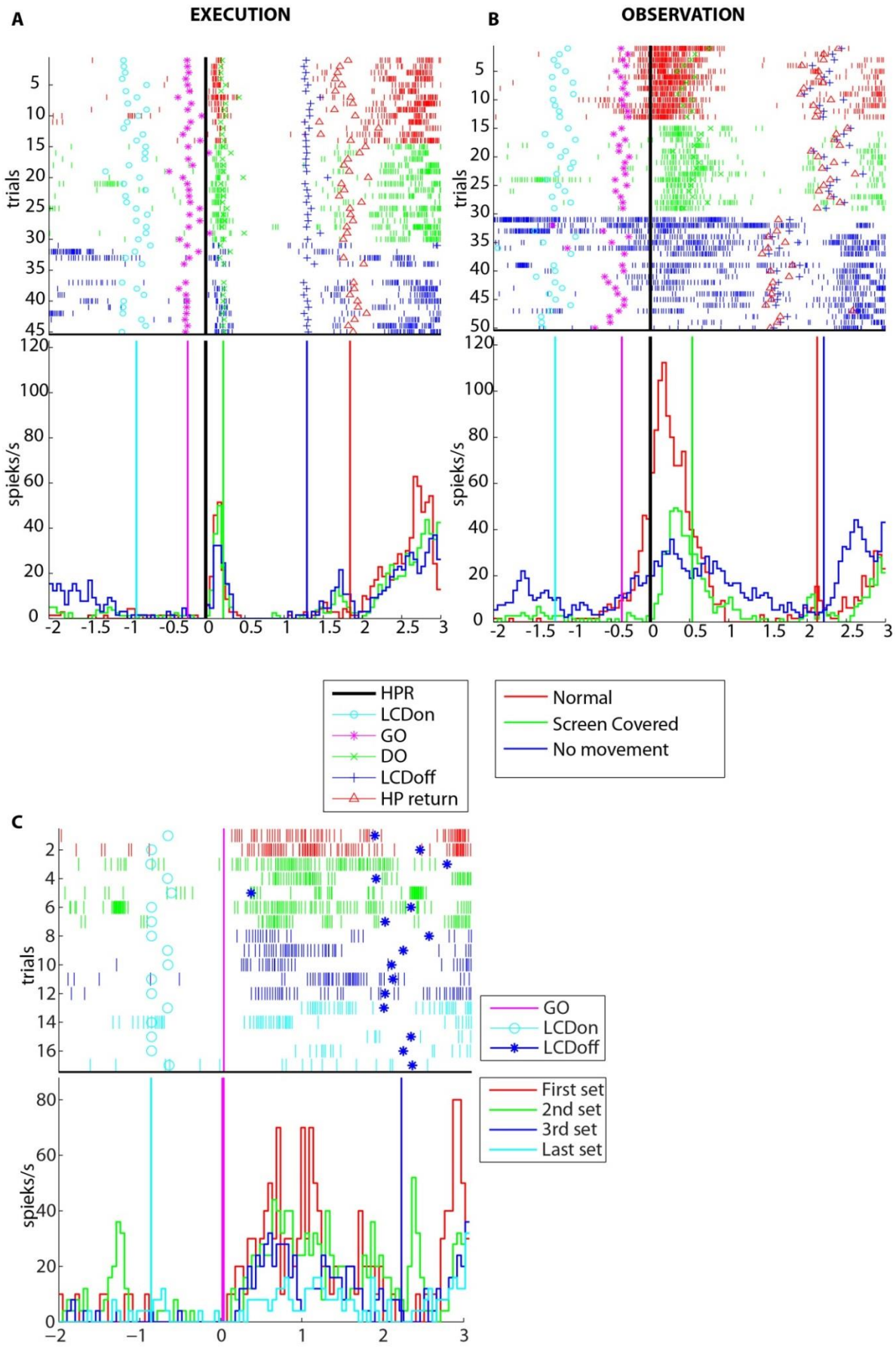

A
EXECUTION
B
OBSERVATION
trials
spieks/s
HPR
LCDon
GO
DO
LCDoff
HP return
Normal
Screen Covered
No movement
C
GO
LCDon
LCDoff
First set
2nd set
3rd set
Last set

Figure 4.6 Additional properties of F5 Mirror Neurons

F5 Mirror PTN tested under additional mirror tests (screen covered and no movement conditions).
(A-B) Raster and histogram plots are aligned to homepad release (HPR) for execution **(A)** and observation **(B)** trials. All execution trials were carried out in the normal way under full vision, but each coloured trace corresponds to the execution trials paired with various observation tests shown in **(B)**, red traces correspond to the normal mirror test as described previously, green traces corresponds to the screen covered test and blue traces corresponds to no-movement trials in which there was no reach to grasp action (only release of the homepad but **no movement** towards the object). Other behavioural events are indicated by coloured markers for each trial on raster plots and with vertical lines on histograms (LCDon (cyan circle) indicates the start of the presentation period, in which the object was visible, GO (magenta asterisk) indicates the signal to reach and grasp, DO (green cross) indicates the first displacement of the object, LCDoff (blue vertical dash) indicates the time at which the screen was turned off and therefore the object became invisible, HP return (red triangles) indicates the time at which the hand returned to the homepad. **(C)** Shows the data from no movement trials (blue trace, B) in an expanded format. Adjacent trials have been grouped together in sets (first two trials in red, next five trials in green, next five trials in dark blue and the last five trials in cyan). Data are aligned to the GO cue (magenta vertical line).

The F5 PTN described above was also recorded in a different experimental condition: the No-movement condition (**Fig. 4.6B**, blue traces), see methods for description of task design, essentially there was no reach to grasp action, only a slight movement to allow release of the homepad whilst the hand remained above the homepad). The neuron actually continued to fire even though there was no reach to grasp action made by the experimenter. On a closer look at single trials (see blue coloured rasters) we find that the PTN showed decreasing activity on successive trials.

Fig. 4.6C shows the same activity shown in **Fig. 4.6B** (blue trace) except that adjacent trials have been grouped together in sets (first two trials in red, next five trials in green, next five trials in dark blue and the last five trials in cyan). The first two trials (shown in red) showed a firing rate close to 70 spikes/s, however by the last five trials

(cyan trace, **Fig. 4.6C**) the activity was barely modulated. This was even when these trials were completely randomised with respect to execution trials, and therefore it is unlikely that the reduction in activity was directly related to repeated exposure to the stimulus from previous trials. Of the three mirror PTNs in M47 recorded during these additional tests, two showed these effects.

4.3.4 Decoding Grip type using Observation data

Much work has been done on decoding grasp types during execution of a skilled grasping task (Townsend et al., 2011). However, since the idea would be to try and implement decoding of grasp configurations with brain machine interfaces in patients without any residual function of the arm or hand, it might be hard/not feasible to train the decoder on execution movements. Instead, decoding of grasp configurations using observation data might be beneficial if the patient is unable to make any movement. In order to test this hypothesis, we trained a linear classifier to decode grip types (precision, hook and whole-hand-grasp) using the single units we recorded in the mirror task (observation condition) in M47. We then tested the decoder on the grip types on single trials during the execution task (see section **4.2.3**). We wanted to see if the **observation** data could be used to classify the grasp type during **execution** trials. In other words, we were testing whether observation and execution of grip types were similarly coded, which might be expected from mirror neurons.

Both identified PTNs and unidentified units (UIDs) were used for this analysis. We found that only 20/135 (15%) units within M1 and F5 had neuronal activity that our linear classifier was able to decode the grip types during execution trials, using observation data as training data. These were units that contained information that our classifier was able to achieve a decoding performance above the chance level (33%, 3 grips used) and the significance level (mean significance level 40% using binomial test (see section **4.2.3**)).

In table 4.1 we show the relative proportions of neurons that had neuronal activity that we were able to use to decode grip type using observation trials as training data. The data is split for unit type (PTNs vs UIDs) and also area (M1 vs F5).

	M1	F5
PTNS	8/46 (17.4%)	1/13 (7.7%)
UIDS	5/29 (17.2%)	6/47 (12.8%)

Table 4.1 Proportion of neurons with significant decoding

For neurons that we were able to achieve a significant decoding performance using a linear classifier, there was a 40-53% (range) decoding accuracy. This means that there was a 40-53% chance of correctly identifying the object being grasped on any given trial.

This indicates that, for a minority of units, there was similar coding of grasp across execution and observation conditions.

We found that there are generally a greater proportion of units in motor cortex (M1) compared with premotor cortex (F5) that we were able to use to achieve significant decoding (17% vs 12%, respectively). Both PTNs and UIDs had similar outcomes with our decoder. In F5 we found a smaller number of units (PTNs) performed well with our decoder (only 1 unit). However, the sample size of F5 PTNs is quite small (n=13) and requires further data and subsequent analysis.

4.4 DISCUSSION

4.4.1 Types of mirror neurons in F5

We have shown that during observation of a precision grip, corticospinal neurons within area F5 or premotor cortex show mirror activity. Moreover, this activity amongst classical type or F-F type mirror neurons is similar in amplitude during execution and observation of a grasp. This is in contrast to the findings discussed in Chapter 3 for primary motor cortex, where we found a much reduced response during observation compared with execution.

In monkey M43, we found significant modulation during observation of a precision grip in almost half (56%) the PTNs recorded, and evidence of both facilitation (13/32, 41%) and suppression type (greater proportion, 19/32, 59%). However, more data is required to validate these findings since they are largely based on data obtained from one monkey performing the simpler precision grip task, since not many mirror neurons were recorded from monkey M47 that had been trained on the more complex version of the task.

This study highlights the importance of suppression mirror neurons. Since classical mirror neurons in area F5 fire equally during execution and observation, then the disfacilitation or inhibition during observation that is probably required in order to prevent any unwanted movement during action observation can result from the reversal in activity of suppression mirror neurons and the non-mirror neuron populations.

4.4.2 Comparison of mirror PTN activity in F5 vs M1

From our analysis of F5 PTNs, we have shown that there is an equal amount of activity of FF type neurons across execution and observation; this confirms the findings of many other studies of F5 mirror neuron activity (Gallese et al., 1996, Rizzolatti et al., 1996, Kraskov et al., 2009). However, this is clearly not the case for M1 PTNs (**see Fig. 4.8**) where F-F type neurons have a much higher firing rate during execution compared with observation. M1 PTNs seemed to be more active during execution compared with F5 PTNs (cf. red bar (E) for M1 and F5).

The raw firing rate analysis (see **Figs. 4.5 & 4.8**) shows that the level of firing in F5 PTNs during execution (~25 spikes/s) is much lower compared with M1 PTNs (~45 spikes/s, see **Fig. 4.8**). These factors might mean that the equally high firing rate we find during observation (compared with execution) for F5 PTNs might not lead to a strong facilitation of downstream spinal networks controlling hand and digit muscles. In addition, the suppression mirror neurons could disfacilitate spinal targets during observation and could directly oppose the activity of the classical type mirror neurons if they terminate on the same spinal targets. Knowing the spinal targets for these two populations of neurons is of great interest but has yet to be investigated.

It is of importance to discuss differences in the characteristics of corticospinal mirror neurons in F5 vs M1, and the likely difference in impact of the classical mirror neurons described here for area F5 and area M1 (described previously in Chapter 3). PTNs from F5 tend to terminate on the upper cervical cord and contribute only 4% of the total frontal lobe corticospinal projection (Borra et al., 2010, He et al., 1993). M1 contributes 50% of the descending corticospinal projection from the frontal lobe (Dum and Strick, 1991), terminates heavily in lower cervical cord (Maier et al., 1993) and includes direct cortico-motoneuronal projections influencing digit muscles (Lemon, 2008).

Interestingly, at the population level, the F5 suppression mirror population appears to have maximal suppression at an earlier time point compared with the maximal facilitation seen from classical mirror neurons (see **Fig. 4.4A**), in this way it might be that the suppression mirror neurons have an earlier influence on downstream targets to counteract the effect of the facilitation type. This seems to be different from the temporal activity of mirror neurons found in primary motor cortex (see Chapter 3, **Fig. 3.2C**), where we find that the maximal suppression and facilitation are around the time of displacement onset. A fuller analysis of the temporal activity is necessary using the more controlled version of the task.

4.4.3 Additional properties of F5 mirror neurons

The preliminary experimental data also brings to light some interesting areas for future research. By manipulating the amount of visual information seen by the monkey, namely by concealing the grasp, mirror neurons in F5 continue to fire. This is in keeping with the findings of a previous study (Umilta et al., 2001), in which the

last part of the action was obscured; the authors argued that the mirror neurons fired because they encoded the goal of the action (presumably the grasp).

In keeping with the idea that F5 might encode predictions of grasping movements, we find that the same neuron shows a trial-by-trial temporal decline in activity when the experimenter did not move, even though the monkey expected the experimenter to grasp the object. **Fig. 4.6B** (cyan trace) shows that at the start the neuron fired even though there was no movement, this might be because the monkey expects to see a movement based on its previous experience, however over trials (see **Fig. 4.6C**), the neuron loses its mirror response, presumably because it does not have anything to mirror (i.e. no grasp was carried out). The idea that mirror neurons might encode predictions has been previously suggested by Kilner et al. (2007); by minimising prediction errors during action observation a prediction about the goal of the action can be achieved.

Our findings support the predictive coding hypothesis, but this certainly warrants further investigation and more neuronal recordings. Interestingly, when the same test was conducted whilst recording mirror neurons in M1 (see Chapter 3 for further details), we have found many PTNs that suppressed or abolished their activity altogether when the grasping action was hidden by covering the screen (see **Fig. 4.7**). **Fig. 4.7A** shows the activity of a mirror PTN recorded in primary motor cortex. During execution trials (**Fig. 4.7A**, left) there was modulation in activity around the times of HPR, DO and HP return, with maximal activity just before HPR (~35 spikes/s). During observation trials (**Fig. 4.7A**, right) there is one main peak of activation around the time of the experimenter's HPR (~15 spikes/s). **Fig. 4.7B** shows the activity of this

same neuron during execution and observation trials when the experimenter's screen was covered. Note that the neuron completely lost its mirror activity when the last part of the action was hidden (**Fig. 4.7B**, right), whilst it continued to fire as normal during the interleaved execution trials (**Fig. 4.7B**, left).

