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PHD THESIS

FUNCTIONAL PROPERTIES OF CEREBRAL CORTICAL NEURONS IN AND AROUND THE CENTRAL SULCUS OF THE MONKEY BRAIN DURING EXECUTION AND OBSERVATION OF FORELIMB MOVEMENTS

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Στη Σοφία

SUMMARY

In the first part of this thesis we investigated the response properties of mirror neurons (MirNs) in the ventral premotor cortex (PMv) of the macaque brain. The fundamental feature of these cells, characterized "tiny miracles", is that they respond both when a monkey reaches for an object and when the monkey watches someone else reach for the same object. This simple response pattern led to the formulation of a wide-reaching theory concerning the neural basis of motor cognition, according to which we understand the actions of others because our MirNs are activated to represent these actions. Existing evidence on the properties of MirNs is limited, and often the theories taking advantage of MirNs to explain aspects of motor cognition are not grounded on empirical facts.

We examined key assumptions of the MirN theory, used to support the involvement of MirNs in action understanding, and found them to be invalid. In contrast to the claim that MirNs respond exclusively to object–directed actions, we demonstrate that MirNs are activated by the observation of both transitive and intransitive actions. Furthermore, our finding that the activity of MirNs is correlated with the kinematics of actions challenges the notion that these neurons encode the goal of actions. Finally, we demonstrate that, in contrast to previous notions, MirNs and non-MirNs of area F5 use different codes to represent actions during execution. Our results dictate a re-evaluation of the function of MirNs. Our finding that MirNs start discharging shortly after the onset of observed movement, combined with the finding that they encode movement kinematics, indicate that they may be involved in the rapid detection and monitoring of others' actions as they unfold. Moreover, the fact that during execution kinematics are encoded almost in synchrony with the movement suggests the involvement of MirNs in the online control of action.

In the second part of the thesis we investigated whether neurons that respond to action observation also exist in the dorsal premotor cortex (PMd) of the macaque brain. MirN theory holds that action understanding is sub-served exclusively by the fronto-parietal circuit consisted of the ventral premotor area F5 and the inferior parietal area PF/PFG which, according to Rizzolatti, are the only ones containing MirNs. Until recently, these were the only areas in which the existence of MirNs was investigated using direct methods such as the extracellular recording of single cell activity, mainly by members of the Rizzolatti's team. However, there is a large and constantly increasing body of evidence indicating that the observation of an action induces widespread cortical

activation, as does its execution. Several brain imaging studies from our lab demonstrate that the system activated during the observation of actions of other subjects encompasses the entire brain circuitry that supports action execution, rather than just a couple of cortical areas supposed to be the only ones containing MirNs. We employed the same paradigm used for the study of MirNs in area F5 to search for MirNs in the PMd, in order to conclusively verify the existence of MirNs in this part of the brain.

We identified several neurons in the PMd that respond both during action execution and action observation, thus proving the existence of MirNs also in this part of the brain. Similarly to F5 MirNs, the dorsal premotor MirNs were action selective and responded to the observation of both transitive and intransitive actions. The discharge of the dorsal premotor MirNs started earlier than the discharge of F5 MirNs during both observation and execution. Moreover, the activity of the dorsal premotor MirNs was correlated with the action kinematics and this correlation preceded the corresponding one between the activity of F5 MirNs and action kinematics. Finally, actions were encoded similarly in the dorsal and ventral premotor cortices, either when the execution or the observation discharges of the two areas were considered. During observation, the activity of the dorsal premotor MirNs occurring at the initial phases of the movement was correlated with the activity of F5 MirNs occurring at the final phases of the movement. The properties of the dorsal premotor MirNs are fully compatible with its long standing involvement in the online control of actions and with our proposal that MirNs may be involved in the rapid detection and monitoring of self and others' actions as they unfold.

ΠΕΡΙΛΗΨΗ

Στο πρώτο μέρος αυτής της διατριβής μελετήσαμε τις λειτουργικές ιδιότητες των νευρώνων κατόπτρων (ΝΚ, mirror neurons) της περιοχής F5 του κοιλιακού προκινητικού φλοιού των εγκεφαλικών ημισφαιρίων του μακάκου. Οι ΝΚ ενεργοποιούνται τόσο όταν το ζώο προσεγγίζει και συλλαμβάνει αντικείμενα με την άκρα χείρα όσο και όταν παρακολουθεί τον πειραματιστή να εκτελεί την ίδια πράξη. Αυτό το πρότυπο ενεργοποίησης οδήγησε στη διατύπωση της θεωρίας των ΝΚ που αφορά το νευρωνικό υπόστρωμα της κατανόησης των πράξεων των άλλων. Σύμφωνα με αυτή, ο παρατηρητής αντιλαμβάνεται την πράξη που βλέπει επειδή ενεργοποιούνται στον εγκέφαλό του οι ΝΚ όπως όταν ο ίδιος εκτελεί την ίδια ή παρόμοιες πράξεις. Δυστυχώς, τα υπάρχοντα στοιχεία σχετικά με τις λειτουργικές ιδιότητες των ΝΚ είναι περιορισμένα και συχνά οι θεωρίες που βασίζονται στον υποτιθέμενο ρόλο τους για να εξηγήσουν πτυχές γνωσιακών λειτουργιών στερούνται θεμελίωσης σε εμπειρικά δεδομένα. Χρησιμοποιήσαμε τη μέθοδο της εξωκυττάριας καταγραφής της δραστηριότητας μονήρων νευρώνων από τον εγκεφαλικό φλοιό μακάκων που είτε εκτελούν μία συμπεριφορά που έχουν μάθει είτε παρακολουθούν τον πειραματιστή να εκτελεί την ίδια συμπεριφορά. Η μέθοδος αυτή έχει υψηλή χρονική διακριτική ικανότητα και είναι η καταλληλότερη για την μελέτη του τρόπου με τον οποίο κωδικοποιούνται ανώτερες εγκεφαλικές λειτουργίες.

Εξετάσαμε τις βασικές παραδοχές της θεωρίας συμμετοχής των ΝΚ στην κατανόηση των πράξεων των άλλων και αυτές δεν επαληθεύθηκαν. Σε αντίθεση με τον ισχυρισμό ότι οι ΝΚ αποκρίνονται αποκλειστικά κατά την παρατήρηση μεταβατικών πράξεων (σύλληψη αντικειμένων), δείξαμε ότι οι ΝΚ αποκρίνονται και κατά την παρατήρηση αμετάβατων κινήσεων (έκταση άκρου και δακτύλων). Βρήκαμε επίσης ότι η δραστηριότητα των ΝΚ συσχετίζεται με τις κινηματικές παραμέτρους των κινήσεων, εύρημα που αμφισβητεί την υπόθεση ότι οι ΝΚ κωδικοποιούν τον σκοπό των παρατηρούμενων πράξεων. Τέλος, δείξαμε ότι, σε αντίθεση με την επικρατούσα άποψη, οι ΝΚ και οι μη-κατοπτρικοί νευρώνες της περιοχής F5 κωδικοποιούν με διαφορετικό τρόπο τις εκτελούμενες πράξεις. Τα αποτελέσματά μας δείχνουν την ανάγκη επαναξιολόγησης του ρόλου των ΝΚ. Το γεγονός ότι οι ΝΚ ενεργοποιούνται μετά την έναρξη της παρατηρούμενης κίνησης, σε συνδυασμό με την ύπαρξη συσχέτισης μεταξύ των αποκρίσεων και των κινηματικών παραμέτρων, υποδεικνύει ότι οι ΝΚ είναι πιθανό να συμμετέχουν στην ανίχνευση και την παρακολούθηση της εξέλιξης των πράξεων των άλλων. Επιπλέον, το γεγονός ότι κατά τη διάρκεια της εκτέλεσης κινήσεων οι ΝΚ

κωδικοποιούν τις κινηματικές παραμέτρους σχεδόν ταυτόχρονα με την κίνηση υποδηλώνει τη συμμετοχή τους στον υπό οπτική καθοδήγηση κινητικό έλεγχο.

Στο δεύτερο μέρος της διατριβής εξετάσαμε την ύπαρξη ΝΚ στον ραχιαίο προκινητικό φλοιό των εγκεφαλικών ημισφαιρίων του μακάκου. Σύμφωνα με την κυρίαρχη άποψη, το νευρωνικό κύκλωμα που είναι υπεύθυνο για την αντίληψη της κινητικής συμπεριφοράς των άλλων περιλαμβάνει αποκλειστικά την περιοχή F5 του κοιλιακού προκινητικού φλοιού και τις περιοχές PF/PFG στο πρόσθιο τμήμα του κάτω βρεγματικού λοβού. Μέχρι πρόσφατα, αυτές ήταν οι μόνες περιοχές στις οποίες διερευνήθηκε η ύπαρξη ΝΚ με τη χρήση άμεσων μεθόδων όπως η εξωκυττάρια καταγραφή της δραστηριότητας μονήρων νευρώνων. Ωστόσο, ολοένα και περισσότερα δεδομένα δείχνουν ότι η παρατήρηση κινήσεων ενεργοποιεί πολλές περιοχές των εγκεφαλικών ημισφαιρίων. Μελέτες λειτουργικής χαρτογράφησης του εγκεφάλου με τη μέθοδο της δεοξυγλυκόζης από το εργαστήριό μας δείχνουν ότι οι περιοχές που ενεργοποιούνται τόσο κατά την παρατήρηση όσο και κατά την εκτέλεση πράξεων δεν περιορίζονται στις περιοχές F5 και PF/PFG αλλά περιλαμβάνουν το σύνολο των περιοχών που ενεργοποιούνται κατά την εκτέλεση πράξεων.

Εφαρμόζοντας την δοκιμασία συμπεριφοράς που χρησιμοποιήσαμε και για τη μελέτη των ΝΚ στην περιοχή F5, αναζητήσαμε ΝΚ στον ραχιαίο προκινητικό φλοιό, έτσι ώστε να εξακριβωθεί η ύπαρξή τους σε αυτή την περιοχή των εγκεφαλικών ημισφαιρίων. Εντοπίσαμε νευρώνες που αποκρίνονται τόσο κατά την εκτέλεση όσο και κατά την παρατήρηση πράξεων, αποδεικνύοντας έτσι την ύπαρξη ΝΚ σε αυτή την περιοχή. Όπως και οι ΝΚ της περιοχής F5, οι ΝΚ του ραχιαίου προκινητικού φλοιού απαντούν επιλεκτικά στην παρατήρηση και στην εκτέλεση μεταβατικών κινήσεων σύλληψης. Επιπλέον, απαντούν και κατά την παρατήρηση αμετάβατων κινήσεων. Οι ΝΚ του ραχιαίου προκινητικού φλοιού ενεργοποιούνται νωρίτερα από τους ΝΚ της F5, τόσο κατά την παρατήρηση όσο και κατά την εκτέλεση κινήσεων. Επίσης, η δραστηριότητα τους συσχετίζεται με τις κινηματικές παραμέτρους και αυτή η συσχέτιση προηγείται της αντίστοιχης των ΝΚ της F5. Τέλος, βρήκαμε ότι οι ΝΚ της F5 και του ραχιαίου προκινητικού φλοιού κωδικοποιούν τις κινήσεις με όμοιο τρόπο, τόσο κατά την εκτέλεση όσο και κατά την παρατήρηση. Κατά τη διάρκεια της παρατήρησης, η δραστηριότητα των ΝΚ του ραχιαίου προκινητικού φλοιού στα αρχικά στάδια της κίνησης συσχετίζεται με τη δραστηριότητα των ΝΚ της F5 στα τελικά στάδια της κίνησης. Οι ιδιότητες των ΝΚ του ραχιαίου προκινητικού φλοιού είναι πλήρως συμβατές με την προταθείσα

συμμετοχή της περιοχής αυτής στον υπό οπτική καθοδήγηση κινητικό έλεγχο καθώς και με την πρότασή μας ότι οι NK συμμετέχουν στην ανίχνευση και την παρακολούθηση της εξέλιξης των πράξεων των άλλων.

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Abbreviation	**Meaning**
NK	νευρώνας κάτοπτρο
MirN	mirror neuron
PMv	ventral premotor cortex
PMd	dorsal premotor cortex
PF, PFG	subdivisions of inferior parietal lobule
F5	anterior PMv
F5c	F5 convexity
F5a	anterior sector of posterior bank of inferior arcuate sulcus
M1/F1	primary motor cortex
AIP	anterior intraparietal area
SII	secondary somatosensory cortex
F2	posterior PMd
F2vr	ventrorostral part of F2
F4	posterior PMv
FEF	frontal eye fields
SEF	supplementary eye fields
STS	superior temporal sulcus

MST	medial superior temporal area
FST	fundus of superior temporal area
MT	middle temporal area
V6A	visual area 6a
MIP	medial intraparietal area
PI	preference index
AI	amplitude index
tAI	task amplitude index
RDM	representational dissimilarity matrix

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1 INTRODUCTION

Which is the neural substrate of social cognition, in other words which are the processes in our brain when we watch conspecifics acting, and how do we achieve efficient understanding of their actions? The discovery of mirror neurons (MirNs) in the ventral premotor cortex (PMv) of the macaque offered a simple answer that gave rise to a theory concerning the neural basis of motor cognition. MirNs fire both when a monkey reaches for an object and when the monkey watches someone else reaching for the same object. According to the MirN theory, we understand the actions of others because our MirNs in cortical areas F5 and PF/PFG are activated to represent these actions.

This simple idea sprang an avalanche of speculation about the potential importance of these MirNs. They have been implicated in a number of human abilities, phenomena and disorders, and have profoundly influenced not only neuroscience (basic and clinical) but also psychology, philosophy, computer science and robotics. In fact, in numerous scientific publications in prominent journals, MirNs have been dubbed the neural cause of action understanding, empathy, emotion recognition, imitation, mind-reading, language, aesthetic experience and, when malfunctioning, autism, schizophrenia, and aspects of Down's syndrome (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996; Rizzolatti and Arbib 1998; Gallese 2003; Arbib 2005; Iacoboni 2005; Dapretto et al. 2006; Iacoboni and Dapretto 2006; Keysers and Gazzola 2006; Enticott et al. 2008; Savaki 2010). In turn, the publicity of MirNs led to controversy and criticism, based both on theoretical grounds and on inconsistencies that appeared in the published

data (Csibra 2008; Jacob 2008; Hickok 2009; Jacob 2009; Heyes 2010; Kosonogov 2012; Hickok 2013; Cook et al. 2014; Steinhorst and Funke 2014). It is worth noting that, almost twenty five years after the discovery of MirNs, very few independent studies have replicated or reexamined the original data. The available reports concerning the original work on MirN contain mostly qualitative description of the properties of individual neurons, leading to unsubstantiated speculations. Key findings are not replicated, leading to frequent revisions of the mirror neuron theory. In this chapter, I will attempt to provide a thorough review of the available MirN data, considering only the primary literature on MirNs: reports on monkey cortical neurons that modulate their activity during both execution and observation of a motor act.

1.1 Mirror neurons in the PMv

The first description of MirNs in the monkey brain (di Pellegrino et al. 1992) reported 29 neurons in area F5 (rostral part of the PMv) that discharged both when the monkey performed and observed a hand action. In this and the two other original papers (Gallese et al. 1996; Rizzolatti et al. 1996), the responses of MirN to various observed or performed actions were described qualitatively. Prior to these reports, F5 was considered to be a part of the premotor cortex involved in the control of distal movements (Kurata and Tanji 1986; Rizzolatti et al. 1988).