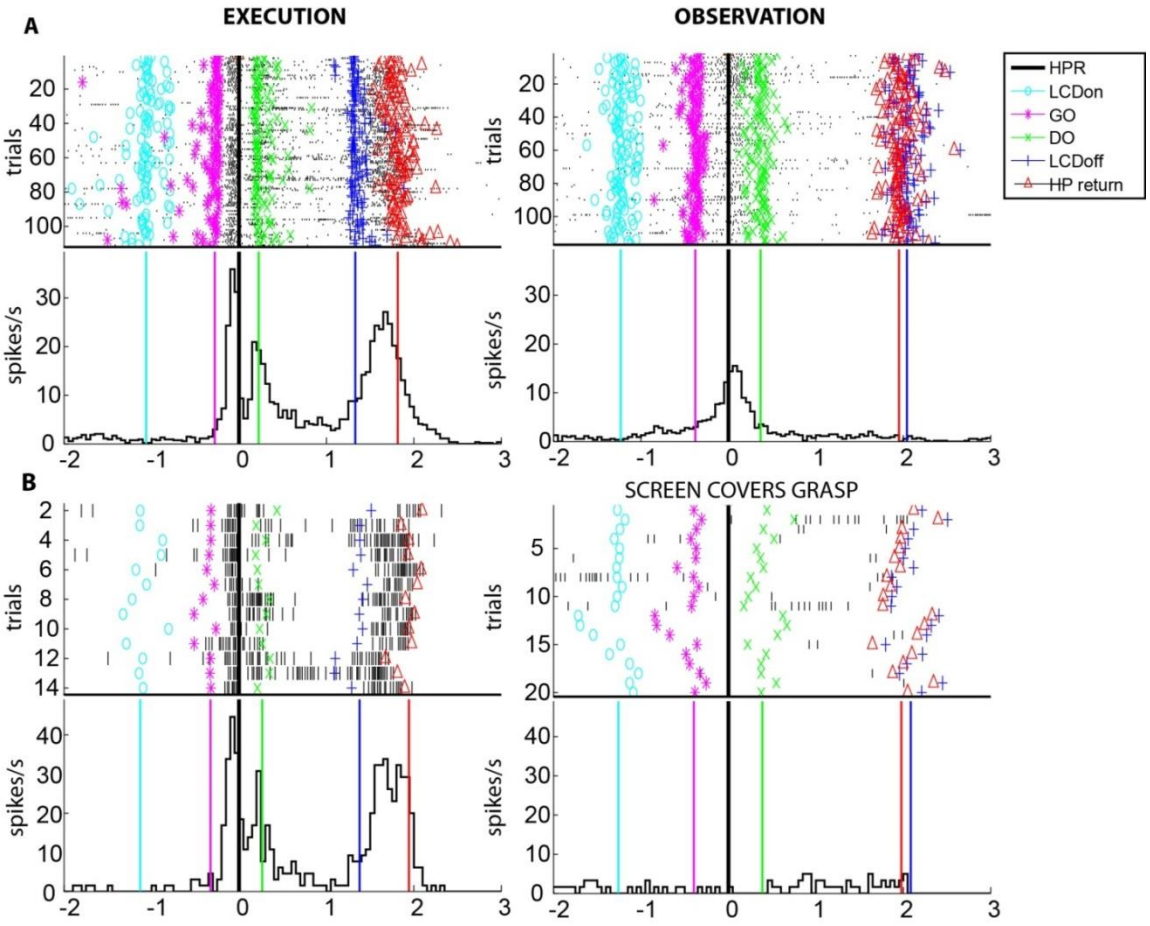


Figure 4.7 M1 mirror PTN loses its mirror activity following covering of the grasp

(A-B) Raster and histogram plots are aligned to homepad release (HPR) for execution (left) and observation (right) trials. **(A)** Shows the activity of an M1 mirror PTN during execution (left) and observation (right) trials during the normal version of the mirror task. **(B)** Shows the activity of this same neuron for normal execution trials (left), and observation trials, (right), a screen covered vision of the experimenter's grasp. Other behavioural events are indicated by coloured markers for each trial on raster plots and with vertical lines on histograms (LCDon (cyan circle) indicates the start of the presentation period, in which the object can be viewed, GO (magenta asterisk) indicates the signal to reach and grasp, DO (green cross) indicates the first displacement of the object, LCDoff (blue vertical dash) indicates the time at which the screen was turned off and therefore the object became invisible, HP return (red triangles) indicates the time at which the hand returned to the homepad.

This might suggest that mirror neurons in F5 have a different role to those in M1. F5 might be coding more of a prediction of the grasp (higher level action representation), whilst M1 purely reflects the action that is seen (low level action representation). The difference between these two signals might then be used to update an internal model of action prediction, i.e. the difference between what is expected (F5 mirror activity) to what is actually observed (M1 mirror activity) might be used to update a model which can be used on subsequent trials.

4.4.4 Encoding of grasp by units in F5 and M1

Grip type is well defined in execution activity in both F5 and M1 (Umilta et al., 2007), but it is unclear whether this relationship exists during observation. By using observation data to train a classifier for subsequent discrimination of grip types during execution, we showed that both M1 and F5 have a proportion of units (both PTNs and UIDs) that have grasp related information. M1 seems to carry more units that a linear classifier was able to correctly decode grip type (using observation data) compared with F5 (17% vs 12%). Whilst we did not note any differences in our decoding of grip type dependent on unit type (PTNs and UIDs) in M1, there was a small trend towards UIDs having a higher performance. More data is required to validate these findings. These results suggest that some units do show similar differences in firing across observation and execution conditions (these might be classified as strictly congruent neurons). However, we were unable to decode grip type in the vast majority of units; indicating that grip type is not represented in the same way across execution and observation. It might not be surprising that not all

PTNs respond in the same way across execution and observation, namely in one condition there is production of movement and no movement in the other.

However, It is interesting that the primary motor cortex contained more units with similar coding across execution and observation compared with premotor cortex (see **Table 4.1**). This might be because the primary motor cortex is closer to the output of the motor system responsible for movement (Porter and Lemon, 1993). Nonetheless, it means that single unit data from M1 might be a useful target for inputs used in BMIs, considering in many patients, there might not be any residual function of the hand or arm. Even though the proportion of units that provided successful classification of the three different grasp types was quite low, observation of movements might be a feasible option in such patients.

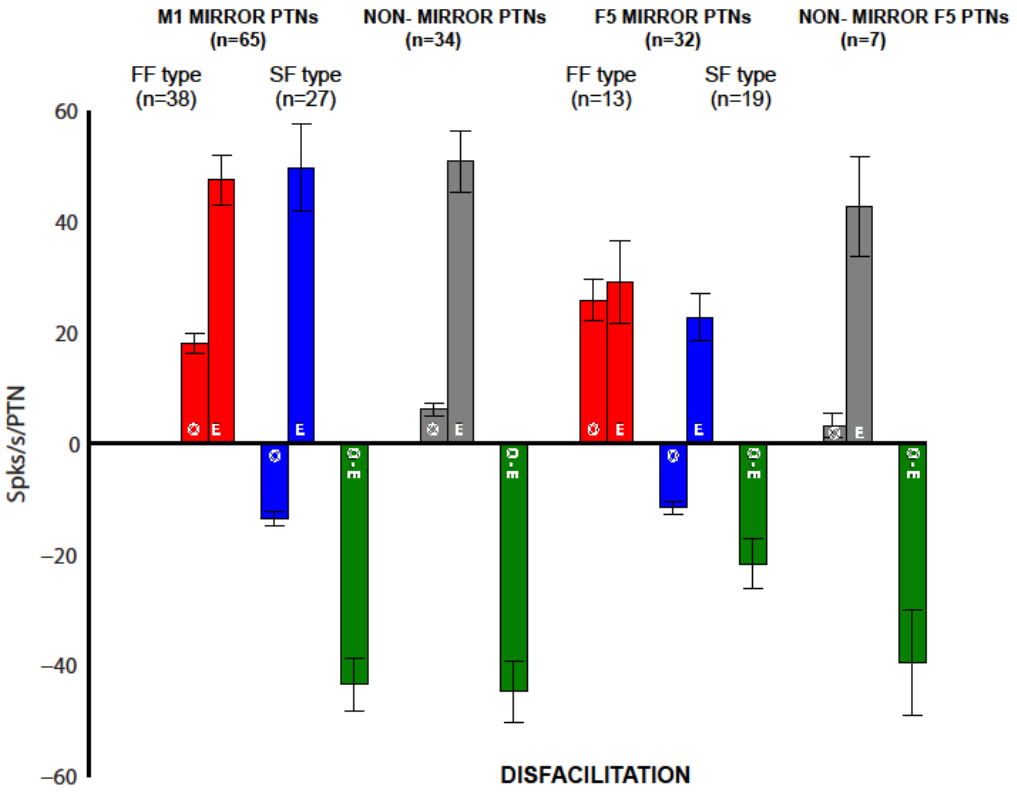


Figure 4.8 Summary of M1 and F5 PTNs

Summary of data recorded in M1 and F5. (See **Figure 3.2D** and **Figure 4.5** for more details).

CHAPTER 5:
Corticospinal excitability during a Go/No-go grasping task

5.1 INTRODUCTION

Thus far, Chapters 3 and 4 have addressed the role of mirror neurons as part of the corticospinal system in areas M1 and F5 of the macaque monkey by means of electrophysiological recordings from single cells. In humans, the corticospinal excitability during action execution and action observation can be measured indirectly using transcranial magnetic stimulation or TMS. Fadiga and colleagues were the first to show a facilitation of MEPs in the FDS and FDI muscles during action observation using TMS (Fadiga et al., 1995). From our knowledge on PTN mirror neurons in M1 we indeed expect that MEPs during action observation of a reach-to-grasp task might also be modulated. However, since people do not imitate everything that we see during action observation, there must be a level of suppression at some stage along the corticospinal pathway. In Chapters 3 and 4 we suggested that there are at least three ways in which this might occur: 1) disfacilitation of facilitation mirror PTNs during observation (facilitation mirror neurons have a low level of activity above baseline), 2) suppression mirror neurons suppress their activity below baseline during action observation, 3) Non-mirror PTNs do not modulate their firing rate during action observation.

Suppression within this system might be measured by probing cortico-cortical interactions during an observed movement. Paired pulse TMS protocols might be able to elucidate any inhibition in the system during action observation. Work by

Strafella & Paus (2000) showed that during action observation of a hand writing task and arm movements there was a muscle specific reduction in both SICI (short intracortical inhibition, which is thought to reflect activity of GABAergic interneurons (Brown et al., 1996, Ridding et al., 1995, Ziemann et al., 1996)) and SICF (short intracortical facilitation, thought to reflect facilitatory cortical activity (Ziemann et al., 1996)). This finding is unusual as it represents a conflict in cortical processes, in that there was both reduced inhibition *and* reduced facilitation. This might be because the MEP amplitude represents the net effect of many simultaneous processes acting on the corticospinal output.

TMS can also be used to measure corticospinal excitability during inhibition of a movement (Sohn et al., 2002). Since we embedded a Go/No-go paradigm within our task, we also wanted to compare the inhibition on No-go trials with action observation. In this study, using the same factorial design of Go/No-go and execution/observation we wanted to find evidence for an increase of cortical excitability, and/or suppression related to action execution and action observation.

5.2 METHODS

5.2.1 Participants

6 right-handed subjects (19-33 years old) participated in the experiment after providing informed consent and screened for adverse reactions to TMS.

5.2.2 Transcranial Magnetic Stimulation

To investigate the corticospinal excitability in the left hemisphere, we used a figure-eight coil (8 cm outer diameter) connected to two single-pulse monophasic Magstim stimulators. The conditioning (C) and test (T) pulses were set at 80% and 120% of the

resting motor threshold (rMT), respectively. The resting motor threshold is defined as the minimum intensity that induced motor evoked potentials (MEPs) ≥ 50 µV peak-to-peak in the first dorsal interosseous (FDI), abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles in 5 out of 10 trials (Rossini et al., 1994). The rMT was measured at the beginning of the experiment by using a coil connected to a single-pulse Magstim stimulator and was on average 43±8 % of the maximal stimulator output (mean ± SD, n = 6).

The stimulation site over motor cortex (M1) was determined by trial and error, and the final position was where the TMS caused the largest MEPs in all three muscles (FDI, APB, ADM).

We chose to investigate the overall corticospinal excitability using single pulse TMS over the hand area of Motor cortex. In addition, we carried out paired pulse regimes to measure the cortical excitability. We used a delay of 2 ms for SICI and 12 ms for SICF as these timings have been shown to produce inhibition and facilitation, respectively (Kujirai et al., 1993).

5.2.3 Experimental Design

In training, subjects first had to perform one block of 30 trials. This was so that the reaction time could be calculated in order to adjust the time that TMS was delivered to the subject during the full experiment. In the full experiment, subjects had to perform six blocks of ~50 trials. In between trials the test pulse was delivered, the MEP amplitudes measured at these time points were used as baseline values (10 baseline trials were collected in each block).

Figure 5.1 Task Apparatus

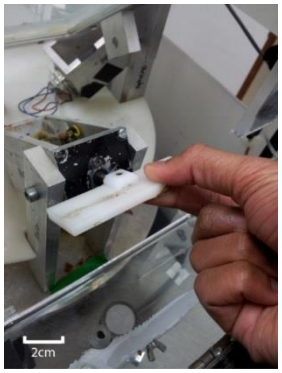


Plate object. Grasp required the subjects to supinate the wrist and grasp the object between the thumb and index finger.