1.1.1 The role of area F5 in the execution of grasping actions

In the modern map of the monkey motor cortex, area F5 is the rostral part of the ventral division of the frontal agranular cortex of the macaque (Matelli et al. 1985). Kurata and Tanji showed that this part of the PMv is functionally related to distal rather than proximal arm movements (Kurata and Tanji 1986). The functional properties of F5 neurons were further described by Rizzolatti (Rizzolatti et al. 1988) who reported that F5 neurons discharged during specific goal-directed actions performed with the hand and/or the mouth such as grasping, holding, tearing or otherwise manipulating. Grasping neurons could be classified according to grip configuration in "*precision grip neurons*", "*finger prehension neurons*" or "*whole hand prehension neurons*". Subsequently, it was shown that F5 neurons also respond to the mere presentation of graspable objects and that this response follows a "*grasping code*" (i.e. that objects of different shape that require the

same hand configuration to be grasped are coded similarly (Murata et al. 1997; Raos et al. 2006)). The encoding of objects in motor terms in F5, as well as its role in the visuomotor transformation required in grasp actions was further established recently (Fogassi et al. 2001; Fluet et al. 2010; Vargas-Irwin et al. 2015). Moreover, PMv is considered to encoe extrinsic parameters of performed actions. Directionally tuned neurons in the PMv encode the direction of movement regardless of the initial hand posture (Kakei et al. 2001; Ochiai et al. 2005). Schwartz (Schwartz et al. 2004) used a visual illusion to dissociate the perceived trajectory from the actual movement during figure drawing and found that most of the PMv neurons represented the perceived trajectory and not the actual movement. Interestingly, almost half of these neurons also responded to movements of the experimenter's hand within the animal's visual field. Umilta (Umilta et al. 2008) trained monkeys to use normal and reverse pliers to grasp an object. They showed that a subset of F5 neurons responded equally well when using the two kinds of pliers, even though the use of the two tools required different movements (closing or opening of the hand, respectively). This finding indicated that non-MirNs encode movement information in an extrinsic frame of reference, independently of the muscles used, and was interpreted by the authors as encoding of the goal of the performed actions.

1.1.2 Responses of F5 neurons during the observation of grasping actions

In the first short report on MirNs, di Pellegrino reported 184 F5 neurons that responded during distal movements (grasping, holding or tearing) (di Pellegrino et al. 1992). Out of them, 29 responded also to the observation of movements. The authors divided these neurons in groups according to the matching between the selectivity of their discharges during action execution and action observation (termed as motor – visual congruency): in 12 neurons the action for which the neuron displayed the best response during execution elicited also the best response during observation. Neurons of the second group (n = 6) responded during execution to one action and during observation to this and also to other similar actions. In the third group (n = 11), different actions evoked the maximum response during execution and observation. None of the recorded MirNs responded to intransitive movements of the hand, even when the object was present in the scene. It was reported that an interaction between the hand and the object is required to

evoke the response of MirNs during observation. Moreover, MirNs didn't respond to the observation of actions performed with a tool. The visual responses were "*usually stronger*" when the observed movement was in the peri-personal space of the monkey and the response of "*some units*" was affected by the laterality of the presented action.

In a subsequent extensive report, the properties of 92 MirNs were described (Gallese et al. 1996). Fifty one of them were selective for a single action during observation (such as grasping, placing, giving a piece of food to another individual) while the rest responded to two or three different actions. Out of the 30 neurons that preferred grasping, 18 were selective to the grip type (e.g. precision grip or finger prehension). The responses of MirNs were also modulated by other factors such as the hand used (12/32) or the direction of the observed movement (30/47). The motor responses of MirNs in area F5 were reported to be similar to the responses of non-MirNs because "*they (MirNs) also showed a clear specificity for particular motor acts*". A subset of the MirNs (n = 14) were also tested for both grasping in the light and in the dark and were found to be responsive in both conditions. As in the previous report (di Pellegrino et al. 1992), the authors classified the recorded neurons according to their motor – visual congruency. In 29 neurons (the so called "*strictly congruent neurons*") the action evoking the strongest activity during execution also evoked the strongest activity during observation. The neurons displaying action selectivity during execution but not during observation (n = 56) were classified as "*broadly congruent*". "*Non-congruent neurons*" were selective for different actions in execution and observation. The authors reported the absence of MirNs in the primary motor cortex because they did not find any visual response in 49 tested neurons. As in (di Pellegrino et al. 1992), none of the MirNs responded to pantomimed actions (the experimenter's hand mimicking an object-directed action while the object was absent), or to actions performed with a tool. The lack of effector selectivity (hand or mouth) displayed by the majority of neurons was considered to support the view that these neurons encode the goal of the action. The motor properties of MirNs and non-MirNs during action execution were reported to be indistinguishable. The accompanying theoretical paper (Rizzolatti et al. 1996) used the same data to argue that the human verbal communication system evolved from a gesture recognition system subserved by MirNs.

As reported in the first three papers, MirNs did not respond to pantomimed actions, solidifying the view that the effector-object interaction is necessary for their

activation. However, this result did not fit well with the proposed function of MirNs in understanding others' actions since we are able to understand observed actions even when the target object is hidden. To investigate this issue, Umilta recorded 37 MirNs of area F5 while the monkey performed and observed hand actions (Umilta et al. 2001). In the observation task, an object-directed and a pantomimed action were presented in two different conditions: in the first the action was fully visible whereas in the second the final phase of the grasping was hidden. In the hidden condition, the monkey knew whether the object was behind the opaque screen or not. The authors found that 19 neurons responded to the observation of actions in both conditions. These neurons were then separated in three groups, depending on the relationship between the discharge in the full vision and hidden conditions (higher, equal or lower). For most neurons (9/19) the response to the full vision condition was higher than to the hidden condition. These results were also verified in the normalized population activity. Hence, the authors concluded that mirror neurons respond to the observation of grasping actions, even when the target object and the final movement phase are not visible. The effect of object presence, in both hidden and visible conditions, was only reported for two neurons in which cases it was found to be significant. To rule out the possibility that this effect was due to differences in hand movements, the authors analyzed the hand kinematics of the observed movements and found them to be similar in the two conditions. However, the alleged kinematic similarity between the object-directed and the pantomimed movements was not adequately tested. The reported similarity was restricted to the distance between the travelling hand and a stationary marker and was only qualitatively assessed. Therefore, it is not clear whether the observed action kinematics affected the neuronal responses.

The results of the above four papers were the basis of a highly influential opinion article in Nature Reviews Neuroscience (Rizzolatti et al. 2001). The authors introduce the "direct matching hypothesis" according to which "*we understand actions when we map the visual representation of the observed action onto our motor representation of the same action"*. The evidence for action understanding was that MirNs were found in "*motor sectors that code actions (PF and premotor areas)*". Since MirNs are activated both during observation and execution, the direct matching hypothesis was supported, and these neurons are responsible for action understanding. In contrast, the alternative "visual hypothesis" which states that understanding comes only with visual analysis, without

involvement of the motor system, was weakened. This syllogism has two weak points that would be later used by MirN theory opponents: Firstly, the "visual" and "direct-matching" hypotheses are not mutually exclusive. In particular, activation of a component of the motor system cannot be solely responsible for understanding actions, as the authors themselves point out. Also, what part of understanding is "visual" and what is "motor" and how can one dissociate between the two? Second, the MirN responses merely correlate with observation. To claim that MirNs are specifically involved in action understanding, one should try to directly correlate MirN responses to the subject's understanding or to prove that the specific component of the motor system is necessary for action understanding.

In 2005, Ferrari described F5 neurons that responded to both the execution of action and to the observation of actions performed with tools (Ferrari et al. 2005). This finding was in contradiction to the original description of MirNs according to which observation of actions with tools was ineffective in triggering MirN discharge. The authors mentioned that these MirNs were found after a long exposure of the animals to the observation of actions with tools. They concluded that, after training, MirNs generalize the goal of the observed actions. Similar neurons that responded to both the execution and observation of actions performed with tools were reported in a later study (Rochat et al. 2010). The difference between the two studies is that in the latter study the monkeys had been trained to use tools for grasping objects whereas in the former one the monkeys could not use the employed tools, even after months of watching the experimenters using them.

In a series of experiments, Caggiano et al studied the responses of F5 MirNs to various characteristics of observed actions that had the same goal. They found that the discharge of 55 out of 105 MirNs depended on the distance between the monkey-observer and the object grasped by the experimenter (Caggiano et al. 2009). Thus MirNs were considered to encode the space (peri-personal or extra-personal) around the observer. In a different experiment, the same authors reported the preference of MirNs to the observer's viewpoint (Caggiano et al. 2011). The monkeys were shown videos of grasping actions displayed in one of three views (0, 90 or 180 degrees with respect to the first person perspective). Sixty out of 201 neurons preferred a single viewpoint, 89/201 preferred more than one viewpoints whereas the responses of the remaining 52 neurons

were view-independent. Finally, in a third paper (Caggiano et al. 2012), the monkeys observed the experimenter grasping two different objects: a small cylinder (in which case the monkey was rewarded at the end of the trial) and a large cylinder (in which case the monkey was not rewarded. Out of the 87 recorded MirNs, 40 preferred the observation of grasping the object that led to reward whereas 11 preferred the observation of action that did not lead to reward (the rest showed no preference). It was concluded that MirNs mostly prefer actions that have a value for the observer and moreover that this value is encoded in their discharge.

In another experiment, Bonini used a task in which the monkey or the experimenter grasped a piece of food and either ate it or placed it in a container that was located near the actor's mouth (Bonini et al. 2010). The aim of this task was to examine whether MirNs can dissociate between seemingly similar actions (grasping the object) with different goals (to eat or to place). Nine of the 23 recorded MirNs responded differentially to the two variations in both execution and observation. The authors concluded that this response pattern enables the understanding of the goal of the observed action. However, the similarity of the two observed grasping actions in terms of kinematics was not assessed. Thus the differential discharge of the MirNs could be due to the observation of actions with different kinematics. Moreover, the visual scene was not identical in the two conditions. The absence or presence of a container in the grasp-to-eat and grasp-to-place conditions, respectively, informed the monkey about the upcoming action and thus could also affect the discharge of MirNs.

Bonini also reported MirNs that not only discharged when an actor performed a grasp in front of the monkey (action) but also when the actor refrained from acting (inaction) (Bonini et al. 2014b). Seventy nine out of the 188 MirNs discharged during the observation of the "inaction" condition. Compared to the responses to "action" observation, the discharge during the observation of "inaction" was weaker and occurred earlier in time. Because these neurons responded to both "action" and "inaction" conditions, the authors suggested that they represent actions at a highly abstract level, even when these actions are not actually performed. However, the response during the inaction condition could be due to the presence of instruction cues and thus it may reflect learned associations.

In a subsequent study, Maranesi reported that MirNs associated with "inaction" started firing 480ms before the cue instructing the movement (go cue) whereas MirNs associated with "action" started firing 100ms before the go cue (Maranesi et al. 2014). The authors interpreted the early onsets of activity as "predictive activations". Nevertheless, they never defined what is meant by "predictive", nor what these neurons predict. In fact, the early activations of the inaction neurons (the ones that started discharging earlier) were not selective for the upcoming movement. In other words, the neuronal responses could not be used to detect if an action was about to happen or not.

In the original studies (di Pellegrino et al. 1992; Gallese et al. 1996) the sight of a graspable object alone did not suffice to trigger the response of MirNs. This led to the claim that MirNs are different from "canonical neurons" (Raos et al. 2006) which discharge upon the sight of an object to-be-grasped (Rizzolatti and Kalaska 2013). This claim changed in 2014, when Bonini reported that MirNs also discharge, albeit weakly, upon the presentation of graspable objects (Bonini et al. 2014a). The authors reported three separate functional groups of neurons: 137 MirNs, 46 canonical neurons and 60 canonical-MirNs; each group spatially intermingled in area F5. Most canonical (mirror or not) neurons responded to object presentation only when the object was in the peri-personal space. On the other hand, most mirror (canonical or not) neurons responded to action observation regardless of whether the action occurred in the peri- or extra-personal space. Moreover, the authors reported that MirNs neurons were found not only in the cortical convexity of area F5 (F5c), as previously thought (Rizzolatti and Kalaska 2013), but also in locations deep in the posterior bank of the inferior arcuate sulcus (F5p).

Maranesi and colleagues examined the influence of gaze on the discharge of monkey MirNs (Maranesi et al. 2013). The monkey was not required to fixate on the observed action, so the authors were able to separate trials in "fixation" and "no fixation" groups. The "fixation" trials were further subdivided in two groups, depending on when the monkey fixated to the observed action or not (before or after the hand-target contact). Thirty-eight out of the 71 MirNs responded stronger when the monkey looked at the action, whereas the responses of the remaining ones were independent of gaze. When the monkey looked at the object before the hand-target contact (proactive gaze), the responses were stronger than when the monkey looked at the object after the hand-target contact (reactive gaze). The discharge of the gaze independent neurons demonstrates that many

MirNs respond to action observation irrespectively of whether the monkey focuses on the action (the experimental setup did not control for the monkey's attention). The gaze dependent neurons also discharged in both "fixation" and "no fixation" trials, with weaker activations in the latter case. This showed that the foveation of the observed action is not a necessary condition for the activation of these neurons.

In the early studies, MirNs were reported to respond when the monkey grasped objects in the dark (without visual feedback), although this report came without statistics and example figures. The role of the visual feedback in the response of MirNs during execution was formally assessed in 2015 (Maranesi et al. 2015). The authors examined the role of visual feedback in both mirror and non-MirNs of area F5. In both classes of neurons, the responses were mostly equal when the monkey grasped in the light and in the dark. One third of the MirNs (30.3%) discharged more during grasping in the light whereas the respective percentage for non-MirNs was 11.4%.

In all of the above studies, researchers carried out extracellular recordings from F5 neurons that responded to grasping execution and observation. This method does not provide much information about the cell type of the recorded cells (e.g. if a recorded neuron is a pyramidal cell or an interneuron). In a different approach, Kraskov first used antidromic stimulation to identify 64 pyramidal tract neurons in F5 and then tested these neurons for mirror properties (Kraskov et al. 2009). About half of them (31/64) modulated their activity during observation: 14 showed a facilitation of activity and 17 showed a suppression of activity (compared to baseline). It was suggested that the suppressed MirNs are involved in the inhibition of self-movements during action observation.

Table 1: Properties of F5 MirNs during action observation

Report	**Properties**
(di Pellegrino et al. 1992)	Variable levels of selectivity and congruency, no response to intransitive, preference for peri-personal space, left or right visual field preference
(Gallese et al. 1996)	Hand preference, direction preference, responding during execution in the dark, no response to intransitive

(Umilta et al. 2001)	Response to partially occluded actions, as long as an object is present
(Ferrari et al. 2005)	Response to the observation of actions executed with tools (monkeys were not trained to use tools). Various levels of effector preference.
(Rochat et al. 2010)	Response to the observation of actions executed with tools (monkeys were trained to use tools). Various levels of effector preference.
(Caggiano et al. 2009)	Preference to distance of the object from the observer
(Caggiano et al. 2011)	Preference of action viewpoint
(Caggiano et al. 2012)	Preference for presence or absence of reward
(Bonini et al. 2010)	Preference for the final part of a two parts action (grasp to eat vs grasp to place)
(Maranesi et al. 2013)	Gaze dependent modulation
(Bonini et al. 2014a)	Response to mere object presentation
(Bonini et al. 2014b; Maranesi et al. 2014)	Response to inaction (occurs earlier than response to action)
(Maranesi et al. 2015)	Response during execution in the dark
(Kraskov et al. 2009)	Suppression of some MirNs' activity during action observation

1.2 Mirror neurons in other brain areas

1.2.1 Mirror neurons in the primary motor cortex

In the early papers in the MirN literature, the authors were unable to find neurons in the primary motor cortex (area M1, Brodmann area 4) that responded during action observation (di Pellegrino et al. 1992; Gallese et al. 1996). Since most neurons in area M1 are active during action execution, the lack of activity in M1 during observation excluded the possibility that the monkey was making small, undetectable by visual inspection, movements that could account for the F5 activity recorded during action observation. However, evidence for visual responses in the primary motor cortex has been presented repeatedly in the literature. In 1989, Wannier reported M1 neurons that responded to "*…movements of the experimentator's hand while the monkeys were obviously immobile*" (Wannier et al. 1989). They reported 36 such neurons that were located in both the rostral bank of central sulcus and the convexity of precentral gyrus. In a series of papers, Georgopoulos and colleagues reported neurons in the primary motor cortex that responded to visual stimuli such as moving targets or optic flow of different directions and speeds (Merchant et al. 2001; Port et al. 2001; Merchant et al. 2004b, 2004a; Merchant and Georgopoulos 2006). Would these neurons respond to the observation of transitive or even intransitive actions? Since they are supposed to be tuned to low level characteristics of motion, the answer is likely positive. Nevertheless, they are usually ignored in the MirN literature.