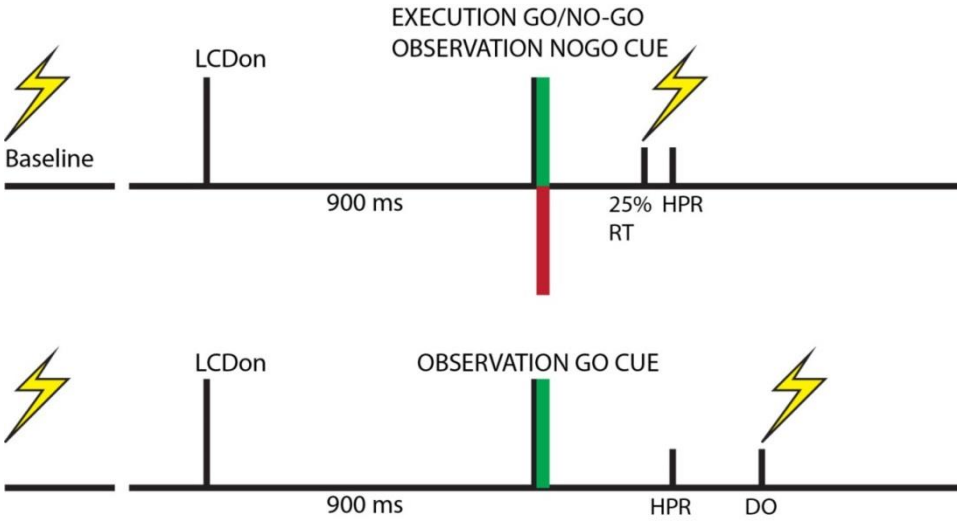


Figure 5.2 TMS paradigm schematic

Top: During execution Go/No-go and observation No-go trials TMS was triggered at 25th percentile of the reaction time (calculated from training data). On these trials, the screen would turn on allowing direct view of the object (LCDon) for 900ms, subsequently, the cue (green or red LED for Go and No-go trials, respectively) would signal the subject or the experimenter to respond by either grasping the object in Go trials or not keeping still on No-go trials. Bottom: During observation Go trials, the TMS was triggered at the time of displacement of the object. Baseline TMS was triggered in between trials.

The task design was intended to be similar to the monkey experiment described in Chapter 2.

Changes to the task design included: only one object (Plate) was to be grasped (see **Fig. 5.1**). This object required the subject to supinate the wrist and subsequently grasp the plate between the thumb and index finger.

In addition, the task program was changed so that the cue (LEDs) were only briefly presented (50 ms flash) instead of a constant illumination of green or red light around the object. This was changed in order to encourage the subject to keep paying attention to the task at all times.

We also incorporated the presence of a 'rare object'. This object was the sphere, and would appear in some observation trials. The subject had to correctly count the number of times the rare object appeared within one block and was rewarded £2 for successfully answering, with a bonus if they were able to correctly answer over all the blocks. This was in order to encourage the subject to pay close attention to the observation conditions.

In short, subjects were instructed to keep their hands relaxed on the homepads but remain focused. Once the right hand was placed on the homepad, it initiated a trial, at which point, one of the two screens (see Chapter 2, **Fig. 2.1**) became transparent. After a short delay (900 ms) a flash of green (Go trials) or red (No-go trials) illuminated the object for a short time (50 ms). On execution Go trials, subjects had to lift their right hand off their homepad, grasp the object, pull the object into an electronically defined window and maintain the grasp (~1s) until another auditory cue would signal that the object had been successfully grasped for the correct time. The subject could then release the object and place the hand back onto the homepad. On No-go trials following presentation of the red LED, subjects had to remain still and withhold their movement by keeping their hand on the homepad for the duration of the trial.

In observation trials, subjects were instructed to focus on the experimenter's actions at all times. More specifically, subjects were instructed to pay attention to the experimenter's grasp. They were also given instructions to pay attention to the presence of 'rare objects' and count the number of times that the rare object appeared. They would then be asked the number of rare objects present at the end of the block. Subjects had the chance to obtain a total of £15 for correctly counting the number of rare objects over the duration of the experiment.

On execution Go trials, TMS was triggered 50 ms before the 25th percentile of the reaction times obtained from the training data. TMS was also triggered at this time on all trials including execution No-go trials and observation No-go trials. However, in observation Go trials, we triggered TMS at the time of the experimenter's object displacement (see **Fig. 5.2** for timeline of experiment).

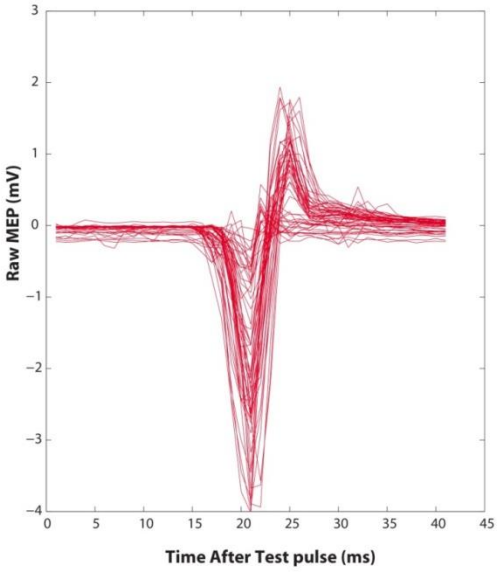


Figure 5.3 Examples of MEPs
Examples of Raw MEPs obtained from FDI muscle from one subject aligned at time 0 to the test pulse.

5.2.4 Data Acquisition and Analysis

The Magstim stimulators were triggered using Spike2 software and the CED data acquisition interface (Cambridge Electronic Design, Cambridge, UK). EMG activity was recorded with bipolar surface electrodes (belly-tendon), one pair positioned over the FDI, another over APB and a final pair over ADM. The raw EMG signals were amplified (1K; Neurolog, Digitimer Ltd, UK) and digitized at 5 kHz for offline analysis. The peak-to-peak amplitude (examples of raw MEPs are shown in **Fig. 5.3**) of each individual MEP was measured and expressed as a proportion of the control (baseline) MEP (test stimulus alone) obtained during the same block. Trials in which the TMS pulse was delivered after the start of movement (detected by home-pad release) were discarded. In addition, trials in which there was modulation of EMG above the mean +/- 2 SD (calculated 150ms before the onset of the MEP) at the time of the detected MEP were also discarded.

5.2.5 Statistical Analyses

In order to compare the MEPs with the baseline we carried out a t-test comparing the MEPs within each condition with the baseline MEPs. For paired pulse data, we normalised the data to the single pulse data within subject and condition. For comparison across conditions (Go, No-go, observation Go and observation No-go) we conducted a one-way ANOVA; $p<0.05$ was deemed significant. Linear regression analysis was performed on reaction times (RT) over trials.

5.3 RESULTS

Following the work we carried out in monkey primary motor cortex in the mirror Go/No-go paradigm we were interested to test whether the modulations at the single neuron level are also detectable in larger neuronal populations in humans using TMS protocols. Namely, we wanted to detect a level of suppression on observation trials, and whether there was a similarity between observation trials and No-go conditions.

5.3.1 Single Pulse Analysis

Fig. 5.4 shows the mean and SEMs of MEPs recorded from FDI, APB and ADM muscles in trials in which single pulse TMS was applied over primary motor cortex across the four conditions. In Go trials (blue bars), we found a strong facilitation of the ADM MEP (MEP amplitude, 4.1 times above the baseline) whilst we found that APB MEP was actually suppressed during grasp (MEP amplitude, 0.5, $p<0.05$) and was significantly different from all other conditions. FDI showed no significant modulation in Go trials (MEP amplitude, 0.92, $p>0.05$).

In execution No-go trials we did not find any significant modulation from baseline in any of the muscles ($p>0.05$). Similarly, in observation Go and No-go trials we do not find any significant modulation of MEPs from baseline. In other words, these experiments revealed only two significant changes: an increase in the ADM MEP and a decrease in the APB MEP for execution Go vs other conditions.

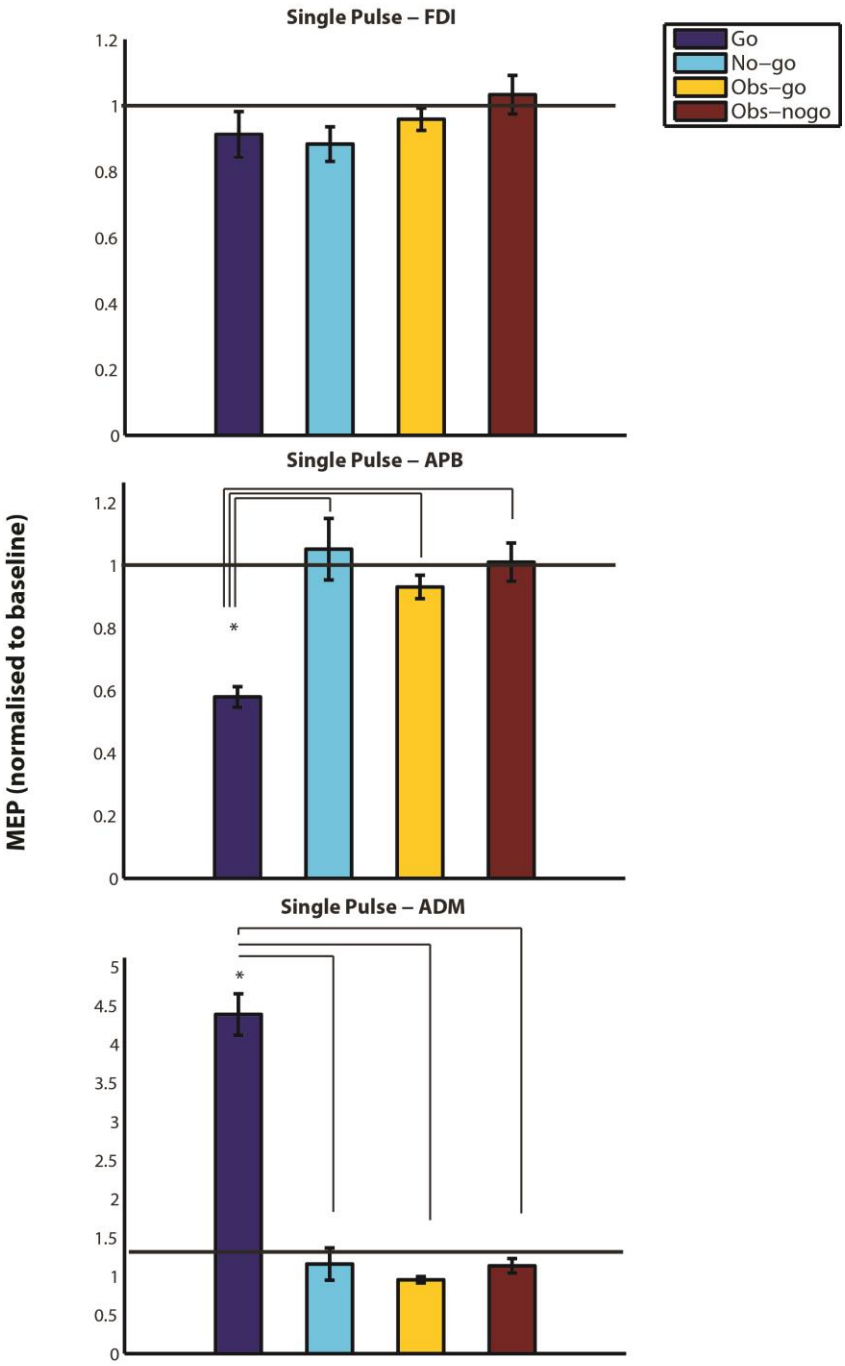


Figure 5.4 Single pulse TMS

MEPs normalised to the baseline MEPs obtained between trials. Mean MEPs with SEMs are shown. Go condition is shown in blue, No-go in cyan, observation Go trials in yellow and observation No-go trials in dark red. Results that are significantly different from the baseline are marked with an asterisk (*).

5.3.2 Paired Pulse Analysis

We also carried out paired pulse techniques to assess the excitability of the intracortical circuitry. **Fig. 5.5** shows mean MEPs and SEM after normalisation (divided by single pulse MEPs from the same condition and subject) when we used a C-T interval of 2ms, which has been shown to elicit SICI (Kujirai et al., 1993).

We found that we had significantly reduced MEPs in the Go (0.74), observation Go (0.65) and observation No-go (0.57) conditions for FDI muscle. In ABP muscle, MEPs were significantly reduced in the observation Go (0.64) and observation No-go conditions (0.62). In ADM muscle, MEPs were significantly reduced in the Go (0.77) and observation Go conditions (0.77). In addition, for this muscle, we found that the amount of SICI in the Go condition was significantly less than that in the observation Go condition.

We also used a C-T interval of 12ms in order to measure SICF during this task (Kujirai et al., 1993). Using this interval, we found a moderately significant facilitation in all conditions and in all muscles except for observation No-go for ADM muscle (see **Fig. 5.6**). For the FDI muscle, MEPs were facilitated to the greatest extent in the No-go condition (1.86). Whilst the least amount of facilitation from baseline was found in the observation Go condition (1.61).

For APB muscle we found the greatest facilitation in Go trials (1.98) whilst the least facilitation was seen in observation Go trials (1.48). This comparison was almost significantly different ($p=0.06$). For the ADM muscle, MEPs ranged from 1.46 to 1.84, but we did not find any of the conditions to be significant from each other.

Retrospectively, we calculated the time when the TMS pulse was delivered in relation to the actual movement. This ranged from 68-206 ms before the movement onset. We also discarded trials with voluntary EMG contamination; since the TMS pulse was triggered at the 25th percentile of the average reaction time we expected contamination on some execution Go trials. In total, we discarded 31.5% of the execution Go trials.

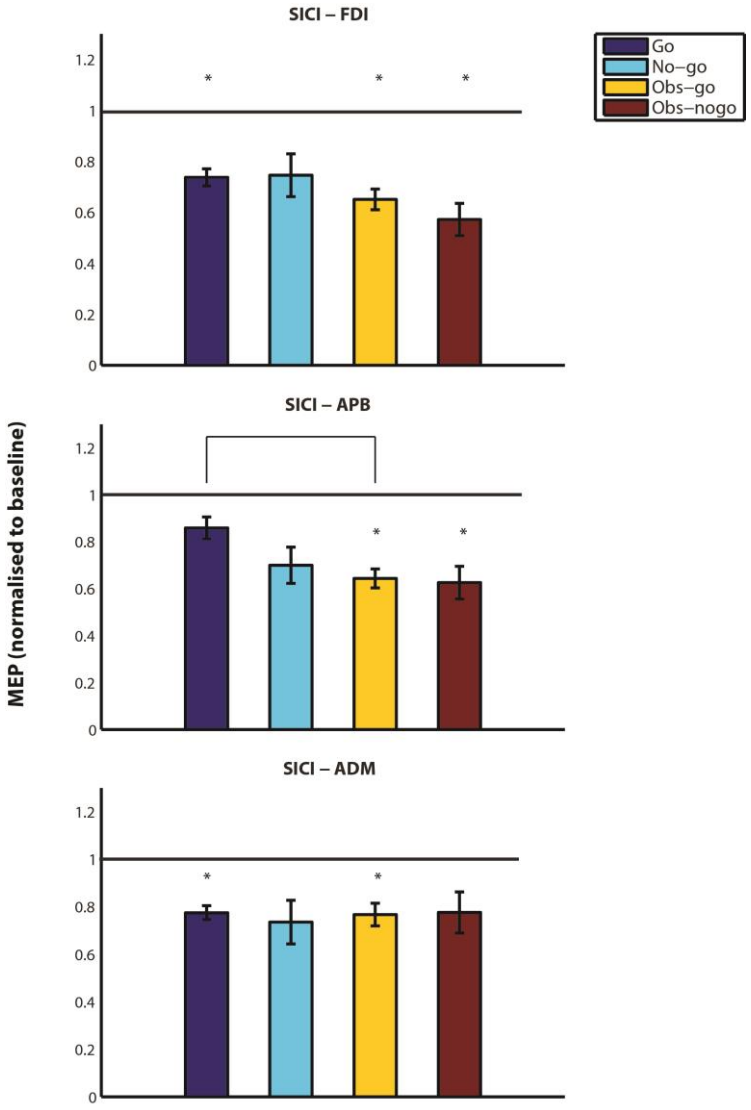


Figure 5.5 Paired pulse TMS, "SICI (2ms)"

MEPs normalised to the single pulse data. Mean MEPs with SEMs are shown. Go condition is shown in blue, No-go in cyan, observation Go trials in yellow and observation No-go trials in dark red. Results that are significantly different from the baseline are marked with an asterisk (*).

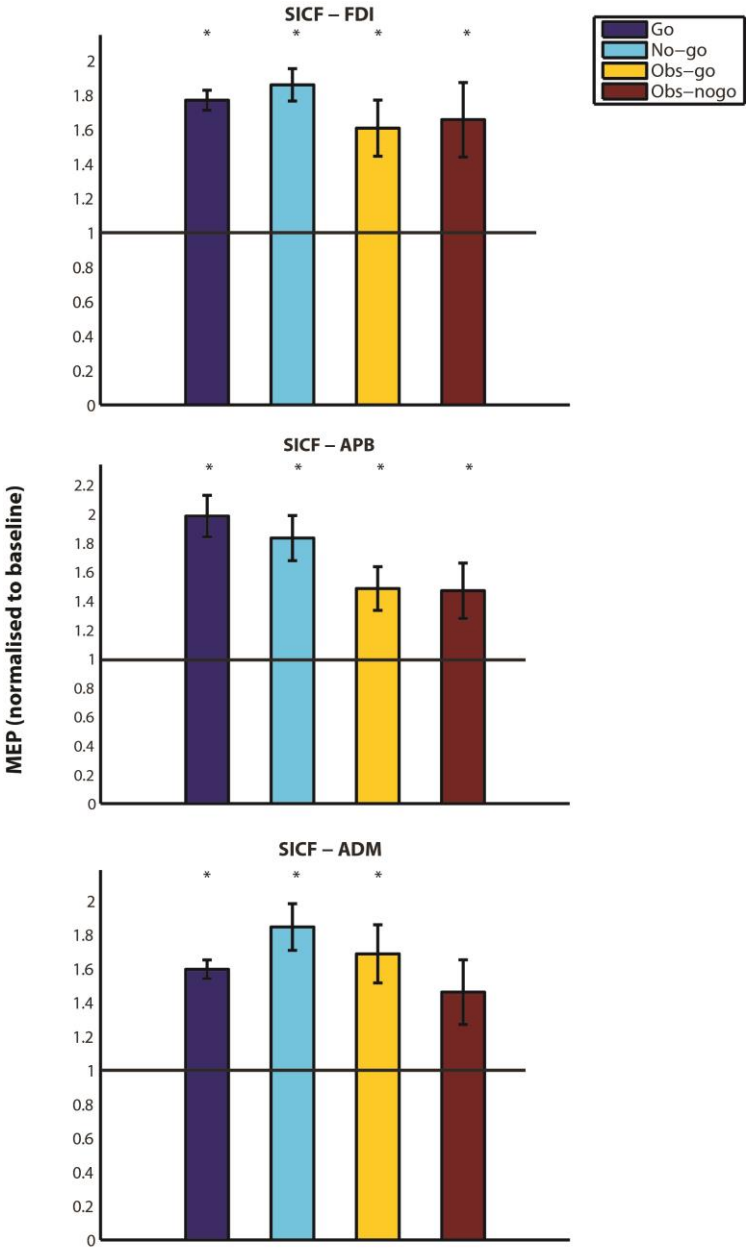


Figure 5.6 Paired pulse TMS, "SICF (12ms)"

MEPs normalised to the single pulse data. Mean MEPs with SEMs are shown. Go condition is shown in blue, No-go in cyan, observation Go trials in yellow and observation No-go trials in dark red. Results that are significantly different from the baseline are marked with an asterisk (*).

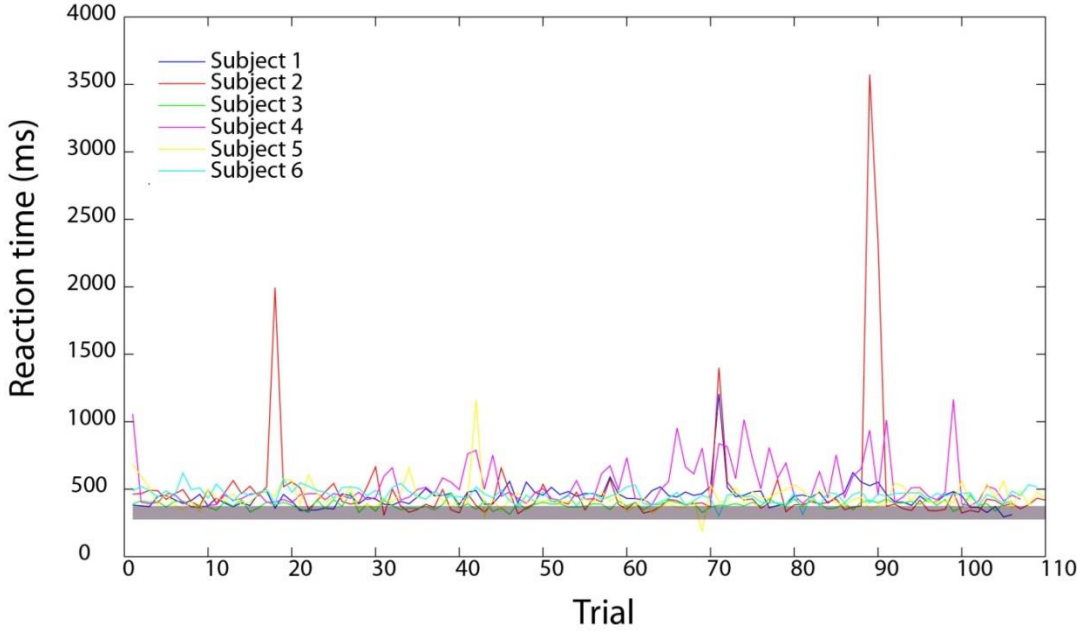
5.4 DISCUSSION

We did not find the results we expected. Firstly, we did not observe a significant 'Fadiga-effect' i.e. a facilitation of the MEP in the two 'prime movers' for the plate task (ABP, FDI) during observation Go trials. Surprisingly, we found that the ADM muscle was the principal facilitated muscle following single pulse TMS rather than FDI and ABP. We actually found a significant suppression of the ABP muscle (0.5). We had expected FDI and ABP to be strongly facilitated since grasping of the plate involves opposition of the index finger and thumb.

There are two possible explanations for these findings. First, the TMS pulse might have been triggered too early, since it had been based on the reaction time data obtained during the training period. Subjects might have slowed down over the course of the experiment and thus, on some trials we might miss the time when the subject prepares to grasp. To investigate this possibility, we investigated the changes in reaction time as the experiment progressed. **Fig. 5.7** shows the reaction times during Go trials for the 6 subjects (all trials). Linear regression analysis showed that subject 4 significantly increased his/her reaction time over the duration of the experiment (coefficient = 1.3, R2 = 0.1, $p<0.05$) and subject 6 showed a significant decrease (coefficient -0.5, R2 = 0.1, $p<0.05$) in reaction times over trials. Second, since grasping the plate involves lifting of the hand and supination of the wrist and then subsequent grasp, ADM might be strongly facilitated because it is involved earlier in the action, i.e. it might be active in flexing the little finger out of the way, allowing the thumb and index better access to the object (see **Fig. 5.1**). In addition, some subjects received the TMS pulse very early compared with their movement onset (>200 ms), they may have started to wait for the TMS pulse before moving.

This strategic delaying may have been compounded by introducing a No-go condition into some of the execution and observation trials. The possibility that one might not need to act at all could have encouraged some subjects to delay their movement preparation until after the TMS pulse. This would result in little or no preparatory activity within M1 and thus no change in corticospinal excitability (Cattaneo et al., 2005, Davare et al., 2008) at the TMS timings that we used. This potential explanation could be tested in a repeat of the experiment without No-go stimuli.

Figure 5.7 Reaction times over trials



Reaction times are plotted over all (includes error trials) execution Go trials in 6 subjects. Each trace corresponds to one subject. Grey shaded area corresponds to the range of times that the TMS pulse was triggered in relation to the movement.

Another reason why we were unable to demonstrate the "Fadiga" effect (see yellow bars, **Fig. 5.4**) might have been due to the noisy baseline data. Corticospinal excitability might be modulated during the presentation of the object, depending on whether the upcoming trial is execution or observation. There is evidence from

monkey data (described in Chapter 4) that when an observation trial begins, neurons decreased their firing rates, presumably because the monkey knows that he will not have to make a movement. A better baseline for the TMS might be within the presentation period rather than between trials, alternatively, the observation trials could be blocked.

We found evidence of SICI at 2ms. Note, that, although all the values in **Fig. 5.5** are below 1, the groups that we were interested in comparing (degree of SICI during action observation and during the execution No-go) were not significantly different from each other.

Surprisingly, we found more SICF in the No-go trials compared with Go trials in FDI and ADM muscles (see **Fig. 5.6**; compare blue and cyan bars), and it is hard to explain these results, since we expected that there would be less facilitation in No-go trials.

Interestingly, there was a trend for less facilitation (detectable through SICF) for observation Go trials vs Go trials and No-go trials for the FDI and APB muscle. This might reflect the greater suppression in observation trials i.e. the presence of inhibition but at an interstimulus interval commonly used to assess facilitation.

It is worth noting that single pulse TMS reflects the excitability of the entire **corticospinal output to the muscle being tested.** While facilitation of MEPs during action observation was expected, it is important to evaluate this in the overall context of action observation. That is, we do not move when we observe an action, indeed, it has been shown that there can be bilateral suppression at the level of the spinal cord during action observation (Stamos et al., 2010).

TMS is non-specific; it will non-specifically stimulate all types of neurons. This might have different effects on each type of neuron that comprises the corticospinal tract. Supposedly during action observation, the MEP will reflect the sum of at least three types of output neurons of motor cortex: facilitation mirror PTNs, suppression mirror PTNs and non-mirror PTNs.

So in summary, several factors might have caused these somewhat surprising and unexpected results. Firstly, the timing of the TMS pulse was not ideal, as subjects tended to get slower over the course of the experiment, this was probably the biggest factor that determined the results of this experiment. Therefore we have not been able to probe the excitability of the corticospinal tract at the best possible time. Secondly, we implemented a Go/No-go paradigm, this may have discouraged subjects from preparing a grasp, and encouraged them instead to wait for the cue to then plan a movement. Lastly, the intensity of the TMS pulse may have not been optimal, or the motor thresholds may have changed over time. If the corticospinal output was already near maximal, any subtle changes due to changing activities in corticospinal mirror neurons may have gone undetected.

CHAPTER 6:
Large identified pyramidal cells in macaque motor and premotor cortex exhibit "thin spikes": implications for cell type classification

6.1 ABSTRACT

Past research has suggested that, in extracellular recordings from awake monkeys, cortical interneurons can be identified by the presence of short, 'thin' spikes while pyramidal neurons have broader spikes. To test this, we investigated the spike duration of antidromically identified pyramidal tract neurons (PTNs) recorded from primary motor (M1, 151 PTNs; median antidromic latency (ADL) 1.1 ms) and ventral premotor cortex (area F5, 54 PTNs, median 2.6 ms) in 4 awake macaques. The duration of PTN spikes, measured from negative trough to positive peak, was 0.15-0.71 ms. There was a highly significant positive linear correlation between ADL and spike duration in both M1 and F5. Thus PTNs with the shortest ADLs (fastest axons and probably largest somas) had the briefest spikes (0.15 to 0.45 ms), which overlap heavily with those previously reported for putative interneurons. This suggests that spike duration alone does not provide a reliable indication of cell type.