Tkach used a visuomotor tracking task to test the visual responses of primary motor cortex (and dorsal premotor) neurons (Tkach et al. 2007). The monkeys were trained to perform a random target pursuit task in which they used a two dimensional manipulandum to move a cursor on a monitor towards different targets. Then, they observed the playback of their own movement on the monitor. The authors found that many of the recorded neurons responded to both the execution and observation tasks. For almost all of these neurons, the preferred direction was the same in execution and observation, indicating a high degree of visual and motor congruency (not typical of grasping MirNs). Using Mutual Information, the authors examined the latency at which the neuronal modulation was most related to the cursor movement. These latencies were not statistically different between active execution and observation: neuronal activity

preceded cursor movement both in execution (65 ± 13 ms, mean ± standard error of the mean) and observation (22.5 ± 24.5 ms). However, in a variation of the observation task, with the cursor moving in constant velocity, without reaction delay between the targets, the neuronal activity followed the cursor movement by -166 +-20.0 ms. Other variations of the observation task showed that the presence of the target is more important than the presence of the cursor for triggering the modulation induced by action observation. These results were in agreement with the claim that the interaction between hand and object is necessary in order to evoke MirN responses during action observation.

In a similar experiment, Dushanova and Donoghue reported that half of the neurons that responded during execution in M1 were also responsive during observation (Dushanova and Donoghue 2010). The intensity of the responses was lower during observation, but the timing was similar (as assessed by comparison of activity onsets and times of peak of firing). In contrast to the results of Tkach, most of the responding neurons (62%) changed their preferred direction between execution and observation. The authors used a Bayesian classifier to decode the cursor movement using neural data. The classifier was successful only when trained and tested in the same task (execution or observation). Cross-decoding success (model training in execution responses and model testing in observation responses or vice versa) was at chance level, even when the population of neurons with similar preferred directions was used.

The two studies described above used a behavioral paradigm different to the one used in typical MirN studies in F5. Thus, the reported responses to observation are referred as “mirror-like” (Rizzolatti and Fabbri-Destro 2008; Rizzolatti et al. 2014). The existence of grasping MirNs in the primary motor cortex was unequivocally shown recently by Vingeswaran (Vigneswaran et al. 2013). They recorded the activity of 132 pyramidal tract neurons and 58% of these (77/132) modulated their activity both during execution and observation. As with the pyramidal tract neurons of F5, the authors reported both facilitation (58%) and suppression (42%) of the activity of MirNs. In contrast to F5, but in agreement to the reports of Tkach and Dushanova, the activity of MirNs during observation was about half of their activity during execution.

1.2.2 Mirror neurons in the dorsal premotor cortex (PMd)

The PMd is not considered part of the mirror neuron system (Rizzolatti et al. 2001; Rizzolatti and Craighero 2004; Rizzolatti and Sinigaglia 2010; Rizzolatti et al. 2014). Neuronal responses in PMd are typically associated with movement planning and execution (Wise et al. 1983; Mauritz and Wise 1986; Kurata and Wise 1988; Wise and Kurata 1989; Crammond and Kalaska 2000) whereas a part of this area, the ventro-rostral sector, contains grasp selective neurons that also respond to visual stimuli (Fogassi et al. 1999; Raos et al. 2004a). Cisek and Kalaska trained monkeys to use a joystick and perform a center out task on a monitor (Cisek and Kalaska 2004). After extensive training in this task, the monkeys were required to simply watch the monitor while the task was performed by the experimenter. Using this paradigm, they recorded 28 'mirror-like' neurons that discharged similarly during execution and observation. They also found that the preferred directions were the same in execution and observation, a finding similar to that reported by Tkach et al (see 1.2.1). Since the monkey did not observe the actual movement required for the task but only the cursor and targets on the monitor, and because the reported neurons discharged hundreds of milliseconds before the onset of the cursor movement, these results were interpreted by the authors as supporting the engagement of monkeys in the mental rehearsal of the task.

1.2.3 Mirror neurons in the inferior parietal lobule

MirNs have also been described in areas PF and PFG of the inferior parietal lobule which are reciprocally connected with area F5 (Petrides and Pandya 1984; Rozzi et al. 2006). MirNs in areas PF and PFG display functional similarities with MirNs in area F5 (Fogassi et al. 2005; Rozzi et al. 2008; Bonini et al. 2010). Fogassi used the grasp-to-eat vs grasp-to-place task (described in 1.1.2) and recorded MirNs that fired selectively for the two conditions (Fogassi et al. 2005). Out of the 41 recorded MirNs, 23 fired significantly more for 'eating', 8 for 'placing' whereas the remaining 10 did not fire differently between the two conditions. Bonini reported similar selectivity in the 28 MirNs recorded in area PFG using the same behavioral task (Bonini et al. 2010). As with the analogous F5 MirNs, these parietal MirNs were associated with the understanding of action goals. Again, the kinematic similarity of the two actions was never assessed, therefore the influence of the different action kinematics on the neuronal discharge cannot

be excluded. Rozzi recorded 124 MirNs from the inferior parietal lobule during the execution and observation of various hand actions (grasp, place, break, hold etc.) and classified them similarly to Gallese (Gallese et al. 1996) as strictly congruent (29%), broadly congruent (54%), logically related (6%) and non-congruent (11%) (Rozzi et al. 2008). In an interesting study, Fujii recorded from the left parietal cortex of two monkeys in a social setting: the animals observed or performed grasps towards food that was available to either both or only one of them (Fujii et al. 2007). The authors report observation related activity only when the two monkeys were close to each other and the food was also available to the observer. Finally, Yamazaki, recorded the activity of 80 MirNs in the inferior parietal lobule, reporting that they have similar properties to premotor MirNs (Yamazaki et al. 2010).

1.2.4 Mirror neurons in the anterior intraparietal area (AIP)

The anterior intraparietal area is occasionally considered to be part of the mirror neuron system (Rizzolatti et al. 2001; Rizzolatti and Craighero 2004; Rizzolatti and Sinigaglia 2010; Rizzolatti et al. 2014). It has strong projections (Luppino and Rizzolatti 2000; Matelli and Luppino 2001; Borra et al. 2008) and similar functional properties to area F5 (Murata et al. 2000; Baumann et al. 2009). Two recent studies demonstrated mirror responses in many grasp related AIP neurons. Pani recorded 104 neurons that responded during both grasping in the light and in the dark in area AIP (Pani et al. 2014). More than half of them (59%) were also active during the observation of a video that displayed the monkey's movements. These videos were recorded from a camera mounted above the animal's head, therefore showing the action in subjective view. To examine the influence of the visual aspects of the observed action on the neuronal response, the authors tested two simpler variations of the observation task in 51 of the neurons responding to observation. In the first, the background and the object were removed, and the monkey watched an isolated hand moving towards the location of the object, with the same speed and trajectory but without the preshaping of the fingers. In the second, the background and the object were intact, but the moving hand was replaced by a scrambled ellipse-shaped body that approached the object to be grasped. All the tested neurons responded to the view of the isolated moving hand and 76% of them also responded to the move of the ellipse shaped body towards the object. Most of these neurons (26/42) were not

effector selective, i.e. they responded equally well when the hand grasped the object or when an arbitrary shape (the ellipse) approached the object. One could argue that the responses in this experiment are not identical to the original "mirroring" because the monkey was not observing another agent. This possibility was excluded in a subsequent study by Murata and colleagues (Maeda et al. 2015). They recorded 54 neurons in area AIP that responded both when the monkey grasped an object and watched a video of its own action. Thirty three of these neurons also responded when the monkey watched the same grasping action in lateral view, performed by the experimenter. Similar to the results of Pani (Pani et al. 2014), 25 of 54 MirNs also responded in a manipulation of the observed video where the object was removed and only the monkey's hand remained.

1.2.5 Mirror neurons in the secondary somatosensory area (SII)

Recently, Hihara reported mirror responses in the secondary somatosensory area SII which is part of the secondary somatosensory cortex located on the ceiling of the lateral sulcus (Hihara et al. 2015). The authors reported 306 neurons that responded to visual stimuli. Eighty nine of them were selective for the observation of actions and 73 responded to the observation of simple moving stimuli. Out of the 89 neurons responding to action observation, 46 also responded during the execution of grasping movements and had no somatosensory receptive fields. Apparently, these neurons fit the definition of MirNs.

2 AIM OF THE STUDY

This study has a two-fold aim: (i) to thoroughly investigate the response properties of MirNs in area F5 of the PMv and (ii) to examine the presence of MirNs in the PMd. For this purpose, we extracellularly recorded the activity of single neurons from the ventral and dorsal premotor cortices of the macaque brain while the animals performed and observed grasping actions.

2.1 What is encoded by MirNs in area F5?

Effective social interaction builds on the ability to grasp the meaning conveyed by the actions of others. The discovery of MirNs (di Pellegrino et al. 1992; Gallese et al. 1996) inspired several researchers, across many disciplines, to investigate the mechanisms of social cognition. Current theories suggest that the meaning of an action is understood because the motor representation of the seen action, activated in the observer's brain, matches the homologous representation in the actor's brain (Rizzolatti et al. 2001). But, what is encoded in the activity of MirNs in ventral premotor area F5 when they are triggered by others' actions? Given the lack of empirical evidence, the answer was obtained through deductive reasoning based on the following propositions: (a) the non-MirNs of area F5 encode the goal of motor acts during execution, (b) non-MirNs and MirNs of area F5 have similar properties during action execution, (c) whatever it is encoded by MirNs during execution, is also encoded during observation. Accordingly, MirNs have been thought to encode the goal of motor acts during observation (Rizzolatti et al. 2014). However, a syllogism is valid and its conclusion true only if the premises are true. So far, the only piece of evidence somewhat supportive of the first premise is the finding that non-MirNs encode movement information in an extrinsic frame of reference, independently of the muscles used (Umilta et al. 2008). Our present findings challenge the second premise and demonstrate that the discharge of MirNs reflects movement kinematics. In light of our findings, a re-evaluation of the role of MirNs is necessary.

2.2 Do MirNs exist in the PMd?

Mirror neuron theory holds that action understanding is sub-served exclusively by the fronto-parietal circuit and specifically the ventral premotor area F5 and the inferior parietal area PF/PFG which, according to Rizzolatti, are the only regions containing MirNs (Rizzolatti et al. 2001; Rizzolatti and Craighero 2004; Rizzolatti and Sinigaglia 2010; Rizzolatti et al. 2014)**.** Until recently, these were the only areas in which the existence of MirNs was investigated using direct methods such as the extracellular recording of single cell activity, mainly by members of the Rizzolatti's team. The discovery of MirNs in the primary motor cortex (area M1 or F1) (Vigneswaran et al. 2013) has not been sufficient to include this area in the MirN system (Rizzolatti et al. 2014). Recent brain imaging findings from our lab indicate that the system activated during the observation of actions of other subjects encompasses the entire brain circuitry that supports action execution, rather than just two cortical areas, which are supposed to be the only ones containing MirNs (Raos et al. 2004a, 2007; Evangeliou et al. 2009; Kilintari et al. 2014; Raos and Savaki 2016). However, the resolution of the neuroimaging methods does not allow to detect whether the same neurons are activated during execution and observation. In other words it is not clear if the common activations are due to the functioning of MirNs. The conclusive demonstration that neuronal responses in any area encode specific behavioral parameters can be obtained only by employing a direct method such as the extracellular recording of single cell activity.

One of the areas activated during both action execution and action observation in our neuroimaging studies is the forelimb representation of the dorsal premotor area F2 (Raos et al. 2007). Other neurophysiological studies have reported the existence of neurons in this area that discharge both when a monkey executes a conditioned task (moving a cursor to capture targets on a computer screen) and when the animal observes the same task executed by the experimenter (Cisek and Kalaska 2004; Tkach et al. 2007). The activity of these neurons has been suggested to reflect mental rehearsal of a learned motor action (acquired through learned stimulus–response associations) rather than processes related to action recognition/understanding, which are the hallmarks of MirNs activity. The facts that (i) the PMd is essential for conditional learning (its lesion impairs movement selection on the basis of a visual contextual cue, (Petrides 1982, 1985;

Passingham and Wise 2012) and (ii) it is unknown whether the identified neurons in PMd would also respond to an interaction between a biological effector and an object (a criterion necessary for their classification as MirNs), have been used to support the interpretation of mental rehearsal rather than action understanding. Thus, although PMd contains neurons displaying execution and observation neural activity, as MirNs do, a detailed description of their properties and their relevance to previously identified MirNs is missing. As a result, it is still debatable whether PMd should be considered part of the so-called mirror neuron circuit and what its exact role in such a network would be. Here, we conclusively demonstrate the existence of MirNs in the PMd. We show that PMd neurons respond during both the execution and observation of grasping movements. Moreover, we compare their properties to those of MirNs of area F5 in terms of timing, grip selectivity and action representation.

3 MATERIALS AND METHODS

3.1 Subjects and recordings

Experiments were performed in two adult female monkeys (Macaca mulatta) weighing between 5 and 7 kg. Animals were purpose-bred by authorized suppliers within the European Union (Deutsches Primatenzentum, Gottingen, Germany). All experimental protocols were approved by the Veterinary authorities of the Region of Crete and complied with the European (directive 2010/63/EU and its amendments) and National (Presidential Decree 56/2013) laws on the protection of animals used for scientific purposes. For immobilization of the monkeys, a metal bolt was surgically implanted on their head with the use of mandibular plates secured on the bone by titanium screws (Synthes, Bettlach, Switzerland). To monitor eye movements, a scleral search coil (AS633 Cooner wire, Chatsworth, CA) was sutured on the sclera (Robinson 1963; Judge et al. 1980) After completion of the training, a recording chamber was implanted over the cortical area of interest. The estimation of the coordinates for the implantation of the chamber was based on anatomical landmarks of the publicly available atlas of McLaren (McLaren et al. 2009). This atlas is an MRI-based atlas derived from the average of 112 rhesus macaques. Surgical procedures were performed under general anesthesia and aseptic conditions.

The recordings were carried out using glass-coated tungsten microelectrodes (Alpha Omega, Israel) inserted into the dura perpendicularly to the cortical surface (impedance 0.5-1.5 MΩ, measured at 1 kHz frequency; Bak Electronics, MD, USA) with an oil hydraulic micromanipulator (Narishige International, UK). The electrode signal was amplified (gain, 10,000), band-pass filtered (1 Hz to 8 kHz) and recorded digitally at 25 kHz (Cambridge Electronic Design, England) while being monitored on an oscilloscope. The signal was band-pass filtered (0.3 to 5 kHz) offline and spike sorted using the Spike2 software (Cambridge Electronic Design, England). Spike occurrences were stored as binary time series at a 1 ms time resolution.

Stainless steel recording chambers were implanted stereotactically over the left hemisphere of each monkey (contralateral to the moving forelimb). The chamber provided access to a large cortical territory which included ventral and dorsal premotor cortical areas and extended from the primary motor cortex (area F1), caudally, to the posterior part of the frontal eye fields (FEF), rostrally. After chamber implantation, the accessible cortical area was functionally explored by means of single neuron recordings and intracortical microstimulation to assess the location of areas F1, F2, F4, F5 and FEF. The criteria used to characterize the different areas were the following: area F1, excitable with low-threshold currents (<25 μA, train of cathodal pulses, train duration: 50 ms, pulse width: 0.2 ms, pulse frequency 330 Hz), vigorous discharge during active movements, responses to somatosensory stimulation; area F2, excitable with currents of higher intensity (>25 μA), vigorous discharge before and during active movements, located medial to area F5; area F4, rostral to the F1 hand field, bimodal neurons with large tactile receptive fields on the face and body and visual receptive fields mostly driven by moving stimuli in register with the tactile receptive fields encountered frequently, neurons discharging during proximal forelimb and axial movements; area F5, further rostrally, responses to the observation of actions, neurons discharging in association with goal-directed hand movements; FEF: vigorous discharge during saccades.

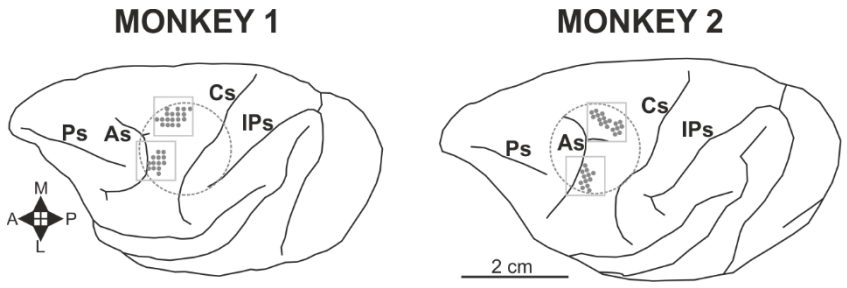

Figure 3.1 Side view of the left hemispheres of Monkey 1 and Monkey 2 . Grey dots indicate loci of penetrations in the dorsal and ventral premotor cortex of each hemisphere with a spatial resolution of 1 mm to avoid cluttering (the spacing of the actual penetrations was 0.5mm). The regions delimited by the grey rectangles medially and laterally to the spur of the arcuate sulcus contain the PMd and F5 penetrations, respectively. Dashed circles indicate the perimeters of the recording chambers. As, arcuate sulcus; Cs, central sulcus; IPs, intraparietal sulcus; Ps, principal sulcus.

Informal testing preceded the selection of neurons tested with the behavioral paradigm. The activity of each recorded neuron was correlated with the execution of

active movements as well as with somatosensory and visual stimulation. Active movements consisted of reaching for and grasping objects of different size, shape and orientation, presented in all space sectors, or trunk movements such as orienting towards interesting stimuli or avoiding threatening stimuli.

3.2 Behavioral apparatus and paradigm

Each monkey was seated in front of the behavioral apparatus which was a rotating turntable on which 3D geometrical solids were accommodated. Depending on whether the monkey or the experimenter was performing the task, the apparatus was positioned in front of the monkey at a distance of 25 cm or 45 cm, respectively. The topographic arrangement of the object, the monkey and the actor (experimenter) in execution and observation tasks is schematically illustrated in Figure 3.2A. The objects (illustrated in Figure 3.3) were presented one at a time, in blocks of trials, always in the same central position. The following objects and grips were used: large sphere (diameter: 40 mm), whole hand prehension with all the fingers wrapped around the object and the palm in contact with the object; cylinder (length: 40 mm, base diameter: 20 mm), finger prehension, using all fingers but the thumb; ring (diameter: 15 mm), hook grip with the index finger inserted into the ring; cube in vertical groove (side: 10 mm), advanced precision grip using the pulpar surface of the distal phalanx of the index finger opposed to the pulpar surface of the distal phalanx of the thumb. The monkeys were trained to use identical hand postures for grasping the same objects and the overall similarity of the grips performed by the two monkeys was confirmed by comparing the video images of their hand postures during grasp. Eye movements were measured with the scleral search coil technique and recorded at 500 Hz (Remmel 1984). Both behavioral tasks were managed by custom built software and all behavioral events were synchronously stored with the neural data.

Execution task: At the beginning of each trial of the execution task, a LED above the selected object turned on and the monkey was required to fixate it and place its hand on a push button. Following a fixation period (delay), a dimming of the LED signaled the onset of the reaching and grasping movement. The monkey was required to reach for, grasp, pull and hold the object while maintaining fixation. After a holding period, the LED was turned off and the monkey had to release the object in order to get the reward. The monkey

was required to keep its gaze within a circular window of 10^{o} diameter, centered on the object. The delay and hold period lengths were randomly chosen from uniform distributions between 800 to 1200 ms and 400 to 900 ms, respectively.

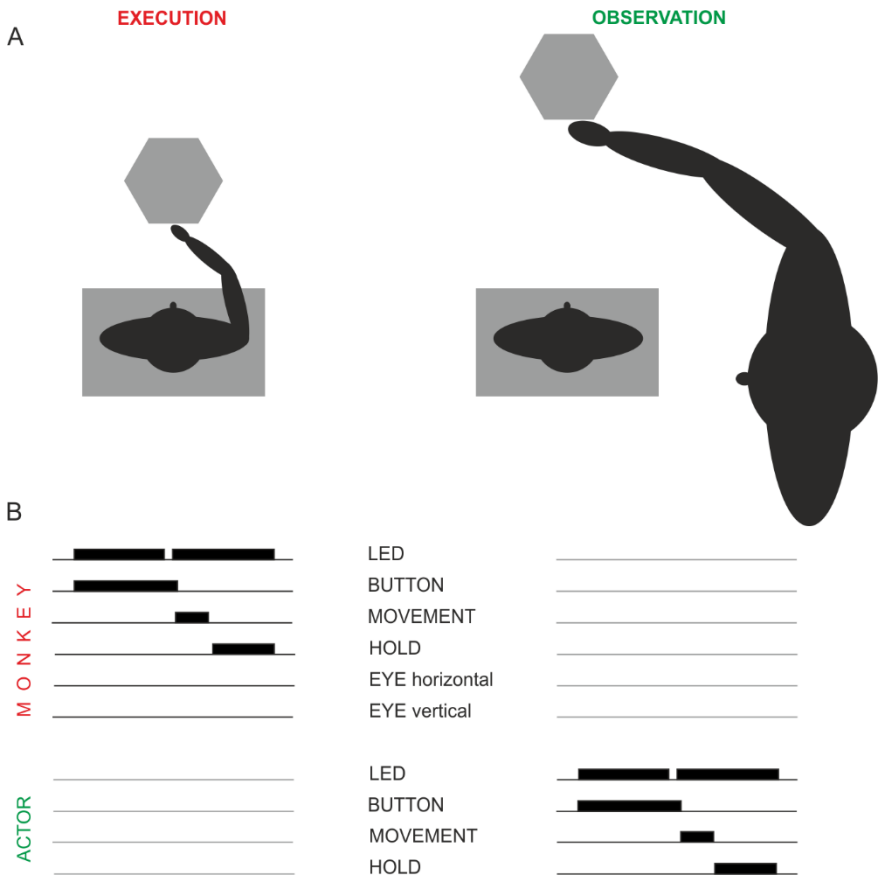

Figure 3.2 . Behavioral task. (A) Topographic arrangement of the apparatus, the monkey and the actor (experimenter) in execution and observation tasks. (B) Diagrammatic representation of the time sequence of the task events in the two tasks. Upward deflection: on; downward deflection: off.

Observation task: The experimenter performed reaching to grasp actions with the right hand, on the same objects, while standing next to the animal on its right side. Transport and hand preshaping of the experimenter's movement were visible to the monkey. The sequence of the task events in the observation task was identical to that of the execution task. The duration of the object presentation/delay period preceding movement and of the holding period were drawn from the same uniform distributions that were used for the execution task. However, the LED above the object was not turned on for the entire duration of the trial and the experimenter was instructed from cues appearing on a screen

out of the monkey's view. In addition to the four transitive actions, the monkey also observed a fifth, intransitive action. The task was performed in blocks of 10 trials for the intransitive as well as for each one of the four transitive actions. When the monkeys observed the intransitive action, the carousel of the behavioral apparatus was positioned in a way that a side with no object mounted on was presented to the monkey. In other words, the monkey knew from the beginning of the block of trials that the actions to follow were going to be intransitive. The intransitive action consisted of an out-reaching non object-directed movement with extended wrist and fingers towards the side of the carousel. A diagrammatic representation of the time sequence of the task events in execution and observation is illustrated in Figure 3.2B.

In the observation task the animals were trained to observe the experimenter performing reaching-to-grasp actions and were rewarded at the end of each one of them. Contrary to the fixation requirement used in the execution task, no restriction was posed on the animals' oculomotor behavior during observation. To verify the animals' engagement in the task, an eye position window of 9.5^{o} in diameter, centered either at the object (transitive actions) or the end point of the intransitive action was used.

3.3 Tracking of actions

Arm and hand movements of the observed actions were tracked using an extension of the electromagnetic method for eye movement recording (Remmel 2006) in 4 different sessions, each consisting of 30 repetitions for each action (total data set: n=120). Small coils, mounted either on elastic bands or on a custom made glove, were positioned on the arm, forearm, hand dorsum, thumb and index finger of the experimenter. Three alternating magnetic fields in the X, Y, and Z directions (at 48, 60, and 80 KHz) were generated by field coils with a side length of 1.5 m (Remmel Labs, TX, USA). The three magnetic fields induce three voltages into each of the small coils by Faraday's law of induction. As the action unfolds, the induced voltages vary with the position and orientation of the small coils. These voltages can be used to estimate the angles of each joint. The following rotation angles were recorded: shoulder joint (3 DoF: flexion/extension in sagittal or horizontal plane, abduction/adduction); elbow joint (2 DoF: flexion/extension, wrist pronation/supination); wrist joint (2 DoF: flexion/extension, abduction/adduction); index finger metacarpo-phalangeal joint (2 DoF:

flexion/extension, abduction/adduction), proximal inter-phalangeal joint (1 DoF: flexion) and distal inter-phalangeal joint (1DoF: flexion); thumb carpal-metacarpal joint (2 DoF: flexion/extension, abduction/adduction), metacarpo-phalangeal joint (1 DoF: flexion) and inter-phalangeal joint (1 DoF: flexion). All coil voltages were digitized and stored at 500 Hz. Bone endpoint trajectories (Figure 3.3) were calculated using forward kinematics. The travelled distance of each endpoint is the magnitude of its position vector as measured from the initial location. Endpoint speed is the rate of change of the travelled distance. The aperture is defined as the Euclidean distance of the endpoint of the index to the endpoint of the thumb.

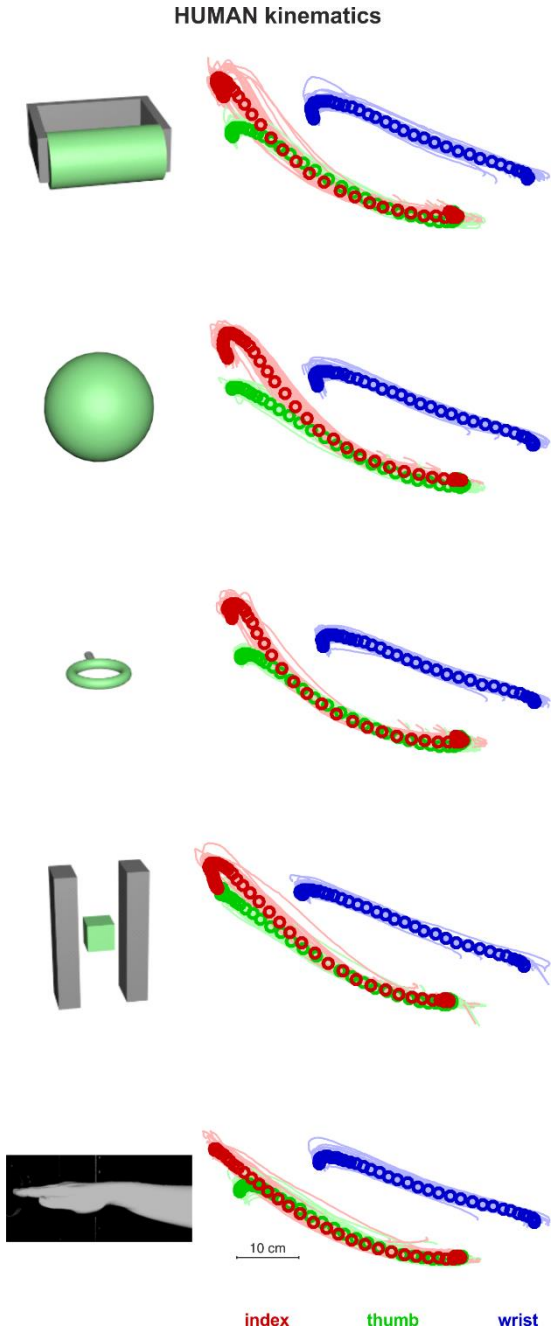

Figure 3.3 Kinematics of the observed actions. Mean trajectories of the ulnar styloid process (blue), tip of the index finger (red) and thumb (green) during object grasping (transitive actions) and the out-reaching non object-directed movement (intransitive action) in a representative session (n=30). Each circle represents the mean instantaneous position of an endpoint with a resolution of 12 ms and each line represents the path of each marker at different trials.

The actions performed by the monkey were recorded at 120 frames per second by a digital camera positioned perpendicular to the trajectory of the forelimb movement. Videos were recorded in 12 different sessions, each consisting of 10 repetitions for each

action (total data set: n=120 for each action). The videos were background subtracted to identify the area occupied by the monkey's moving arm, forearm and hand in each frame. A simple skeletal model of three parts (arm, forearm and hand) was then fitted in each frame. This model allowed the reconstruction of the monkey's hand trajectory during the movements. Our setup did not permit the use of a more detailed hand model that would include the individual fingers due to occlusion of fingers that occurred during the hand-object interactions.

3.4 Analysis of neural activity

Only neurons recorded for at least 8 trials for each action in each task were included in the dataset and were further analyzed. For each trial, spikes were aligned to the movement onset and firing rates were computed in a sliding 200 ms window with a slide step of 5 ms. To account for the varying timings in the firing of the neurons, a dynamic criterion, instead of the behavioral events, was used for the definition of the activity epochs. Consequently, three epochs were defined for each action in each task: *baseline epoch*, 500 ms before trial start; *modulation epoch*, at least 40 consecutive bins (200 ms) with activity above the mean plus one standard deviation of the baseline epoch; *burst epoch*, at least 12 consecutive bins (60 ms) with activity above the mean plus one standard deviation of the modulation epoch (Figure 3.4). Thus, the start and end time of the modulation and burst epochs varied across actions, tasks and neurons. The burst epoch activity, capturing the period displaying the peak activity, was used to rank the transitive actions from preferred to non-preferred for each neuron and task.

The net response of each neuron for each task and action was obtained by subtracting the corresponding baseline activity from each 200 ms sliding window. To account for the different activity amplitudes of the neurons in the population, the obtained net response in each window was divided by the response in the window displaying the highest activity of the neuron across actions and tasks (maximum normalized activity = 1). To calculate the population activity, the obtained net normalized responses of each rank (preferred to non-preferred) were averaged separately for each task. Selectivity among the ranked transitive actions, at the population level, was assessed by a one-way ANOVA ($p<0.01$), calculated separately in each bin. To define the period at which at least one transitive action differed from the intransitive one, the same procedure was used for

the comparison of all five (ranked transitive and the intransitive) actions, followed by a Dunnett test. The intransitive action was used as the single control group in this test.

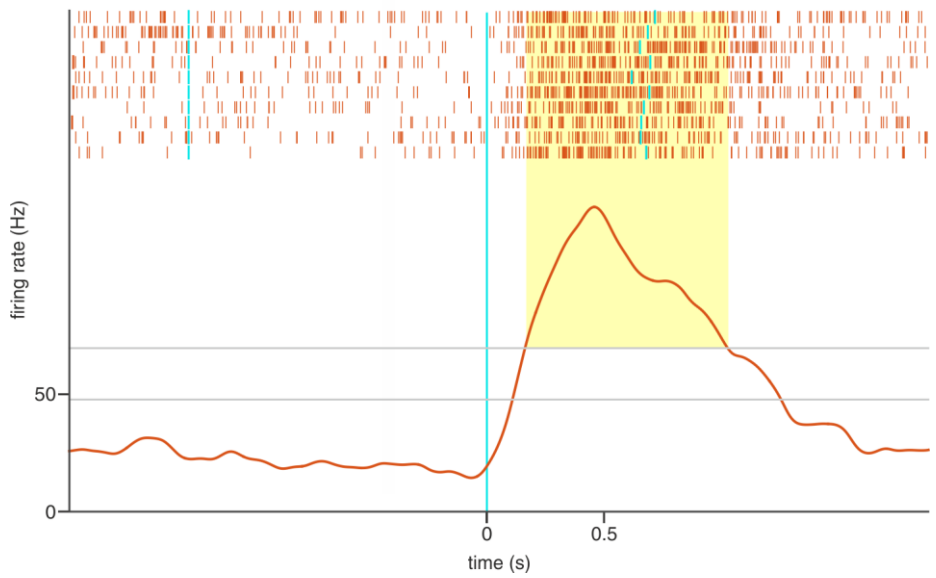

Figure 3.4 Burst epoch definition. Double thresholding process for the definition of the modulation and burst epochs. Activity of one F5 mirror neuron during the observation of a grasping action, presented in spike rasters and corresponding firing rate, both aligned at movement onset (cyan vertical line). In the rasters, cyan marks on the left indicate the start of the trial and cyan marks after the movement onset indicate the end of movement; brown marks indicate action potentials and each line is a different trial. The lower grey horizontal line depicts the threshold for the modulation epoch (baseline epoch mean plus one standard deviation) and the upper grey horizontal line depicts the threshold for the burst epoch (modulation epoch mean plus one standard deviation). The yellow rectangle highlights the burst epoch on the rasters.