6.2 INTRODUCTION

Cell classification is important in determining the function of neurons in the context of the neuronal circuits. The neocortex is broadly composed of two cell types; interneurons and pyramidal cells. Spike duration has often been used as an indicator for cell type (Bartho et al., 2004). "Thin" and "fat" spikes are attributed to

interneurons and pyramidal cells, respectively (Mountcastle et al., 1969). However, much of the data has been collected on studies in the rodent (Connors et al., 1982, McCormick et al., 1985, Contreras, 2004, Bartho et al., 2004), and it is unclear whether the same classification can be made within the cortex of non-human primates. In order to confirm whether the same classification can be used in the awake behaving macaque monkey, we would need to record from an identified cell population. One class of pyramidal cell, the pyramidal tract neurons (PTNs) can be identified by their antidromic discharge in response to stimulation of the ipsilateral pyramidal tract (Evarts, 1964, Lemon, 1984).

In this study we used multiple microelectrode techniques to make extracellular recordings from physiologically identified PTNs in the awake macaque motor (M1) and ventral premotor cortex (area F5) and analysed the distribution of their spike durations. We were particularly focussed on measuring the spikes durations of fast PTNs in order to see if these values overlapped with those claimed for putative interneurons in the awake primate. It is also important to note that a large proportion of the macaque corticospinal tract is derived from the cortex outside M1 (Dum and Strick, 1991), and that corticospinal neurons in secondary motor areas are generally smaller and have slower conduction velocities that those in M1 (Kraskov et al., 2009, Macpherson et al., 1982, Maier et al., 2002, Murray and Coulter, 1981). Therefore comparison of spike durations for PTNs recorded in M1 vs those in premotor cortex (area F5) was of particular importance. We also made comparisons of PTN spikes with those of other unidentified neurons in the same recordings, and also with mean

values of spike durations of interneurons and pyramidal cells reported in the literature.

6.3 METHODS

6.3.1 Recordings

Experiments were performed on two adult purpose-bred Rhesus (*M. mulatta*) monkeys (M43, female 5.5 kg and M44, male 7.1 kg). Additional recorded data from two other purpose-bred Rhesus monkeys (M, female 6.0 kg and L, female 5.3 kg) was kindly provided by Prof Stuart Baker's lab in Newcastle (see Witham and Baker, 2007). All experimental procedures were approved by the respective Local Ethical Procedures committees and carried out in accordance with the UK Animals (Scientific Procedures) Act.

Recordings were made during performance of skilled grasping tasks with the contralateral hand. A full description of the tasks and of the surgical procedures used to prepare the monkeys for recording has been published previously (Kraskov et al., 2009, Witham and Baker, 2007). All monkeys were chronically implanted with a pair of fine tungsten stimulating electrodes in the medullary pyramid for subsequent antidromic identification of PTNs. These electrodes were confirmed to be located in the ipsilateral pyramidal tract by a number of electrophysiological and histological tests (Kraskov et al., 2009, Olivier et al., 2001).

6.3.2 Cortical Recordings

Please see section 2.3.3 for recording parameters used.

Second order high-pass filter with 300 Hz cut off frequency and first order low pass filter at 6 kHz were utilized; some data were recorded using wide band filter settings (10 Hz-6 kHz). Spike data was digitized using a sampling frequency of 25 kHz.

6.3.3 PTN identification

Details of PTN identification are given in section **2.3.2**. In brief, we searched for PTNs by looking for a latency-invariant antidromic response to stimulation of the pyramidal tract (PT) with single shocks of a 250–300 μA (biphasic pulse, each phase 0.2 ms). Once a PTN spike was clearly present in the recording, we determined its antidromic latency (ADL). This was measured from the beginning of the stimulus artifact to the first inflection in the antidromic spike (see example in **Fig. 6.1**, inset). During the same recording sessions, we regularly encountered spikes with good signal-to-noise ratios that did not respond antidromically to PT stimulation; these were referred to as unidentified neurons (UIDs).

6.3.4 Spike duration calculation

We calculated the duration of spontaneous PTN spikes from the negative trough to the succeeding positive peak. This was measured from the averaged spike waveform of the upsampled (1 Mhz) spline interpolated individual spikes aligned to the trough (see examples in **Fig. 6.3**, inset). The median number of spikes we averaged was 1000. This measure was chosen for two reasons. Firstly, the trough and the peak are easily and reliably detectable. Secondly, it has been shown that the unfiltered extracellular spike waveform is approximately the derivative of the intracellular action potential,

i.e. the trough to peak of the extracellular spike is considered the equivalent of the spike duration of the intracellular action potential measured at half amplitude(Henze et al., 2000). As a control, we also calculated the spike duration using the first inflection to positive peak measure ('peak-to-peak') as used in several previous reports (see **Table 6.2**). For this analysis, if there was no clear initial peak, we used the first significant deflection (mean minus 2 S.D.) as a starting point instead of the initial peak. Identical measurements were made on recordings from UIDs.

6.4 RESULTS

6.4.1 Distribution of Antidromic Latencies in Identified PTNs

We analysed data recorded from four monkeys (**Table 6.1**). Recordings were made from M1 in all four monkeys (M43: 67 PTNs and 18 UIDs; M44: 31 PTNs and 97 UIDs; monkey M: 18 PTNs, and monkey L 35 PTNs) and from area F5 in M43 (47 PTNs and 55 UIDs) and M44 (7 PTNs and 51 UIDs). In total we recorded from 205 PTNs, 151 in M1 and 54 in area F5, and from 221 UIDs, 115 in M1 and 106 in area F5).

Table 6.1 Database

Area	Cell type	M43	M44	L	M	Total
M1	**PTNs**	67	31	35	18	**151**
	UIDs	18	97	-	-	**115**
F5	**PTNs**	47	7	-	-	**54**
	UIDs	55	51	-	-	**106**

PTN: pyramidal tact neurons
UID: unidentified neurons
M43, M44, M, L: four Rhesus macaques used in this study

Fig. 6.1 shows a probability density function for ADLs of M1 (blue, n = 151) and area F5 (green, n = 54) PTNs. The M1 ADL distribution was positively skewed towards short ADLs (range 0.5-5.5 ms, median 1.1 ms). However, we also recorded some M1 PTNs

with longer ADLs (> 3.0 ms) indicating that we sampled some PTNs belonging to a slower conducting population. In contrast, the distribution of ADLs in area F5 was shifted towards longer ADLs (range 0.97-6.9 ms, median 2.6 ms) and is significantly different from the M1 population ($p<0.0001$, Wilcoxon rank-sum test). In addition, some area F5 PTNs had ADLs as long as 6-7 ms. Assuming a conduction distance of around 50 mm from cortex to PT stimulating electrode, this equates to an axonal conduction velocity of <10 m/s. These PTNs clearly belong to a slower conducting population which are known to far outnumber large ones but are much less studied due to recording bias (Humphrey and Corrie, 1978, Towe and Harding, 1970).

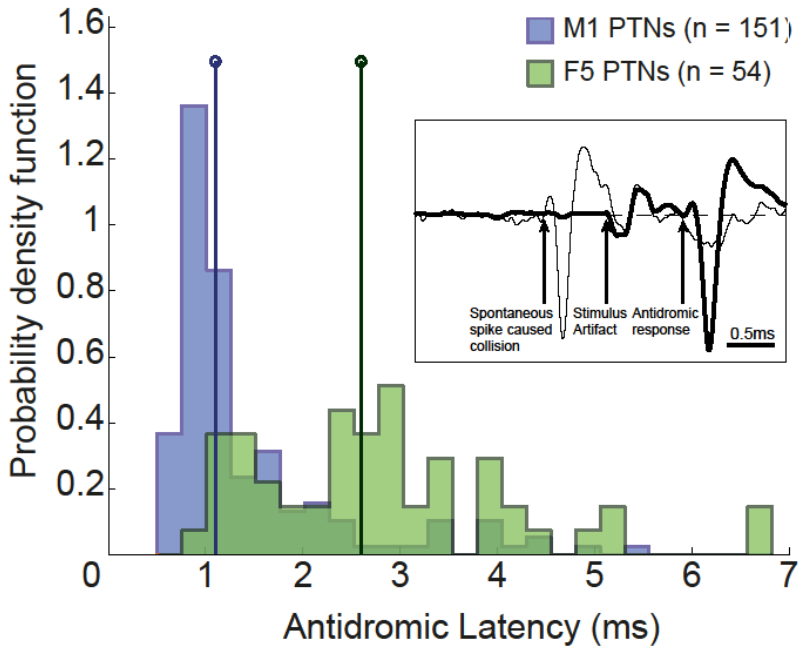


Figure 6.1 Distribution of ADLs

Probability density functions comparing antidromic latencies of identified pyramidal tract neurons (PTNs) in M1 (blue) and F5 (green). Binwidth 0.25 ms. The two vertical lines correspond to the median antidromic latency for each population of PTNs (1.1 ms and 2.6 ms for M1 and F5, respectively). The two median values are significantly different ($p<0.0001$, Wilcoxon rank-sum test). Inset shows a single sweep showing the antidromic response of an M1 PTN. Arrows indicate the onset of the PT stimulus and the onset of the antidromic spike. The antidromic latency of this PTN was 0.9 ms, spike duration was 0.24 ms.

6.4.2 Measurement and Distribution of Spike Duration

Since the high and low pass filter settings can affect the shape of the spike waveform (e.g. (Quian Quiroga, 2009), we performed additional recordings using wide band filter settings (10 Hz-6 kHz) and isolated 25 single units (19 PTNs, 6 UIDs). To estimate the effect of filtering on our measure of spike duration, we digitally filtered the original spike waveforms of the 25 single units (causal, 2 order high-pass Butterworth filter at 300Hz) and plotted the durations of the unfiltered vs filtered spikes (**Fig. 6.2**). The data were fitted using a second order polynomial (R^2=0.99, light blue curve). It is clear from the plot that spike duration was reduced after filtering, and moreover the absolute reduction was much more pronounced for wide spikes than for narrow spikes.

The median difference in spike duration for all filtered spikes longer than 0.30 ms was 0.15 ms, whereas for spikes with durations between 0.20 and 0.30 ms, the reduction was only 0.04 ms. Therefore we concluded that the 300 Hz high pass filter used to acquire the main body of data would not have significantly distorted our measurements of spikes with short durations, which were the main focus of this study.

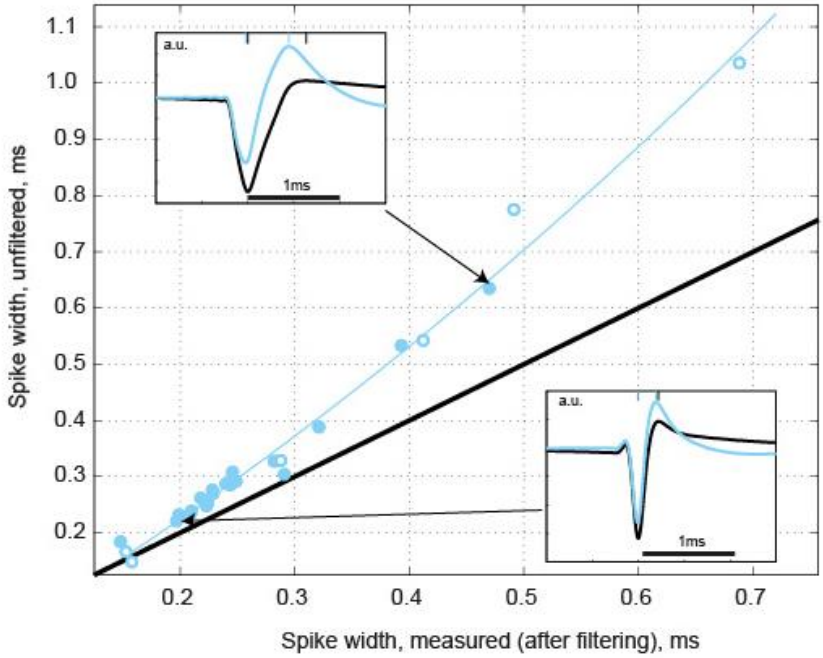


Figure 6.2 Effect of filters on spike duration

Comparison of spike duration for recordings in filtered vs unfiltered conditions (light blue circles), approximated with a second order polynomial (R^2=0.99, light blue curve). Open circles correspond to UIDs and filled ones to PTNs. For filtered recordings we used a second order causal high-pass Butterworth filter with a cut-off frequency of 300Hz (the same filter as used in all the recordings reported here). Thick black line is the line of unity. The two insets show samples of unfiltered spike waveforms (black traces) from two PTNs, one with a narrow spike duration (0.22 ms) and one with a relatively wide spike (0.64 ms) and their filtered versions (light blue traces). The duration of the filtered narrow spike decreased by 0.02 ms (11% reduction) whereas the filtered wide spike was reduced by 0.16 ms (26% reduction).

6.4.3 Spike Duration of identified PTNs

Fig. 6.3A and B show the distribution of spike durations measured from trough to peak) for PTNs recorded from M1 (blue) and area F5 (green), respectively. The distribution of spike durations in M1 was positively skewed with the majority of spikes having short durations of 0.20-0.25 ms. The shortest values measured were in

the range 0.16 to 0.18 ms. The distribution in area F5 was rather different, with one group of PTNs having short spike durations (0.15-0.30 ms) and the other longer durations (0.35-0.50 ms). Whilst the range of spike durations in M1 (0.16 ms to 0.71 ms see **Fig. 6.3**) was similar to that found in area F5 (0.15 ms to 0.71 ms), the median spike duration of PTNs in M1 (0.26 ms) was significantly shorter compared with PTNs in area F5 (0.43 ms) ($p<0.001$, Wilcoxon rank-sum test). The median value for all 205 PTNs was 0.29 ms.

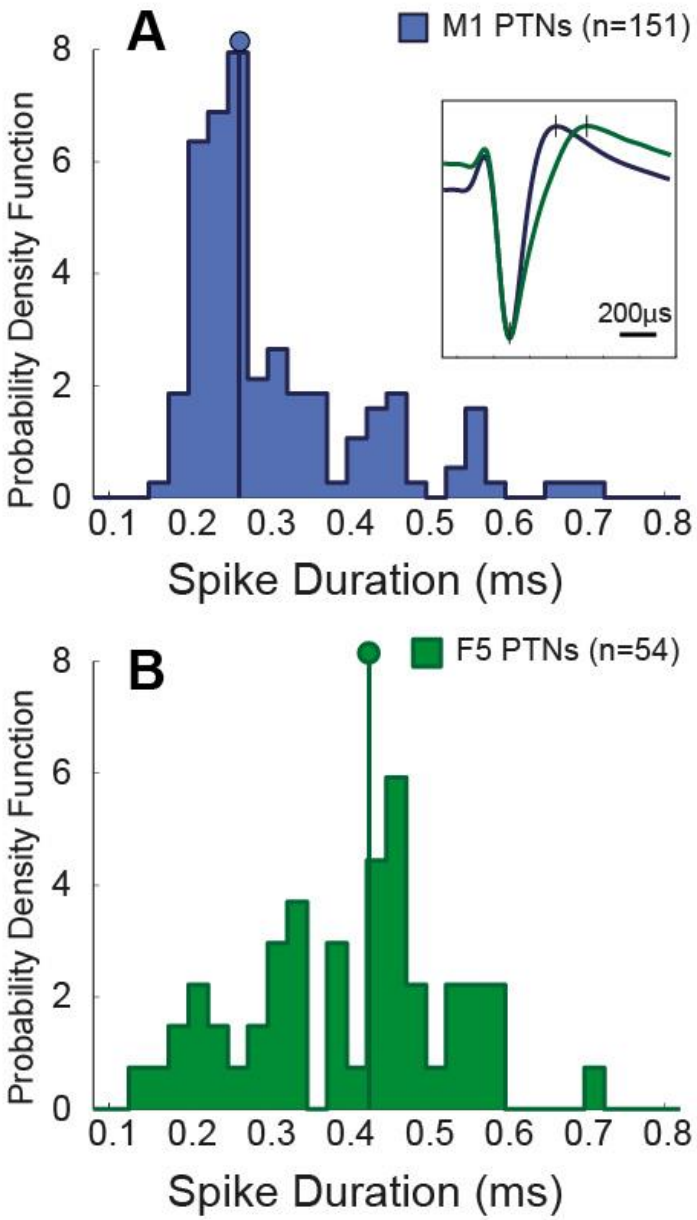


Figure 6.3 Distribution of spike duration in M1 and F5

(A) Probability density function of spike durations of identified PTNs in M1 (blue). Binwidth 0.025 ms. Vertical line corresponds to the median spike duration. (**B)** Probability density function of spike durations of identified PTNs in F5 (green). The median spike duration of PTNs in M1 (0.26 ms) was significantly shorter than that for PTNs in F5 (0.43 ms) ($p<0.001$, Wilcoxon rank-sum test). Inset shows splined averaged waveforms for two PTNs from M1 (blue) and F5 (green). These waveforms have spike durations closest to the medians of their respective populations indicated in the main figure.

6.4.4 PTNs vs unidentified neurons

We next compared the spike duration of the PTN population with a population of unidentified neurons (UID, see methods 6.3.3), many of which were recorded simultaneously from other microelectrodes whose tips were located not more than 1 mm away from the sampled PTNs. The combination of these two distributions should closely resemble a typical population of neurons recorded without PT identification being applied, and potentially contain some interneurons. Since PTN spike durations were found to be different in area M1 and area F5, we compared PTNs and UIDs within the same area. **Fig. 6.4A** shows the probability density function of spike duration distribution of PTNs (n =151) vs UIDs (n =115) in M1 recordings. Importantly, these distributions are not statistically different ($p>0.8$, Wilcoxon rank-sum test) with very similar median values (0.26 ms and 0.27 ms, PTNs and UIDs, respectively) and range (0.13-0.70 ms for UIDs vs 0.16-0.71 ms for PTNs). Thus, although the distribution suggests that there was a larger population of UIDs with very short spike durations, there was almost complete overlap in terms of actual spike duration. We also compared area F5 PTNs (n=54) with UIDs (n=106) (**Fig. 6.4B**). The distributions of these two populations were also overlapping (0.14-0.80 ms for UIDs vs 0.15-0.71 ms for PTNs). Although the median value for the PTNs (0.43 ms) was slightly longer than for the UIDs (0.35 ms) they were not significantly different ($p>0.2$, Wilcoxon rank-sum test).

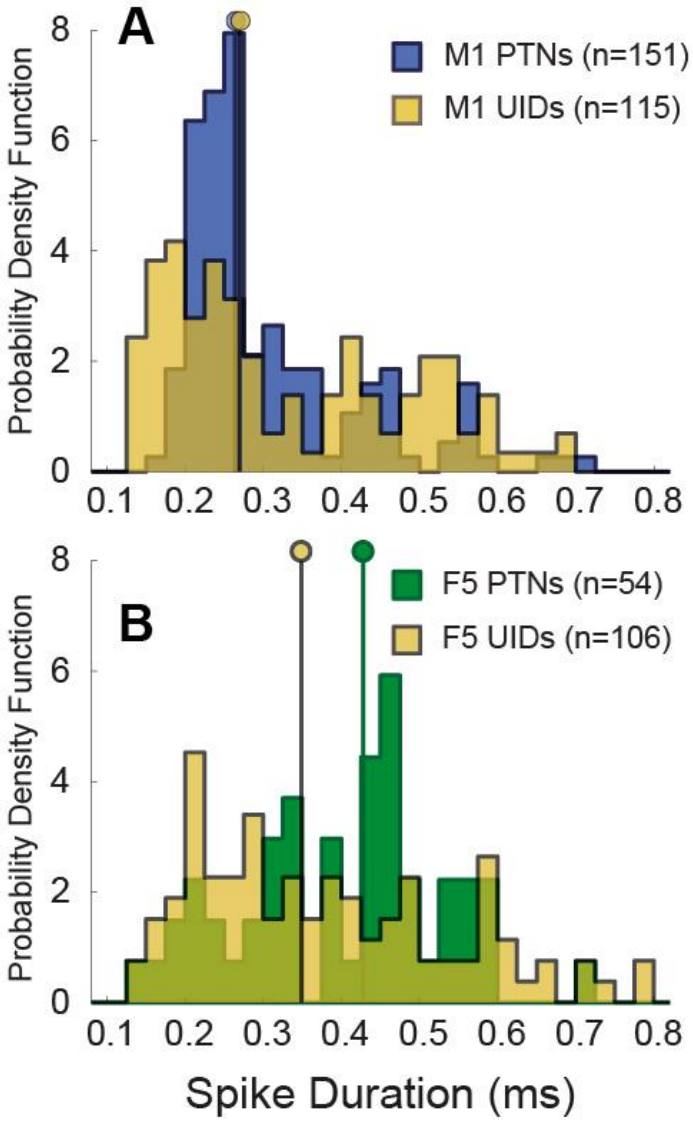


Figure 6.4 PTNs vs UIDs

(A) Probability density function comparing spike durations of identified PTNs in M1 (blue) and M1 UIDs (yellow). Binwidth 0.025 ms. The two vertical lines correspond to the median spike duration for each population. The median spike duration of PTNs in M1 (0.26 ms) was not significantly different from that of UIDs in the same area (0.27 ms) ($p>0.8$, Wilcoxon rank-sum test). Note that the UID population appears bimodal with a trough in the distribution at around 0.4 ms and the pyramidal population overlaps with the UID population.

(B) Probability density function comparing spike durations of identified PTNs in F5 (green) and F5 UIDs (yellow). The two vertical lines correspond to the median spike duration for each population. The median spike duration of PTNs in F5 (0.43 ms) was again not significantly different from that of UIDs in the same area (0.35 ms) ($p>0.2$, Wilcoxon rank-sum test). Note that there is considerable overlap between the distributions.

6.4.5 Positive correlation of antidromic latency with spike duration

We subsequently performed a linear regression analysis between spike duration and antidromic latency (**Fig. 6.5**), since the latter is known to reflect PTN soma size (Deschenes et al., 1979, Sakai and Woody, 1988). We found a strong significant positive correlation for both M1 and area F5 PTN populations (M1, $R^2 = 0.40$; F5, $R^2 = 0.57$, $p<0.001$). **Fig. 6.5** shows the scatter plot and regression line for all the PTNs in the sample (n=205). M1 and area F5 populations shared the same linear relationship between antidromic latency and spike duration. A linear regression for each individual monkey and on the combined data were also highly significant ($R^2 = 0.51$, $p<0.001$).

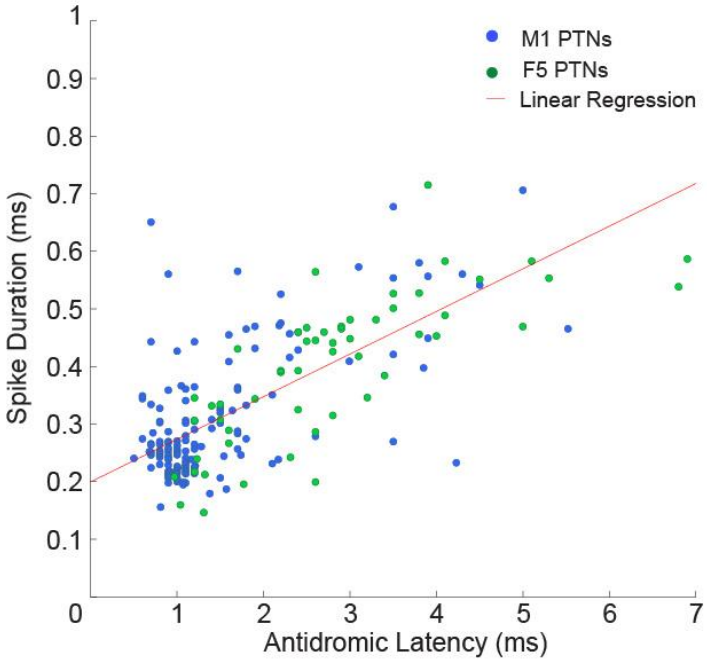


Figure 6.5 Spike duration vs ADL

Scatter plot showing the linear relationship between antidromic latency (a surrogate for axonal conduction velocity and cell size) and spike duration for identified PTNs in areas M1 (filled blue circles) and F5 (filled green circles). The data have been fitted with a linear regression line shown in red. The correlation was highly significant ($R^2 = 0.51$, $p<0.001$).

The relationship implies that the cells with the shortest ADLs and thus the fastest axons and probably largest somas exhibit the shortest spike durations, as assessed by the trough-to-peak measure. We also found a significant correlation between ADLs and spike durations measured from peak-to-peak (R^2 = 0.41, p<0.001), which is unsurprising given that these two measures are highly correlated (Fig. 6. 6; R^2 = 0.80, p<0.0001). The slope of linear regression is, 1.2, [1.12-1.29 95% CI], with an intercept of 106 µs; we used this to estimate the average trough to peak spike duration from peak to peak analyses reported in the literature (**Table 6.2**).

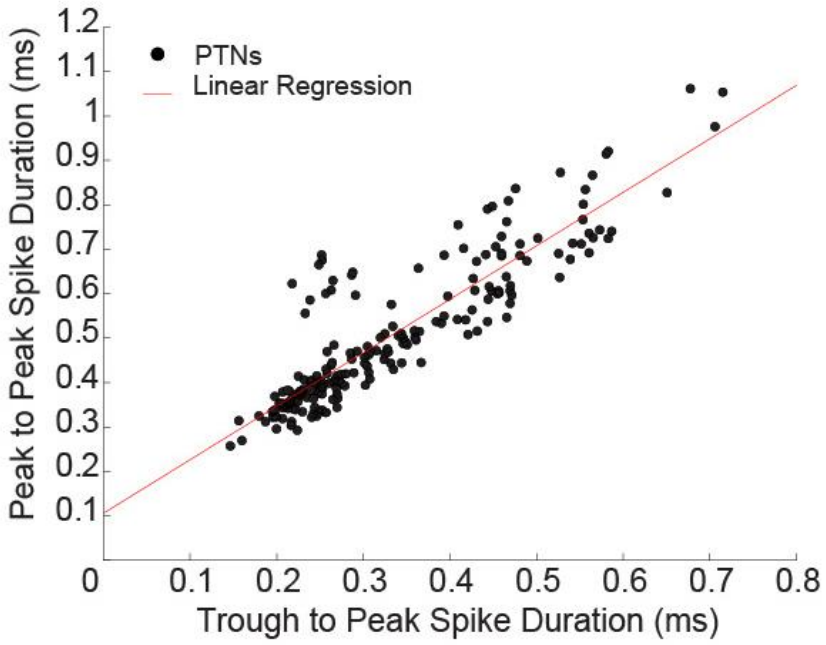


Figure 6.6 Peak to peak vs trough to peak

Scatter plot showing the relationship between spike duration as measured from the first negative trough to the subsequent peak of the extracellular waveform (trough-to-peak, as used in previous figures) and as measured from the first positive peak to the subsequent peak. Data from all identified PTNs in areas M1 and F5 (filled black circles). There was a significant correlation between the two measures of spike duration (R^2 = 0.80, p<0.0001). Note that the slope of the regression line (1.2, shown in red) and the intercept (106 µs) can be used to compare our measure of spike duration with others in the literature (see Table 6.2).

6.5 DISCUSSION

Our results demonstrate that physiologically identified PTNs, recorded in the motor cortex of the awake monkey, exhibit a wide range of spike durations. PTNs with short antidromic latencies generated the narrowest spikes, in the order of 0.15 to 0.17 ms, while those with longer latencies have much broader spikes, up to 0.70 ms. PTNs with very narrow or 'thin' spikes were not confined to M1 but some were also found in a 'secondary' motor region, area F5 of the ventral premotor cortex (**Fig. 6.3**). There was a significant positive correlation between ADL and spike duration for the whole sample of PTNs (**Fig. 6.5**), but also for the two sub-populations of PTNs recorded from M1 and PMv. To our knowledge, this is the first study in the awake monkey highlighting the fact that pyramidal neurons can exhibit 'thin' spikes.