3.5 Neural activity representational similarity analysis

Representational dissimilarity matrices (RDMs) were used to compare the representations between (a) execution and observation activity of MirNs (either at the population or single neuron level), (b) execution activity of MirNs and non-MirNs (c) the activity of F5 and PMd MirNs or non-MirNs during execution or observation and (d) execution activity of non-MirNs from two datasets. The RDM (Figure 3.5) is a distance matrix, that is, a two-dimensional array containing the pairwise distances between the elements of a set, which in our case was the set of actions. The Euclidean distance between each action pair is given by:

$$d(a_1, a_2) = \sqrt{\sum_{i=1}^{n}(a_{1i} - a_{2i})^2} \quad (1)$$

where a_k is action k $(k = 1,..,4)$ and n is the number of neurons (neural RDM). For example, when computing the RDM of a neural population during a given task, a_{ki} equals to the normalized burst epoch activity of neuron i during action k. The RDM of a single neuron is calculated similarly. By definition, RDMs are symmetrical and the entries of the main diagonal are all zero.

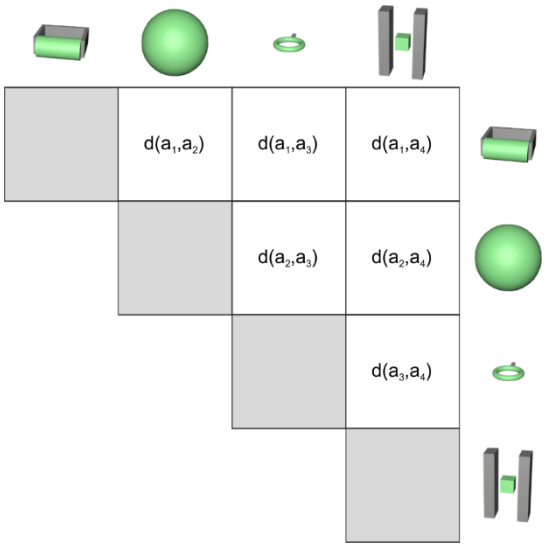

Figure 3.5 Representational Dissimilarity Matrix. Illustration of a generic representational dissimilarity matrix. In each cell, $d(a_1, a_2)$ is the Euclidean distance between actions a_1 and a_2 and can be calculated in either neuronal or kinematics spaces.

The relation between two dissimilarity matrices can be inferred by classical correlation measures. However, such methods assume independent measurements for the two variables and this cannot be assumed for pairwise distances as they are derived from common points. To overcome this, the significance of the correlation coefficient between a pair of RDMs was assessed by the Mantel test (Mantel 1967). Briefly, correlation between RDMs was evaluated by calculating the Pearson product-moment correlation coefficient between the upper triangular parts of each matrix. Then, the correlation null distribution was estimated by permuting the rows and columns of the distance matrix 1000 times (equivalent to permuting the action labels). The significance of the observed correlation coefficient is equal to the proportion of such permutations that result to a coefficient higher than the observed one. To verify that the resulting correlations were not disproportionately affected by certain individual neurons, the distributions of the r values were approximated by 1000 bootstrap resamplings (that is, with replacement) of the neural populations under consideration.

To investigate representational similarity across time, the same procedure was used to evaluate correlations between RDMs at leading, synchronous and lagging time points along the trial. Neural responses were aligned at the movement onset, binned in 50 ms sliding windows with a step of 10 ms and net normalized. For example, to test the correlation between the activity of MirNs during observation and execution, the RDM of each observation activity bin was separately correlated with the RDMs of all the execution activity bins. These correlations can be visualized in two dimensional heat maps (referred as temporally detailed maps) that illustrate periods of representational similarity across time.

3.6 Representational similarity analysis between neural activity and action kinematics

The same analysis was used to investigate representational similarity between the stimulus properties (kinematics) and the activity of MirNs during observation or execution. The position and speed of the ulnar styloid process described the transport of the hand. The aperture and its rate of change described the hand preshaping. The four kinematic features were separately normalized to a range of 0 to 1 and binned in 50 ms sliding windows with a step of 10 ms. Kinematics normalization allows the use of combinations of kinematic features (where each feature is regarded as a different dimension of the kinematics space) and at the same time does not affect individual feature correlations.

The construction of the kinematics RDM for a given bin is similar to the construction of the neural RDM and is done by using eq.1 where n is the number of kinematic features (either one for the individual features or four for the full kinematics space). Similarly to the temporally detailed neural maps, we evaluated correlations between the kinematics and neural RDMs at leading, synchronous and lagging time points along the trial. This way, two dimensional heat maps of RDM correlations were constructed for each kinematic feature and for the full kinematics space (resulting in a total of five maps).

In each map, the count of points with significant correlations in a certain reference period (score) was used to quantify the degree of representational similarity between each kinematic feature f and the activity of MirNs:

$$c_f = \sum_i \sum_j [p_{ij} < 0.05] \quad (2),$$

where [] is the Iverson bracket ($[A] = \begin{cases} 1 \; if \; A \; is \; true; \\ 0 \; otherwise; \end{cases}$) and p_{ij} is the p value of the comparison between points i and j.

The reference periods (denoted by the range of i and j in eq. 2) were constructed to contain all the points with significant correlations in all five maps of each region (F5 or PMd). For the neural population of area F5, this region extended from the diagonal to the end of the movement in the axis of the neural space (abscissa) and from 150 to 450 ms after the movement onset in the axis of the kinematics space (ordinate; total number of points = 1132). For PMd, it extended from start to the end of the movement in the axis of the neural space (abscissa) and from 150 to 450 ms after the movement onset in the axis of the kinematics space (ordinate; total number of points = 1798). The regions are outlined in Figure 4.11 and Figure 4.25. To verify whether the score of each feature was higher than chance, the same procedure was used to compare the kinematics representations with the neural RDMs that were now calculated for the baseline activity. For each feature, the baseline and movement activity scores were compared with the Fisher's exact test. The movement activity scores of the features were compared with each other with a Tukey-type multiple comparison test for proportions (Zar 1999).

To explore the effect of the neuronal population size on the representational similarity between the kinematics and the responses during observation and to verify that the resulting correlations were not disproportionately affected by certain individual neurons, neuronal populations of different sizes (n<=120 for area F5 or n<=140 for PMd) were created by random selection with replacement. The representational similarity analysis was run for each population size and the mean r of a reference period was estimated. This process was iterated 1000 times to approximate r distributions for each subpopulation. The r distributions of the different neuronal populations were compared with an ANOVA followed by a Tukey-Kramer post hoc test. The reference period for this analysis extended from the diagonal to the end of the movement in the axis of the neural

space (abscissa) and from 250 to 350 ms after the movement onset in the axis of the kinematics space (ordinate) for area F5. For PMd, it extended from the start to the end of the movement in the axis of the neural space (abscissa) and from 100 to 500 ms after the movement onset in the axis of the kinematics space (ordinate). Each reference period included all the points with significant correlations in the map of the original population of each area.

3.7 Selectivity assessment

A preference index (PI) was computed for each neuron and task according to the formula $PI = \left(n - \left(\sum r_i / r_{pref}\right)\right)/(n-1)$ where n is the number of actions, r_i the burst activity for action i, and r_{pref} the burst activity for the preferred action The PI ranges between 0 and +1; a value of 0 indicates the same amplitude of response for all actions and a value of 1 indicates preference for only one action. Statistical significance of the selectivity was assessed by a permutation test. The PI null distribution was approximated by permuting the action labels of the trials 1000 times and computing the corresponding PIs. A PI was deemed significant if the observed value lied in or above the top 5% of the null distribution.

To assess the selectivity between the four transitive actions through time, a one way ANOVA was performed at each time bin, comparing the means of the four different actions. The onset of transitive action selectivity was defined as the time stamp of the first of at least 12 consecutive bins (60 ms) with p-value<0.01. To define the time at which at least one transitive action differed from the intransitive one, the same procedure was used for the comparison of all the five (transitive and intransitive) actions, followed by a Dunnett test with the intransitive action being the single control group.

3.8 Amplitude comparison

To quantify the difference between the amplitude of the response to the observation of transitive and intransitive actions, the following amplitude index was calculated: $AI = \frac{r_{tr} - r_{intr}}{r_{tr} + r_{intr}} \cdot \frac{max(r_{tr}, r_{intr})}{r_{pref}}$ where r_{intr} is the burst activity for the observation of the intransitive action, r_{tr} is the burst activity for the observation of a given

(preferred or non-preferred) transitive action and r_{pref} is the burst activity for the preferred action (over all five actions). The AI ranges between -1 to +1 with negative values corresponding to a higher response for intransitive than transitive action and vice versa. To quantify the difference between the amplitude of the response of MirNs during action observation and action execution, the following task amplitude index (tAI) was calculated: $tAI = \frac{r_{per} - r_{obs}}{r_{per} + r_{obs}}$ where r_{per} is the mean activity for the execution of the four grasping actions and r_{obs} is the mean activity for the observation of the four grasping actions. The tAI ranges between -1 to +1 with negative values corresponding to a higher response for observation than execution and vice versa.

3.9 Gaze-related modulation of activity

To investigate the dependence of premotor neurons on gaze position we performed the analysis described by Boussaoud et al (Boussaoud et al. 1998) and Cisek and Kalaska (Cisek and Kalaska 2002) on 122 PMd and 120 F5 MirNs of our dataset during both execution and observation conditions. Briefly, gaze fixation episodes with a duration of at least 100 ms were identified as time periods in which eye speed did not exceed $50\ °/s$. Long lasting episodes were divided in fragments of 100 ms. For each 100 ms fragment of a fixation episode the average gaze direction and the average firing rate was calculated within each epoch of interest. Three epochs were considered for this analysis: an 800 ms period prior to movement onset, the movement epoch from movement onset to the beginning of object pulling and the holding epoch from the beginning of object pulling to object release. The gaze related modulation was studied separately for each cell, epoch and condition using planar regression (Boussaoud et al. 1998) in which the neuronal activity in each fragment of a fixation episode was expressed as a function of the horizontal and vertical components of gaze direction in the two-dimensional linear regression model: response (spikes/s) = c + a*$gaze_{horizontal}$ + b*$gaze_{vertical}$ (where c is the intercept, a and b the slopes along the horizontal (X) and vertical (Y) axes, respectively).

The strength of the modulation was assessed by the coefficient of determination (r^2) of the regression ($p<0.01$). The results of this analysis are summarized in Table 3.

3.10 Location of recordings

Histological analysis was carried out in both monkeys. Before perfusion, pins were inserted at the periphery of the chamber and at points delimiting the studied area. The animals were euthanized with a lethal dose of barbiturate [pentobarbital sodium (Dolethal), 50 mg/kg] and perfused transcardially with buffered saline followed by fixative. The brain was then removed and photographed with and without a grid superimposed over the area of the chamber. Penetration entry points were estimated and transferred on the drawings of the hemispheres Figure 3.1.

4 RESULTS

4.1 Mirror neurons in area F5

4.1.1 Neuronal properties of MirNs and non-MirNs in area F5

Two monkeys were trained initially to grasp objects with specific grips (execution task), and subsequently to observe the experimenter performing the same object-directed reaching-to-grasp actions as well as an outreaching intransitive movement (observation task). We recorded the activity of 192 neurons from a cortical strip extending 3 mm in the anteroposterior and 4 mm in the mediolateral dimensions, oriented parallel to the inferior limb of the arcuate sulcus, and thus corresponding to the ventral premotor area F5 (Figure 4.1). The majority of the recorded neurons (77 %) were located in the first 3 mm of cortex, as measured from the most superficial point at which neuronal activity was encountered. Neurons from both monkeys displayed similar properties and thus data from both monkeys were combined.

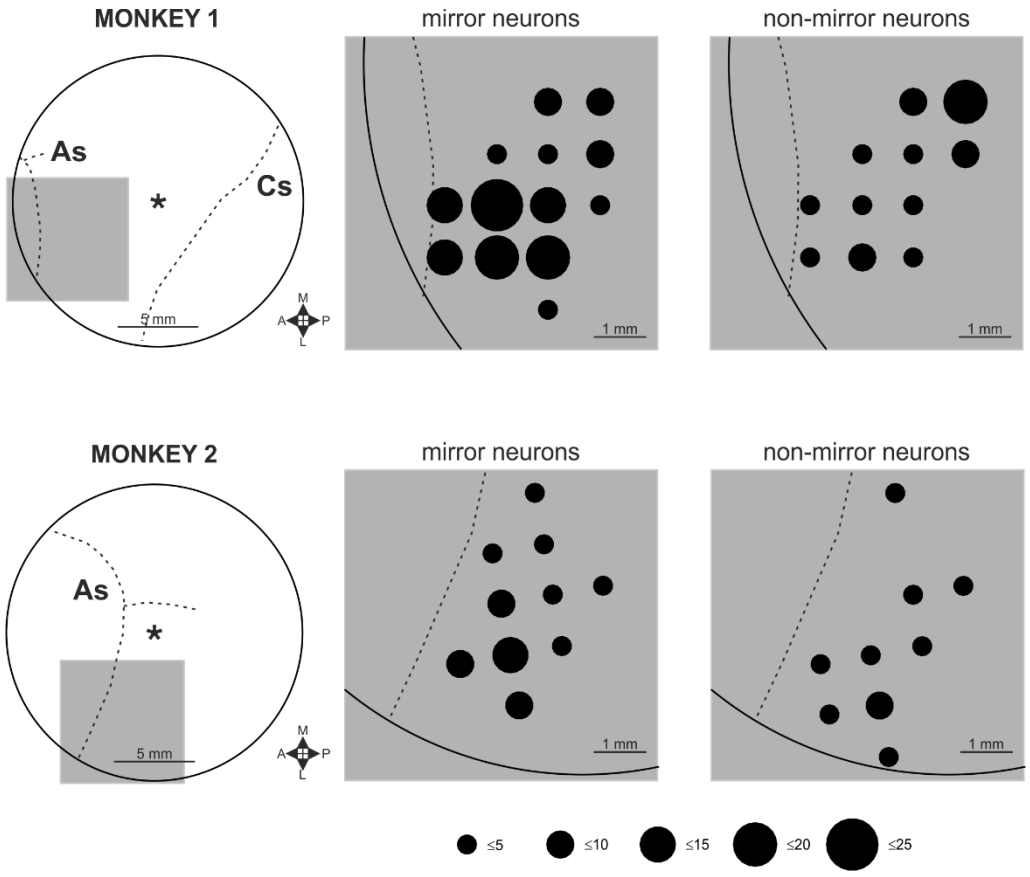

Figure 4.1 Location of recorded neurons in F5. Map of the cortical area exposed under each recording chamber. Grey rectangles at the left panels include the area of each hemisphere from which neurons were recorded. These areas are enlarged in the central and right panels of the figure and the number of mirror and non-mirror neurons recorded in each location is illustrated by the size of the black filled circles. Dashed lines indicate the location of the arcuate sulcus (AS) and the spur based on penetration lengths and functional criteria. The asterisks at the left panels indicate the chamber's centers (anteroposterior stereotaxic coordinates at 16 in monkey 1 and 21 in monkey 2). A, anterior; L, lateral; M, medial; P, posterior.

Statistical analysis demonstrated that 122 neurons responded to both tasks whereas the remaining 70 were active only in the execution task. For each neuron of the population, we calculated a task amplitude index (tAI) to quantify the difference between discharges for execution and discharges in response to observation. The distribution of the tAIs of all neurons (Figure 4.2) was not unimodal (Hartigan's Dip Test, null hypothesis of unimodality rejected, p=0), thus indicating that the cells we found belong to two different groups. The 122 neurons that were active in both tasks were classified as MirNs, whereas the remaining 70 were classified as non-MirNs.