6.5.1 Previous studies comparing spike durations of neocortical neurons

Spike duration has been suggested as one means of distinguishing putative neocortical interneurons from pyramidal neurons. **Table 6.2** summarises the results from a number of studies in which spike duration has been reported, including the type and conditions of recording and the spike features measured. From the data provided in these papers we have attempted to derive average values for the trough-to-peak spike durations of putative pyramidal and interneurons. Some of the studies listed were carried out in awake macaques, and involved extracellular recordings from unidentified neurons in a variety of cortical motor (M1, PMd), visual (V1, V4) and prefrontal (DLPFC) areas. All of them used the 'trough-to-peak' measure of spike duration and all concluded that it was possible to distinguish interneurons on the basis of their short spike duration, although the boundary value varies from 0.19 ms

(Kaufman et al., 2010) to 0.40 ms (Gur et al., 1999). This distinction has been based upon a large number of other studies, including *in vivo* recordings from rabbit SI and V1 (Swadlow, 1988, Swadlow, 1989), from rat S1 and prefrontal cortex (Bartho et al., 2004) and cat M1 (Baranyi et al., 1993, Calvin and Sypert, 1976), and *in vitro* recordings from guinea pig brain slices (McCormick et al., 1985). In fact, as we discuss below, both cortical area and species are important factors in analysing the significance of these findings for distinguishing interneurons from pyramidal cells.

Paper	Criterion	Putative Interneurons/FS, ms	Putative Pyramidal/RS, ms	Animal, Brain Area	Condition
Mountcastle, 1969	Unsure	0.1-0.3	0.3-0.5	Macaque,	Awake
Gur, 1999	T2P	<0.4	>0.4	Macaque, V1	Awake
Constantinidis, 2002	P2P (Inv)	0.47	0.64	Macaque, DLPFC	Awake
Mitchell, 2007	T2P	<0.2	>0.2	Macaque,	Awake
Cohen, 2008	T2P	0.22	N/A	Macaque,	Awake
Merchant, 2008	P2P (Inv)	0.42	0.80	Macaque,	Awake
Diester, 2009	T2P	<0.28	>0.28	Macaque, PFC	Awake
Kaufmann (Chronic implant), 2010	T2P	<0.19	>0.22	Macaque,	Awake
Kaufmann (single electrode), 2010	T2P	<0.2	>0.2	Macaque,	Awake
Song, 2010	T2P	0.1-0.3	0.3-0.5	Macaque, PMd	Awake
Krimer, 2005	IHA	<0.4	>0.4	Macaque,	Slices
Zaitsev et al., 2009	IHA	0.32 – 0.74	N/A	Macaque, DLPFC	Slices
McCormick, 1985	IHA	0.32	0.8	Guinea Pig,	In vitro
Swadlow, 1988	P2P	0.47	0.98	Rabbit,	Awake
Swadlow, 1989	P2P	0.43	0.98	Rabbit,	Awake
Bartho, 2004	T2P	0.43	0.86	Rat, S1	Freely moving/ anaesthetised

Table 6.2 Literature Review

The mean value for the spike duration of putative pyramidal cells is ~0.55 ms which has been calculated either using the numbers reported in the paper or estimated from the Figs.
FS = fast spiking
RS = regular spiking
T2P = trough to peak
P2P = peak to peak
Inv = inverted spike
IHA= Intracellular spike duration at half amplitude

6.5.2 Spike durations in identified pyramidal neurons

In view of the possible differences in spike duration between interneurons and pyramidal neurons, it is important to consider the range of durations exhibited by *identified* pyramidal neurons. The early intracellular study by (Calvin and Sypert, 1976) of PTNs in the motor cortex of the anaesthetised cat showed a clear

relationship between spike duration and antidromic latency (their Fig. 1B). Similarly, (Baranyi et al., 1993) recorded intracellularly in motor cortex of awake cats from pyramidal neurons identified as projecting either to the cerebral peduncle or VL thalamus; the briefest spikes from fast PTNs had durations ranging from 0.30 to 0.80 ms (mean 0.41 ms), measured as the duration of the intracellular spike at half-maximum, which is approximately equivalent to the trough-to-peak extracellular measure used in this study. Chen et al. (1996) made intracellular recordings from slices of cat motor cortex and reported 'narrow spiking' in cells that were located in lamina V and which intracellular staining revealed to be large pyramidal neurons. It is remarkable that many of these studies in the cat are not cited by those working with the awake monkey.

In the current study, the median spike duration for identified PTNs in M1 (0.26 ms) and in area F5 (0.43 ms) from this study are considerably briefer than the estimated mean spike duration for the population of 'putative' pyramidal cells in all the studies listed in **Table 6.2** (~0.55 ms), and the M1 value is shorter than any of the listed macaque studies. More importantly, the mean spike duration of 'putative interneurons' listed in **Table 6.2** is longer than the median spike duration of identified M1 PTNs in our study. There clearly exists a population of PTNs with 'thin' spikes having durations smaller than the boundary value between putative interneurons and pyramidal cells reported in any of the cited studies. That is, without PT identification, these PTNs would have been erroneously classified as interneurons.

6.5.3 Pyramidal neurons in M1 vs other cortical areas: the significance of cell size

The distribution of ADLs within M1 (**Fig. 6.1**) is clearly skewed towards short ADLs. There is a well-established relationship between PTN soma size and axon diameter, and therefore to conduction velocity and ADL (Deschenes et al., 1979, Sakai and Woody, 1988), and so this probably represents a recording bias towards neurons with large somas (including Betz cells), as noted in many earlier studies (e.g. Calvin and Sypert, 1976, Humphrey and Corrie, 1978, Towe and Harding, 1970)

Therefore, it could be argued that M1 is a special case and that in recordings from other cortical areas the interneuron-pyramidal distinction based on spike duration could still be applied. The corticospinal tract arises from a large cortical territory including many different frontal and parietal areas (Dum and Strick, 1991), and it is known that corticospinal neurons in areas such as PMv and SMA are smaller than those in M1 (Murray and Coulter, 1981) and have slower conduction velocities (Kraskov et al., 2009, Macpherson et al., 1982, Maier et al., 2002). Two recordings of PTNs encountered in somatosensory (granular) cortex confirmed this impression. In area 3a, one PTN had a long ADL of 3.7 ms and spike duration of 0.36 ms, while another in area 2 had values of 4.8 ms and 0.50 ms, respectively.

However, our results suggest considerable caution even for recordings made beyond M1. Although our area F5 population comprised PTNs with significantly longer ADLs (**Fig. 6.1**), there is some considerable scatter in the regression shown in **Fig. 6.5**, and indeed we did encounter a considerable proportion of area F5 neurons with short-duration (0.15-0.30 ms) spikes (**Fig. 6.3**; green dots in Fig. 6. 5; 12/54=23%). The single population of PTNs in area F5 clearly comprised those with narrow vs broad

spikes (**Figs. 6.3, 4**), and so a bimodal distribution in spike duration *per se* cannot be taken as evidence of recordings from different cell types.

It could be argued that corticospinal neurons represent a special case, and that their large cell bodies and fast-conducting axons are very different to other types of pyramidal neuron, such as cortico-striatal neurons (Turner and DeLong, 2000), callosal neurons (Soteropoulos and Baker, 2007) and cortico-cortical neurons (Godschalk et al., 1984, Kraskov et al., 2011) which make much more circumscribed projections and have much lower conduction velocities (< 20 m/s). These pyramidal neurons have relatively broad spikes (e.g. Soteropoulos and Baker, 2007). However, there are other corticofugal neurons making longer projections to the brainstem and pons, which might be anticipated to have large axons (Turner and DeLong, 2000). These projections far outnumber those in the corticospinal tract and arise from a far wider cortical territory (Glickstein et al., 1985, Tomasch, 1969).

6.5.4 Comparison of PTNs with UIDS

Fig. 6.4 shows a very substantial overlap between the spike durations of PTNs and UIDs, in both area F5 and M1. There is a clear population of UIDs with brief spikes in both areas. There are two extreme interpretations of these data. One interpretation is that the UID sample contained a significant proportion of interneurons (cf. Merchant et al., 2008) in which case it emphasises the almost complete overlap between the spike durations of these interneurons and the identified PTNs. This might seem unlikely, given the small size of interneurons and their relatively small contribution to the total population of cortical neurons (Sloper et al., 1979). A contrasting interpretation is that the UID recordings were from other pyramidal

neurones, whose axons do not travel in the pyramidal tract, which might then suggest that the PTNs we have sampled is rather representative of the pyramidal population in these cortical areas. Recordings from both UIDs and PTNs might be biased towards large neurons (with brief spikes) by being mainly present in recordings from lamina V (where all PTNs are located). However, we know that pyramidal neurons with axons projecting to the pyramid represent only a minority of those in lamina V (see above).

In one of the few studies in which records were made from identified neurons in monkey prefrontal cortex slices, (Krimer et al., 2005) pointed out that there may be some overlap between the spike durations of regularly-spiking pyramidal cells and at least one type of interneuron in prefrontal cortex. The same authors reported that morphologically identified cortical interneurons can themselves show a wide range of spike durations (0.32-0.74 ms) (Zaitsev et al., 2009).

6.5.5 Comparative biology of pyramidal neurons

Our data suggest that macaque PTNs can have briefer spikes than those found in the cat (Baranyi et al., 1993, Sakai and Woody, 1988). This is probably partly explained by the presence of a population of PTNs in the monkey that are larger and faster conducting than in the cat (Evarts, 1965, Humphrey and Corrie, 1978, Nudo et al., 1995). Likewise, the relatively broad spikes recorded *in vivo* and *in vitro* from rodent and rabbit cortex (**Table 6.2**) probably reflect the smaller size of pyramidal neurons in these species (Donoghue and Kitai, 1981, Landry et al., 1984, Nudo et al., 1995). For example, in the rat the largest corticospinal axons have conduction velocities of < 20 m/s (Mediratta and Nicoll, 1983) and relatively small somata (Landry et al., 1984,

Nudo et al., 1995). This might explain the results of (Bartho et al., 2004) who defined the interneuron population as having mean spike durations of 0.43 ms while that for the pyramidal population was 0.86 ms, and of (McCormick et al., 1985). Swadlow recorded from antidromically identified pyramidal neurons in visual (V1) and somatosensory (SI) cortex (Swadlow, 1988, Swadlow, 1989). All of them had wide spikes (mean peak-to-peak duration 0.98 ms) whose duration did not overlap with the narrow spikes from 'suspected interneurons' (0.47 ms). However, judging from the low axonal conduction velocity (max 18 m/s; most < 10 m/s) of the sampled pyramidal neurons, these recordings were dominated by small cells.

6.5.6 What is the underlying mechanism of the fast spike duration in large pyramidal neurons?

The trough to peak of the extracellular spike waveform encompasses the repolarisation phase of the membrane potential (Henze et al., 2000). It has previously been shown that the difference in spike duration between interneurons and pyramidal cells is partly due to a different level of expression of Na^+ and K^+ channels (Erisir et al., 1999, Martina and Jonas, 1997, Martina et al., 1998). Recent work has shown that fast-spiking properties reflect the presence of Kv3 and Kv1 channels, and these channels make repolarisation faster and allow subsequent firing of the cell. Kv3.1b mRNA and protein are associated with fast spiking interneurons in rodents (Hartig et al., 1999, Kawaguchi and Kubota, 1997), but, in keeping with our results, these markers are also expressed by large pyramidal cells of layer 5 in macaque motor cortex(Ichinohe et al., 2004). We speculate that large and fast PTNs might be expressing more Kv3.1b allowing for shorter spike durations and the higher firing rates of fast vs slow PTNs that was first reported by (Evarts, 1965). However, the

range of firing rates exhibited by PTNs in awake animals is heavily influenced by the recording conditions and experimental task being performed.

6.5.7 Conclusion

In summary, our study confirms for the awake monkey previous findings in the cat motor cortex that 'thin' spikes can originate from pyramidal neurons, and extends this observation to PTNs recorded in a secondary motor area. We conclude that spike duration alone may not provide a reliable indication of cell type, at least in areas which contain pyramidal tract neurons, but more likely reflects discharge properties shared between cortical interneurons and pyramidal neurons.

CHAPTER 7:
General Discussion and Summary

This thesis has included results from a series of experiments on the characteristic properties of the mirror system, both in the awake, behaving monkey using electrophysiological techniques to record from single cells, and in humans using transcranial magnetic stimulation to measure the excitability of the corticospinal tract. In addition, this thesis has provided an insight into classification of neuronal recordings in the awake, behaving monkey. A discussion of the results obtained in the different projects contributing to this Thesis has already been provided at the end of each Chapter. In this final Chapter, I will discuss the links between these studies and the potential implications of the results.

7.1 THE MIRROR NEURON SYSTEM

Mirror neurons were first discovered in the macaque ventral premotor cortex (area F5). Their characteristic feature is the modulation of their firing rate during both the monkey's own action and during observation of another individual performing a similar action. Some F5 mirror neurons have also been shown to be corticospinal neurons, by identifying them as pyramidal tract neurons (PTNs). This discovery means that downstream spinal targets are also influenced during action observation. The activity of these fascinating cells cannot be explained by any covert movement on the part of the monkey, since EMG recordings from hand and arm muscles during action observation show no evidence of modulation. The question arises as to how

you can have activity in the output cells of the cortex (PTNs) without the generation of overt movement.

Much of my thesis has attempted to address this question by first assessing whether the primary motor cortex (M1) also contains mirror neurons, since this area contains many more PTNs and is classically thought to be more involved in movement generation. In addition, my thesis has included a detailed comparison of the level of activity during execution and observation in M1 and F5. The key finding in Chapters 3 is that over half of the PTNs in primary motor cortex are mirror neurons (modulate their activity during action observation), but the depth of modulation during observation is much less compared with execution.

Since the primary motor cortex (M1) contains over 50% of the entire frontal lobe corticospinal projection to the spinal cord, the discovery that many M1 PTNs have mirror properties is a further reason to re-examine their role as "upper motor neurons" controlling muscles through projections to spinal "lower motor neurons" (Schieber, 2011, Schieber, 2013). Our results show a clear, context-dependent dissociation between the behaviour of cortical output neurons and the activation of the neuromuscular system.