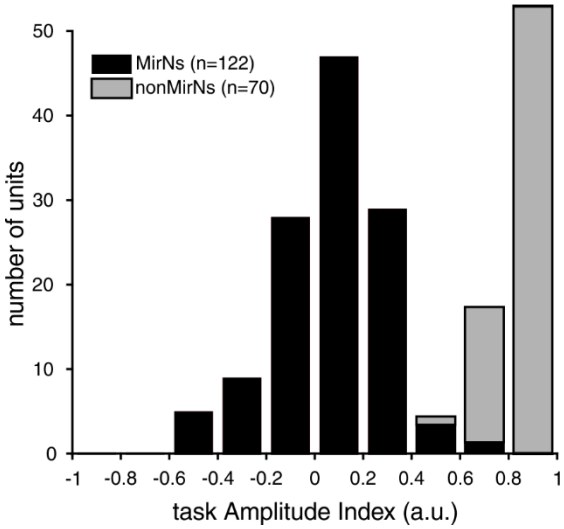

Figure 4.2 Amplitude difference between execution and observation responses of F5 neurons. Histogram of the task amplitude index of the recorded neurons.

Single-neuron and population activity was selective for actions during both execution (MirNs and non-MirNs) and observation (MirNs, Figure 4.3 and Figure 4.4). Both MirNs and non-MirNs displayed action selectivity during execution, and this was already present at movement onset (Figure 4.5). The selectivity of MirNs was lower during observation than during execution, and began 350ms after movement onset (median of selectivity onset distribution; Figure 4.6, A and B). Moreover, activity bursts of MirNs and non-MirNs begun at the same time but the maximum activity and burst end occurred earlier in non-MirNs than in MirNs. The above temporal characteristics of MirN activity occurred later in observation than in execution (Figure 4.6, C to E).

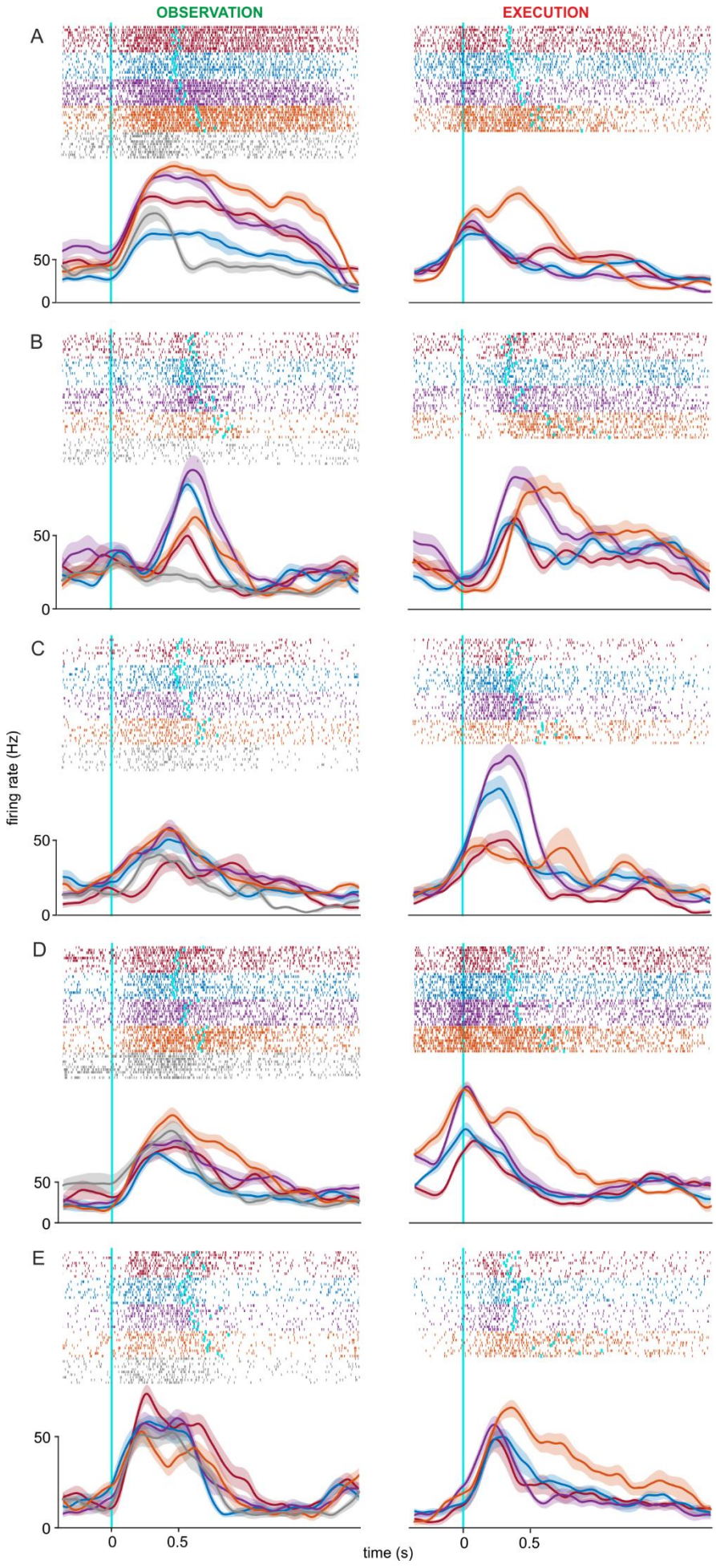
OBSERVATION
EXECUTION
A
B
C
D
E
50
0
firing rate (Hz)
0
0.5
time (s)

Figure 4.3 Examples of F5 mirror neurons. Activity of five F5 mirror neurons (A to E) during observation (left) and execution (right) presented in spike rasters and firing rates, both aligned at movement onset (cyan vertical line). In the rasters, cyan marks indicate the end of movement; colored marks indicate action potentials during observation/execution of a grip (from top to bottom: finger prehension for the cylinder, whole hand prehension for the sphere, hook grip for the ring, advanced precision grip for the cube in groove) and grey marks indicate action potentials during observation of the intransitive action. Firing rates follow the same color code and the bands represent mean ± standard error of the mean (SEM).

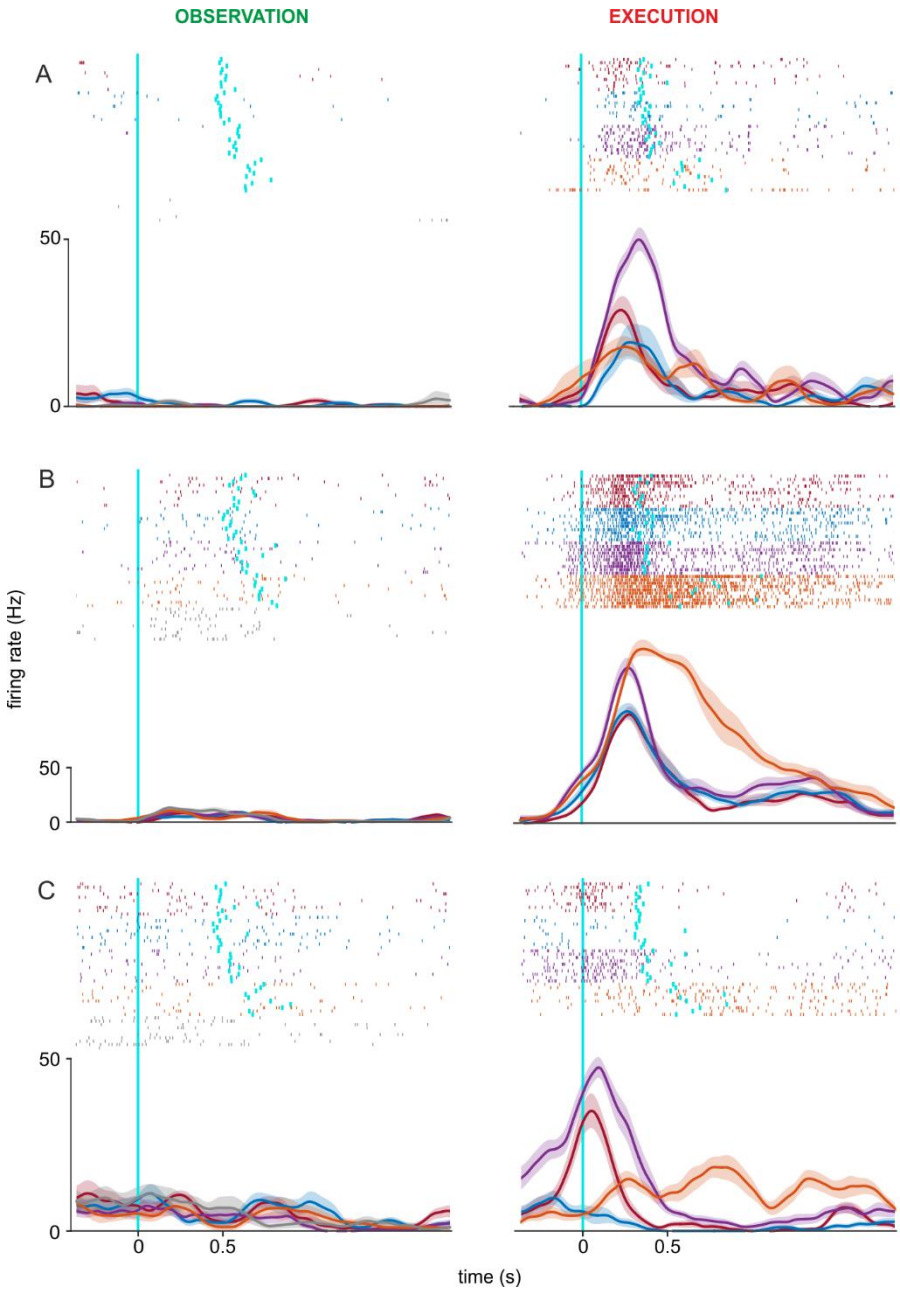

Figure 4.4 Examples of F5 non-mirror neurons. Activity of three F5 non-mirror neurons during observation and execution. Neuron in A was recorded simultaneously with the neuron in Figure

4.3C and neuron in B was recorded simultaneously with the neuron in Figure 4.3E. All conventions as in Figure 4.3.

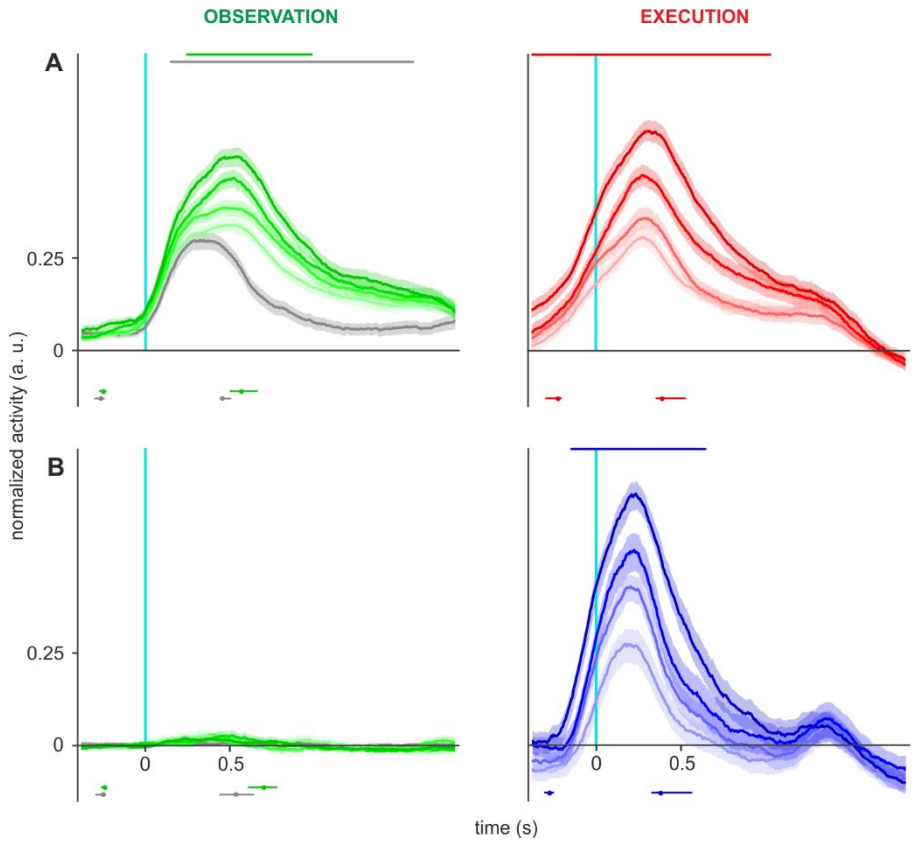

Figure 4.5 Population activity of area F5 during action observation and execution. Ranked net-normalized population activity of mirror (A) and non-mirror (B) neurons during observation (left) and execution (right), aligned at movement onset (cyan vertical line). Ranking of transitive actions is indicated by colored shading (preferred to non-preferred, dark to light). The grey line and band indicate the activity during observation of the intransitive action. Colored marks and horizontal lines below the population activity denote the median and the 25th to 75th percentile times of the behavioral events (from left to right: go cue, movement end). Horizontal lines at the top of each panel denote the period displaying statistically significant selectivity among transitive actions (colored) and between transitive and intransitive actions (grey). Other conventions as in Figure 4.3.

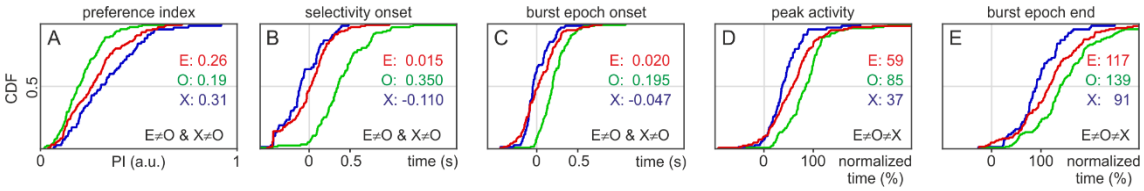

Figure 4.6 Activity characteristics of MirNs and non-MirNs in area F5. (A) Preference index (arbitrary units, a.u.). (B) Onset of action selectivity measured from movement onset. (C) Start of burst epoch measured from the movement onset. (D) Time of peak activity expressed as

percentage of movement duration. (E) End of burst epoch expressed as percentage of movement duration. In all panels, curves represent the empirical cumulative distribution function of MirN activity characteristics during execution (red) and observation (green) as well as non-MirN activity characteristics during execution (blue). Distribution medians and statistical comparisons (Kruskal-Wallis followed by Bonferroni, $p<0.05$) are reported in each panel (E: MirNs execution, O: MirNs observation, X: non-MirNs execution).

4.1.2 Response of F5 MirNs during the observation of intransitive actions

We found that 110/122 (90%) of the recorded MirNs responded to the observation of both intransitive and transitive actions. The response of all recorded MirNs to the observation of transitive and intransitive actions is shown in Figure 4.7 and Figure 4.8. The responses to the intransitive action shared similar characteristics with those to the transitive ones. The onsets of the burst activity for transitive and intransitive actions coincided. The activity seemed to reach its maximum and end earlier during the observation of intransitive than transitive actions (Figure 4.9, A to C) but this difference was abolished when the timing of these temporal landmarks was normalized to the duration of the movement (Figure 4.9, D to F). In addition, both the amplitude and the slope of the response to the observation of the intransitive action were equal to those of the observation of the non-preferred transitive action. (Figure 4.5A, Figure 4.9, G and H). These results suggest that the activity of MirNs during observation may be modulated by the kinematics of the observed actions. For this purpose we recorded the kinematics of both transitive and intransitive observed actions (Figure 3.3). Several kinematic features of the intransitive action were different from those of the transitive actions. We found that movement duration, travelled distance and maximum speed of wrist, maximum aperture of grip as well as maximum speeds of opening and closing of grip for the intransitive action were different from those for the transitive ones. The four transitive actions also differed with each other in terms of the examined kinematic features (Figure 4.10).