We have shown that over half of the PTNs we recorded from in M1 had mirror properties. Although PTNs with mirror properties were already shown to exist in the 'classical' cortical area for mirror neurons, area F5 of the ventral premotor cortex (Kraskov et al., 2009), the contribution of area F5 to the pyramidal tract is quite small (~4%, (Dum and Strick, 1991)) and the corticospinal terminations from F5 are concentrated in the upper cervical cord, and their function is still poorly understood.

In contrast, PTNs within M1 have well-defined physiological and anatomical properties, and they have a strong pattern of terminations in the lower cervical cord, including projections to the motor nuclei in C8 and T1 which innervate the most distal hand and digit muscles (Armand et al., 1997). So it is indeed surprising that some M1 PTNs have mirror properties.

It is even more interesting that some mirror PTNs are CM or cortico-motoneuronal cells. This means that even PTNs within M1 that are monosynaptically connected to α-motoneurons innervating digit muscles can show mirror properties. This means that the excitability of spinal neurons, including α-motoneurons, should be modulated during action observation. Therefore it is predicted that these neurons may also behave like mirror neurons. Indeed, the spinal circuitry during action observation has been investigated through measurements of the H-reflex (Baldissera et al., 2001) and the modulation in the H-reflex directly reflects its activation pattern during action execution (Montagna et al., 2005). Another study involving measuring the metabolic activity (measured glucose utilisation – ^{14}C-deoxyglucose method) in the cervical enlargement of the spinal cord was suppressed bilaterally during observation whilst it was active ipsilaterally during execution trials (Stamos et al., 2010).

In Chapter 3, we proposed that the reason that there is no overt EMG modulation during action observation even though we have modulation of PTNs is because the level of activation of classical mirror neurons during observation is much less compared with execution (see Chapter 3, **Fig. 3.2D**). In addition there are also PTNs that suppress their activity during action observation (S-F or suppression mirror

neurons). This means that, overall, the amount of additional spikes reaching the spinal cord during execution from M1 PTNs is quite small. Although, our analysis is quite simple, since other factors such as the level of synchrony between PTNs might also be important during action observation.

In contrast, the primary finding from Chapter 4 is that PTNs in F5 seem to fire equally during execution and observation. This is very much in keeping with the classical picture of mirror neurons following Gallese et al. 1996. However, the overall firing rate in F5 is much lower compared with M1 meaning that the relative contribution from M1 and F5 being similar during observation. All these points show that a detailed quantitative analysis of the mirror neuron system can reveal important differences.

Of course it is interesting to consider why PTNs should be involved at all in action observation. In a sense this question reprises that which arose after the discovery of mirror neurons: why is the motor system at the heart of the mirror neuron system? The answer must now be that, whatever you consider the function or functions of this system, a strict comparison of its activity during execution and observation must be made. So, for example, the detection, monitoring and even understanding of the actions of others depends upon this matching (Brass and Heyes, 2005, Rizzolatti et al., 2001, Wilson and Knoblich, 2005, Rizzolatti and Sinigaglia, 2007, Gallese et al., 2009). PTNs are intimately connected with the rest of the motor network: their axon collaterals target many other neurons at both cortical and subcortical levels, and they receive thousands of synaptic inputs from local and remote regions of the motor network (Porter and Lemon, 1993, Huntley and Jones, 1991) and they can be shown

to be embedded in the oscillatory assembly that characterises motor cortex in a wide variety of conditions (Jackson et al., 2002, Baker et al., 1999a, Hari et al., 1998, Hari and Salenius, 1999). So one could argue that, if activation of the motor network is in some way essential to the function of the mirror neuron system, then this must involve the output neurons too, including PTNs (Vigneswaran et al., 2013).

So perhaps the question is not so much "Why send information to the spinal cord, via PTNs, if it not concerned with movement generation?" but rather "Can the motor consequences of PTN recruitment during action observation be suppressed?" Interestingly, we found evidence of a No-go signal in M1 (a sharp rise and fall in the firing rate of neurons following presentation of the No-go cue). However, the suppression of movement during No-go and during action observation does not appear to share the same cortical mechanisms apart from a total reduction in input to the spinal cord from PTNs in the no-movement scenario compared with movement.

We did not find the No-go effect to be specific to suppression mirror neurons as we first hypothesised. Instead the effect was found in mirror and non-mirror neurons alike. The evidence points to the conclusion that not all movement suppression is equal. We speculate that suppression of movement might be addressed differently by the brain in an execution vs observation scenario than in a move or do not move scenario. In the Go/No-go situation, the monkey is likely to be preparing for movement (Go trials are much more common (80%) than No-go trials (20%)), in comparison, during action observation, the monkey is not preparing movement but instead knows that it *does not need* to make a movement, watching the action

activates the mirror system (both facilitation and suppression) but as we have suggested, the actual number of spikes reaching the spinal cord overall is not as high compared with during execution. The No-go scenario might pose a stronger sense of suppressing movement compared with suppressing movement during action observation because the emphasis is on not executing during No-go trials whilst during observation the emphasis is on observing, and the subject's motor system is slightly facilitated, but not activated.

Of course, a further possibility is that activation of the motor network, and its PTN output, below the threshold for movement per se, might serve other functions. It might, for example, modulate forms of synaptic plasticity at the spinal level that improve performance during execution (Schieber, 2013). Motor learning might be achieved by facilitating downstream spinal targets during observation even though there is no movement (Vogt et al., 2007, Gatti et al., 2012).

It is somewhat surprising that the TMS data obtained from the human experiment did not directly support the monkey data. We were unable to elicit facilitation during observation above baseline. As discussed in Chapter 5, this is probably because we were unable to obtain a stable baseline and due to a large variability in the data. To put the results into context, our monkey research has shown us that not every cell in M1 is a mirror neuron and many of the neurons that do modulate during observation can be suppression mirror neurons. Maybe it is not surprising that we struggled to find a strong facilitation using TMS in humans.

7.2 CELL CLASSIFICATION

We wanted to classify all neurons recorded in the mirror task as either pyramidal neurons or cortical interneurons. However, when we attempted to verify whether the commonly used technique of spike duration can be used to classify cells into pyramidal cells and interneurons, we found strong evidence that spike duration alone may not be a reliable indicator of cell type in the awake, behaving macaque monkey. Since we had an identified population of pyramidal cells (confirmed by antidromic stimulation from the pyramidal tract at the level of the medulla) we were able to examine their spike durations and distributions. We did not find evidence of the commonly reported bimodal distribution trough-to-peak spike durations; instead we found a strong correlation between antidromic latency (ADL) and spike duration. The idea of using spike duration to separate cell types is well established in the rodent (McCormick et al., 1985, Bartho et al., 2004). However, the rat lacks any of the large corticospinal neurons that are present in the macaque monkey (Mediratta and Nicoll, 1983, Landry et al., 1984, Nudo et al., 1995), and this fits with the well-established finding that, in the rat, these small pyramidal tract neurons have long-duration spikes (Bartho et al., 2004). However, in recordings from M1 in the awake, behaving monkey, spike duration is not a reliable indicator of cell type. Our findings have several implications for the field. Namely, other, additional indicators of cell type will be required to identify reliably different cell types in physiological recordings. This is not least because of the diversity of both pyramidal neurons and interneurons (Krimer et al., 2005). Much of the work carried out in monkey that has used spike duration as the only indicator of cell type needs to be reviewed in light of our findings, with more robust indicators, such as cross correlation analysis to identify inhibitory

interneurons (Merchant et al., 2008, Merchant et al., 2012). It also brings to light the problem of assuming that mechanisms investigated in one animal species can be directly applied to others.

7.3 FUTURE DIRECTIONS

The results described in my thesis pose several questions and possible directions for future research. Since PTNs are mirror neurons and more specifically, CM cells can have mirror properties, it is likely that neurons in the spinal cord also have mirror properties. This is interesting because it would further our understanding of the function of mirror neurons, extending them to more than just a cortical phenomenon. Other subcortical targets of corticospinal tract neurons may also show mirror-like properties such as parts of the basal ganglia network including the STN, where action observation can bring about changes in the beta oscillatory activity (Alegre et al., 2010).

Furthermore, it would be interesting to understand whether facilitation and suppression mirror neurons terminate on different parts of the spinal cord controlling different muscles. We found that the proportion of suppression and facilitation mirror neurons can vary dependent on the grasp, and thus, it might be that these sub-populations of neurons are terminating on very different spinal targets. This might further our understanding of the functional role of these subtypes of mirror neurons. It would also be interesting to examine the synaptic interactions between F-S and S-S type neurons even at the cortical level (tested by cross-correlation analysis). However, this requires simultaneous recordings of pairs of mirror neurons.

The low amount of grasp selectivity during observation found for M1 mirror PTNs needs to be verified in more than one monkey, and compared with selectivity in area F5 for the same task. This is important, because it has several implications for our understanding of the function of mirror neurons. First, it should be stressed that most of these PTNs did show grasp selectivity during execution (86.5%), in agreement with an earlier study from this laboratory (Umilta et al., 2007). Second, the lack of grasp specificity during observation (40%, but very small differences) suggests that these neurons mirror the overall movement but not the specific grasp being used by the experimenter: so they are activated by movement but not in a specific manner. However, it is important to remember that grasp selectivity might change if the monkey had to use the information about the grasp for his reward, that is, if he observed grasp of a sphere the monkey would have to carry out action A, but if he observed grasp of a trapezoid it would mean that he would execute action B. Selectivity during action observation might be more pronounced in a situation where information about the observed action needs to be extracted by the monkey. This remains to be tested.

The results provided on mirror PTNs that demonstrate that they slowly lose their mirror activity following repeated exposure of expecting to see a movement where none occurs (Chapter 4, **Figs. 4.6,7**) is potentially very interesting. This finding is relevant to understanding the predictive capacity of mirror neurons, which has been much debated in the literature (Kilner et al., 2007). A more focused study and data from at least two monkeys is required to answer these questions.

A possible future direction might also be in inactivating area F5 (with a GABAergic agent such as muscimol) and therefore also the mirror neurons within F5, whilst simultaneously recording from M1 mirror PTNs. If the mirror neurons within M1 are purely driven by F5 mirror neurons, the prediction would be that M1 mirror PTNs would lose their mirror activity following administration of muscimol to area F5. This is not a simple experiment, since F5 also contains many other types of canonical neurons, and the inactivation of F5 is expected to affect active grasp (Fogassi et al., 2001) not just action observation. However, it might be a first step in identifying the origin of the input to M1 which reverse the activity of suppression mirror PTNs, a property that now seems fundamental to the operation of the mirror neuron system.

Since we concluded that it is unlikely that suppression of movement during No-go and action observation share the same mechanism, it would nonetheless also be interesting to explore where the No-go response recorded in M1 originates. Simultaneous recording from subcortical structures such as the subthalamic nucleus (STN) and motor cortex might provide more clues since the STN has been known to be involved in initiating/stopping movement.

The data provided on spike duration show that for PTNs in area M1 and to an extent F5, that the neurons with the shortest ADLs have the shortest spike durations, and these significantly overlap with the spike durations of putative interneurons. However, we do not know whether this overlap exists in other brain areas that do not contain neurons that contribute to the pyramidal tract. It would therefore be interesting to carry out the same spike-duration/ADL analysis on PTNs in other

cortical areas, and indeed on other identified pyramidal cells, such as those that have axons that pass through the cerebral peduncle but do not reach the pyramidal tract.

7.4 SUMMARY

In this Thesis I show, for the first time, that the discharge of M1 PTNs is indeed modulated during observation of precision grip by a human experimenter. I compared the discharge of the same population of neurons during active grasp by the monkeys. I found that 'facilitation mirror neurons', which are activated (increased discharge) during both execution and observation, were only half as active for action observation compared with action execution. For mirror neurons that exhibited decreased discharge during action observation ('suppression mirror neurons'), I found a reversal of their activity pattern such that their discharge was actually facilitated during execution. Thus although many M1 output neurons show significant modulation during action observation, M1 direct input to spinal circuitry, as represented by PTN activity, is either reduced or abolished and may not be sufficient to produce overt muscle activity.

In a separate series of studies I investigated similar questions using non-invasive transcranial magnetic stimulation (TMS) in human volunteers. TMS can be used to probe the overall excitability of the corticospinal system. I hypothesised that if human motor cortex contained a significant population of suppression mirror neurons; this might be detectable, at the population level, by examining motor evoked potentials (MEPs) during action observation and might help to explain why we do not move when we observe an action. The results of this series of experiments are inconclusive and require a more thorough investigation.

I also investigated whether it is possible to assign mirror neuron activity to different cell types on the basis of extracellular spike duration, which has been used in previous studies to attempt to differentiate pyramidal neurons with broad spikes from cortical interneurons with 'thin' spikes. To this end, I carried out the first systematic study in the monkey of spike durations of PTNs and other, unidentified neurons recorded in ventral premotor and primary motor cortex. Since all PTNs are by definition pyramidal cells, I was able to test whether the distribution of spike widths of identified PTNs in M1 and F5 corresponded or overlapped with the pyramidal/interneuron boundary described in the literature. M1 antidromic latencies (ADLs) were skewed towards short latencies and were significantly different from that of F5 ADLs. The duration of PTN spikes measured from the negative trough to the positive peak of the spike waveform ranged from 0.15 to 0.71 ms, and there was a positive linear correlation between ADL and spike duration in both M1 and F5. Thus PTNs with the shortest ADL (fastest axons) had the briefest spikes, and since PTN soma size is correlated with axon size and conduction velocity, it is likely that the largest pyramidal neurons (Betz cells in M1) have spikes with short durations. The values found for spike durations in these neurons overlap heavily with those reported for putative interneurons in previous studies in rodents. In summary, one class of physiologically identified cortical pyramidal neuron exhibits a wide variety of spike durations and the results suggest that spike duration alone may not be a reliable indicator of cell type.

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