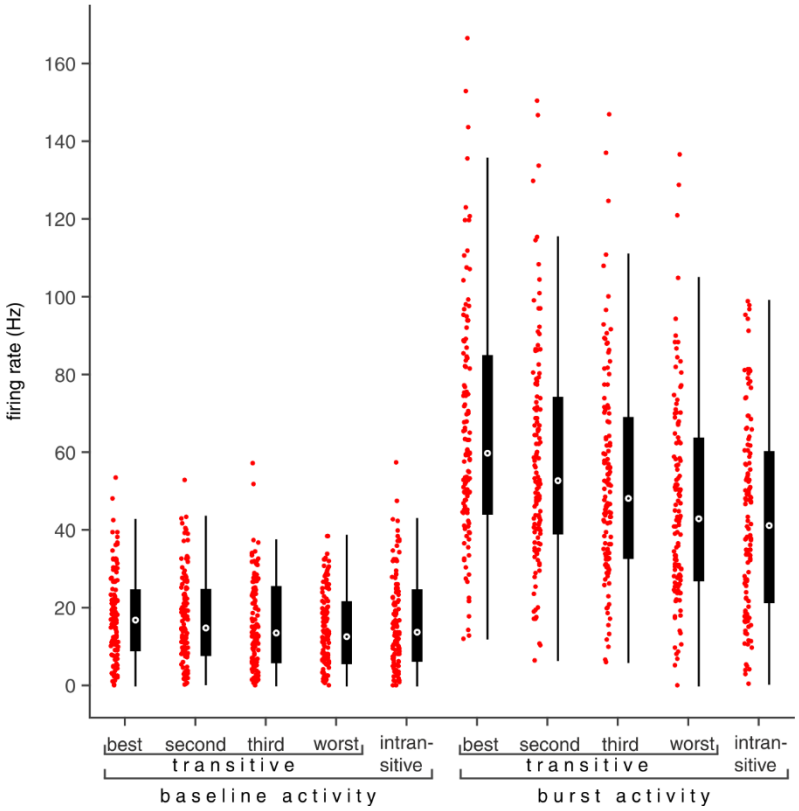

Figure 4.7 Baseline and burst activity of F5 MirNs during observation of actions. Box plots showing baseline and burst activity of MirNs during observation of four transitive and one intransitive actions. The circle in the box marks the median, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points not treated as outliers. The red points next to each box plot represent the activity of individual MirNs

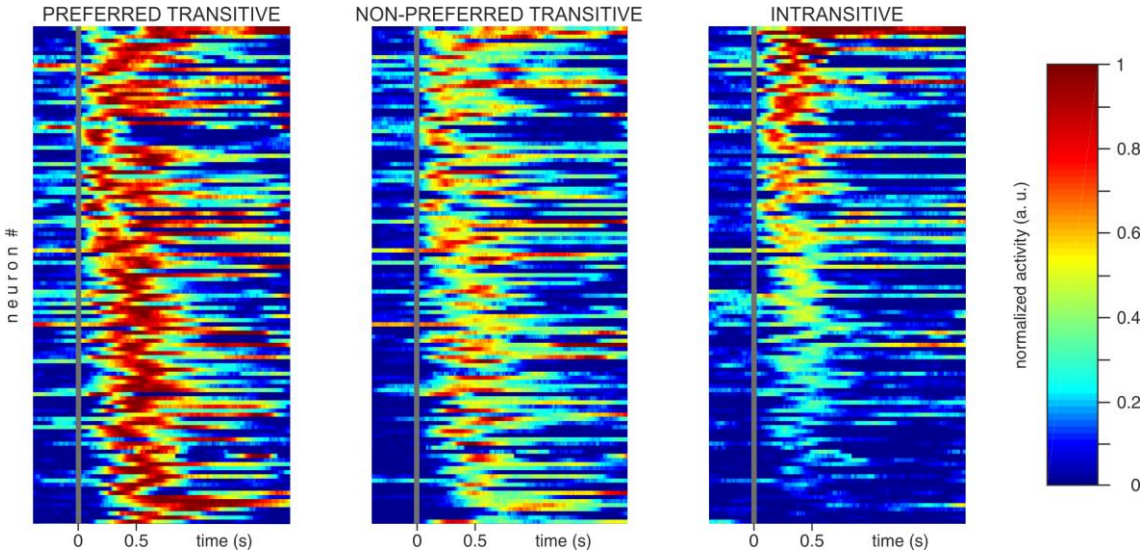

Figure 4.8 Net normalized activity of F5 MirNs during observation of actions. Time course of the net normalized activity of F5 MirNs during observation of transitive (preferred, non-preferred) and intransitive actions aligned at movement onset (grey vertical line). Each horizontal line of the map represents one neuron and the intensity of the activity is represented by color as indicated by the bar at the right side of the figure. Neurons are ordered by the intensity of the activity during the observation of the intransitive action.

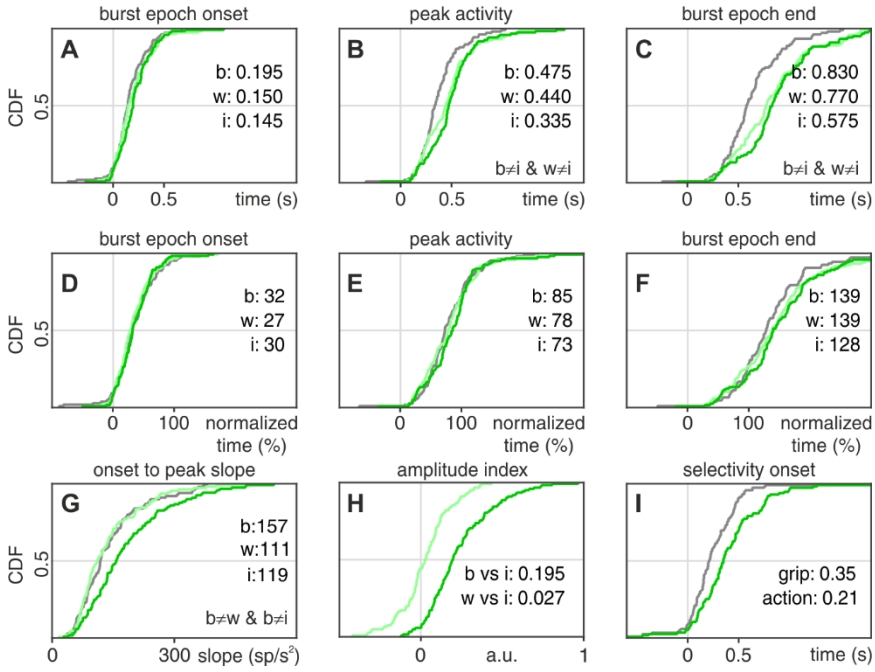

Figure 4.9 Activity characteristics of F5 MirNs during observation of transitive and intransitive actions. (A) Start of burst epoch. (B) Time of peak activity. (C) End of burst epoch. Characteristics in (A) to (C) are measured from the movement onset. (D-F) same as in A-C but relative onsets are expressed as percentage of movement duration. (G) Slope of activity from onset to peak of burst epoch measured by linear regression on the firing rate. In panels (A) to (G), curves represent cumulative distribution functions of activity characteristics during observation of preferred (dark green) and non-preferred (light green) transitive actions as well as during observation of the intransitive action (grey). (H) Amplitude index between the preferred transitive and the intransitive action (dark green) and between the non-preferred transitive and the intransitive action (light green) (I) Onset of action selectivity between transitive actions (green) and between transitive and intransitive actions (grey) measured from the movement onset. Distribution medians and statistical comparisons (Kruskal-Wallis followed by Bonferroni, $p<0.05$) are reported in each panel (b: preferred transitive, w: non preferred transitive, i: intransitive action, a.u.: arbitrary units).

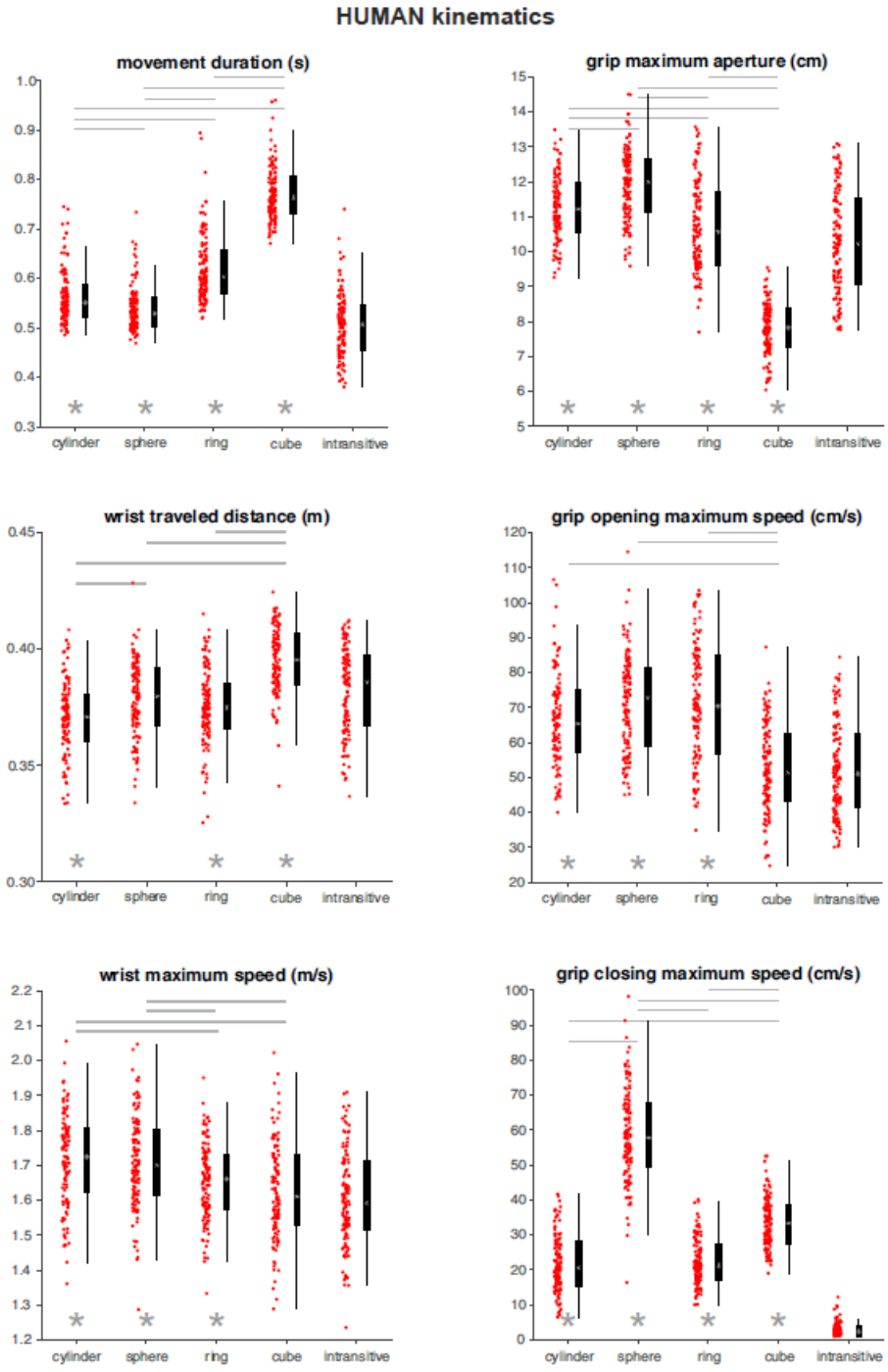

Figure 4.10 Human kinematic features. Box plots showing six kinematic features of intransitive and transitive actions performed by the experimenter. The circle in each box marks the median, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points not treated as outliers. The red points next to each box plot represent the values of kinematic features at different trials. Grey horizontal lines at the top of each panel denote statistically significant differences between the four transitive actions (one-way ANOVA followed by Tukey-Kramer post hoc, $p<0.05$). Grey asterisks at the bottom of each panel denote statistically significant differences between intransitive and transitive actions (one-way ANOVA followed by a Dunnett test, $p<0.05$).

4.1.3 Representational similarity between action kinematics and activity of F5 MirNs

To explore whether the pattern of activity across MirNs during action observation represents the kinematics of the observed actions we used representational similarity analysis (Kriegeskorte et al. 2008). We recorded the kinematics of the observed actions (Figure 3.3) and constructed representational dissimilarity matrices (RDMs) at the kinematics and neural spaces. Two correlated RDMs indicate a structural similarity between internal representations of different origin (Shepard and Chipman 1970). The similarity between pairs of RDMs was assessed by the Pearson correlation and statistically verified by the Mantel test.

The hand transport was described by the instantaneous position and speed of the wrist. The hand preshaping was described by the grip aperture (the distance between the tips of the index finger and the thumb) and its rate of change. These kinematic features were used to construct a four dimensional kinematic space. The representation of actions in this kinematic space around the middle third of the movement was correlated both with the burst activity of MirNs during observation ($r=0.902$, $p=0.024$; Figure 4.11 A and B) and with activity in the period when the population of MirNs displayed selectivity, as illustrated in the temporally detailed map (Figure 4.11 C). Furthermore, the neural representation followed the kinematics, as indicated by the scatter of points displaying statistically significant correlations below the diagonal. The neural RDMs were significantly correlated with the RDMs of each kinematic feature examined individually, as indicated by the proportion of time points with significant correlations ($p<0.05$) in a reference period (wrist position: 11%, wrist speed: 11%, aperture: 17%, aperture rate of change 18%, four dimensional kinematic space: 32%). Statistical comparison revealed that the proportions obtained by the features describing the hand preshaping were significantly higher than those obtained by the features describing the hand transport, and that the proportion achieved at the four dimensional kinematic space was the highest (Fisher's exact test, $p<0.01$). It is important to note that 75 neurons randomly selected from the population sufficed to obtain significant correlations with coefficients as high as those achieved with the entire population (Figure 4.12).

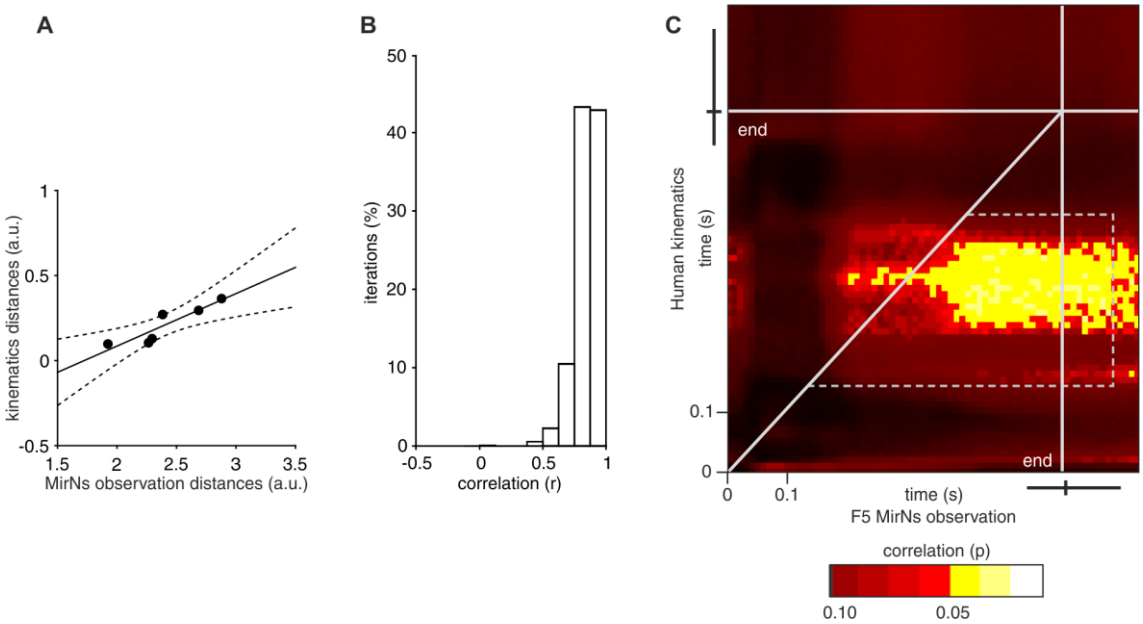

Figure 4.11 Representational similarity between action kinematics and activity of F5 MirNs during observation. (A) Plot of the normalized action distances of the kinematic features (ordinate) versus the normalized action distances of the observation burst activity of MirNs (abscissa). Solid line is the least squares linear regression line through the data and dashed lines indicate the 95% confidence intervals. (B) Distribution of the representational correlation coefficients between the combination of the kinematic features and observation burst activity of MirNs. (C) Map of representational similarity across time between the combination of the kinematic features and observation activity of MirNs. Time points on the representational similarity maps displaying statistically significant correlations are depicted with different shades of yellow as indicated by the color bar. Median and the 25th to 75th percentile times of the movement end are depicted by a black mark and lines parallel to each axis. Dashed lines indicate the reference period.

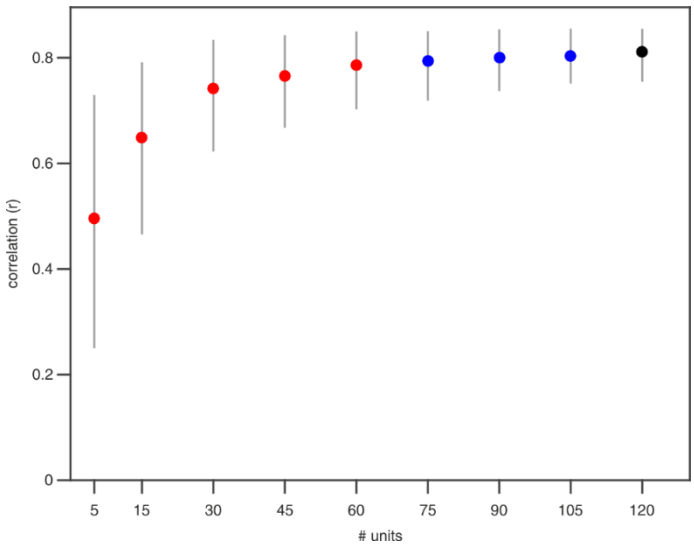

Figure 4.12 Effect of the neuronal population size on the representational similarity between kinematics and neural activity of F5 MirNs during observation. Median and 25th to 75th percentiles of the correlation coefficient bootstrap distribution for neural populations of different

sizes. Distributions of population sizes with less than 120 neurons were compared with the distribution of the full set of 120 neurons. Significant and non-significant differences are represented by red and blue circles, respectively.

Given that MirNs are also active during execution, we explored whether the pattern of activity across MirNs during action execution represents movement kinematics. For that purpose we recorded the monkey's wrist movement during the execution of the four transitive actions. As in the case of the human actor, the four transitive actions differed with each other in terms of the examined kinematic features. (Figure 4.13). In accordance to previous psychophysical results, (Roy et al. 2000), the pattern among the four actions performed by the human was similar to that among the four actions performed by the monkey for each of the examined kinematic features, as indicated by the correlation of their RDMs (movement duration, r = 0.884, p=0.045; wrist travelled distance, r = 0.779, p = 0.040; wrist maximum speed, r = 0.989, p = 0.039). Subsequently, we assessed the representational similarity between these kinematic features and the activity of MirNs during execution. The burst activity of MirNs during execution was significantly correlated with the available monkey kinematics around the middle third of the monkey's movement (r=0.914, p=0.042; Figure 4.14 A and B). In the temporally detailed map, the points displaying statistically significant correlations gathered around the middle third of the movement as in observation (Figure 4.14 C, Figure 4.11 C). Moreover, the period of significant correlations was closer to the diagonal during action execution as compared to action observation.

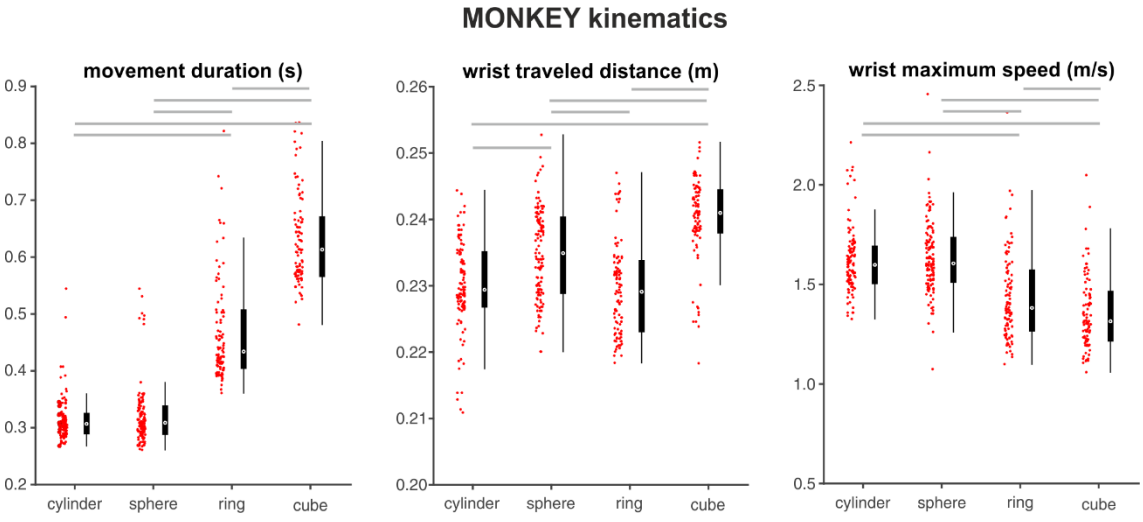

Figure 4.13 Monkey kinematic features. Box plots showing three kinematic features of transitive actions performed by the monkey. Conventions as in Figure 4.10

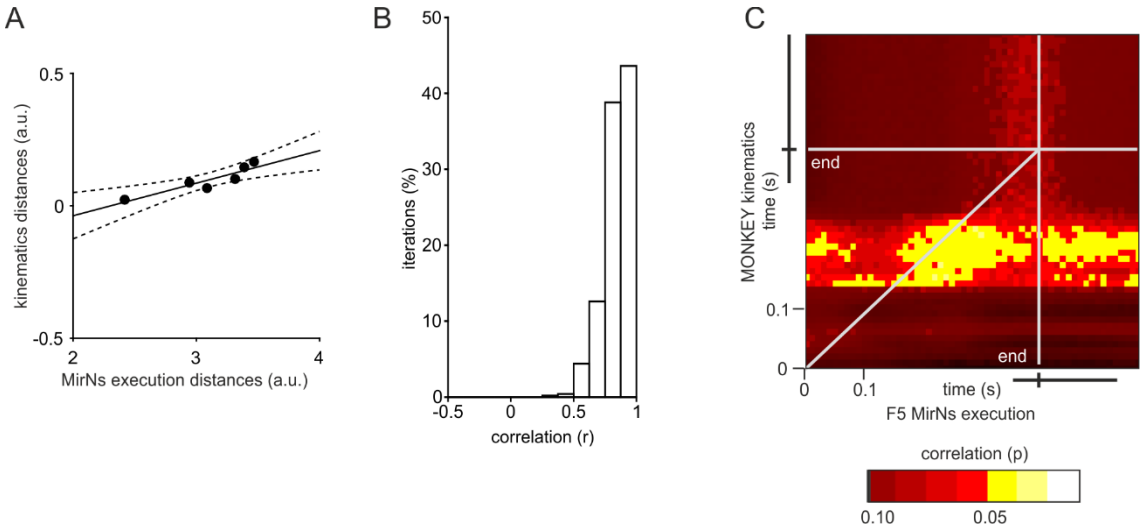

Figure 4.14 Representational similarity between action kinematics and activity of F5 MirNs during execution. (A) Plot of the normalized action distances of the kinematic features (ordinate) versus the normalized action distances of the execution burst activity of MirNs (abscissa). (B) Distribution of the representational correlation coefficients between the combination of the kinematic features and burst activity of MirNs during execution. (C) Map of representational similarity across time between the combination of the kinematic features and execution activity of MirNs. Conventions as in Figure 4.11.

4.1.4 Representational similarity between execution and observation activities of F5 MirNs

The criterion used at the original studies for characterizing a neuron as congruent was whether the action for which the neuron displayed the best response during execution elicited also the best response during observation (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996). With this criterion, the response to other grips performed or observed was neglected. Using this criterion, 27% of our neurons could be considered congruent, a percentage compatible with that reported in the original studies. To take into consideration all grips tested and not only the one displaying the maximum response, we used RDMs to investigate congruency between execution and observation either at the single neuron or the population level. Only 9% of MirNs were found to be congruent when considered individually (Figure 4.15). On the contrary, at the population level the burst activity RMDs of MirNs during execution and observation were significantly correlated (r=0.915, p=0.041; Figure 4.16 A and B). Early phases of the execution activity (before movement onset) were correlated with the period of the observation activity that displayed selectivity as revealed by the detailed two-dimensional map of correlations (Figure 4.16 C). Moreover, during movement, earlier phases of the execution activity

were correlated with later phases of the observation activity as indicated by the concentration of significant points below the diagonal.

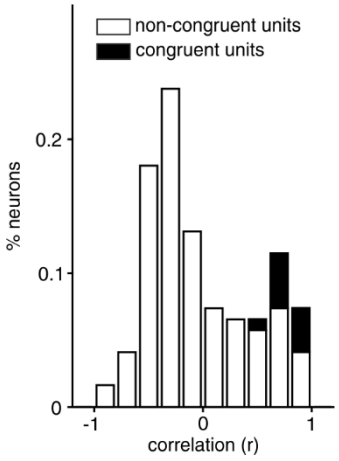

Figure 4.15 Distribution of the representational correlation coefficients as calculated for the burst activity of individual MirNs of area F5. The proportions of congruent units ($p<0.05$) are depicted with black colored bars.

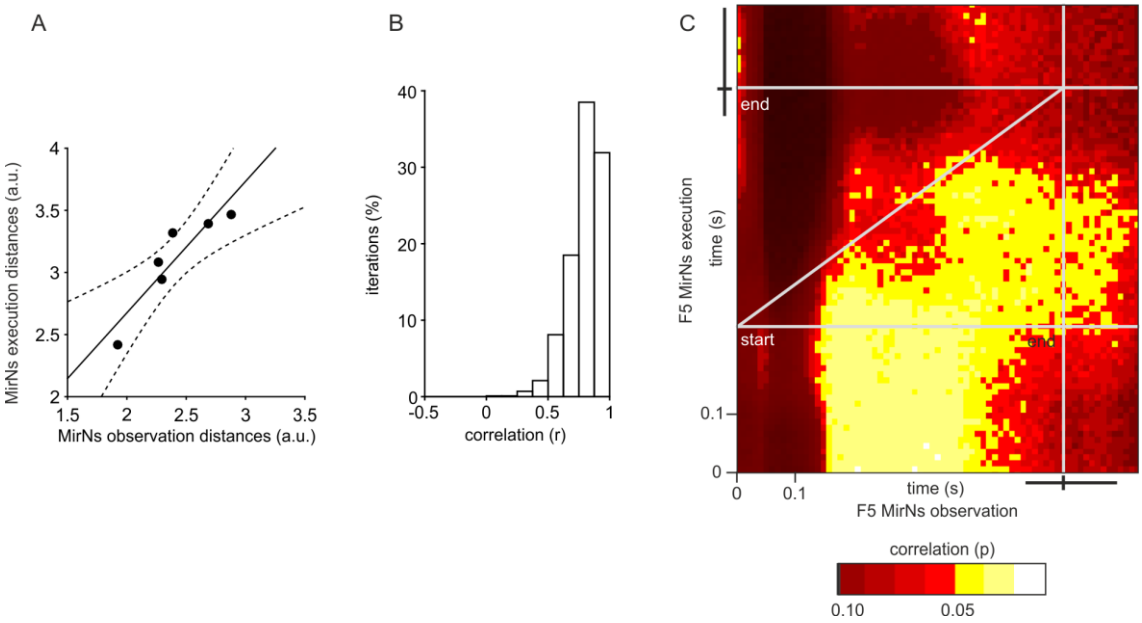

Figure 4.16 Representational similarity between execution and observation activities of F5 MirNs. (A) Plot of the normalized action distances of the execution burst activity of MirNs (ordinate) versus the normalized action distances of the observation burst activity of MirNs (abscissa). (B) Distribution of the representational correlation coefficients between observation and execution burst activity of MirNs. (C) Map of representational similarity across time between observation

and execution activity of MirNs. Conventions as in Figure 4.11.

Representational similarity between the activities of MirNs and non-MirNs of area F5 during execution

We used representational similarity analysis to compare the action encoding of MirNs and non-MirNs during execution. Population RDMs, which contain dissimilarity values for each pair of activity patterns elicited by a given action, characterize the encoding of actions in different populations (Kriegeskorte 2009). Therefore, their similarity indicates similar action encoding. If the claim that the motor properties of F5 MirNs are similar to those of other neurons in this area is correct (Rizzolatti et al. 2014), then the RDMs of the discharge of MirNs should be correlated to those of the discharge of non-MirNs. No correlation was seen between MirNs and non-MirNs when the activity during the burst epoch was considered (r=0.605, p=0.121; Figure 4.17 A and B). At the temporally detailed map of representational similarity, points with statistically significant correlations were concentrated into a period around the last quarter of the movement and extended also to the holding period (Figure 4.17 C). The proportion of time points with significant correlations ($p<0.05$) in a reference period around the diagonal (containing both leading and lagging time points in a 100ms wide strip) was 16%. To obtain a point of comparison, we assessed the representational similarity between the non-MirNs of this study and the F5 non-MirNs of a previous study (Raos et al. 2006). As expected, the burst activity RDMs of the two populations were significantly correlated (r=0.915, p=0.034; Figure 4.18 A and B) and the proportion of time points with significant correlations in the reference period on the temporally detailed map was 85% (Figure 4.18 C).

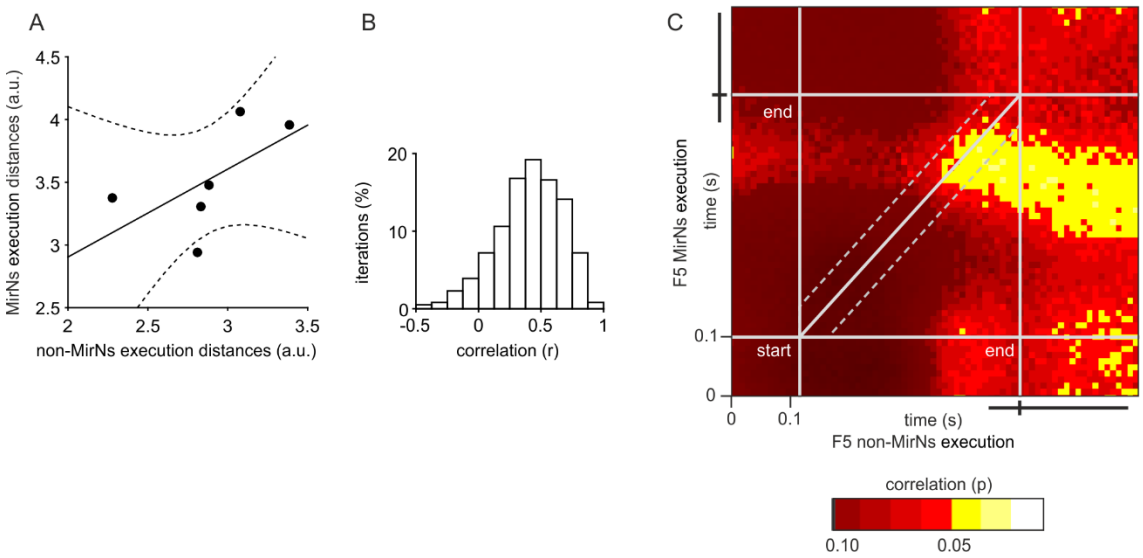

Figure 4.17 Representational similarity between the activities of MirNs and non-MirNs of area F5 during execution. (A) Plot of the normalized action distances of the execution burst activity of MirNs (ordinate) versus the normalized action distances of the execution burst activity of non-MirNs (abscissa). (B) Distribution of the representational correlation coefficients between execution burst activity of MirNs and non-MirNs (C) Map of representational similarity across time between execution activity of MirNs and non-MirNs. .Conventions as in Figure 4.11.

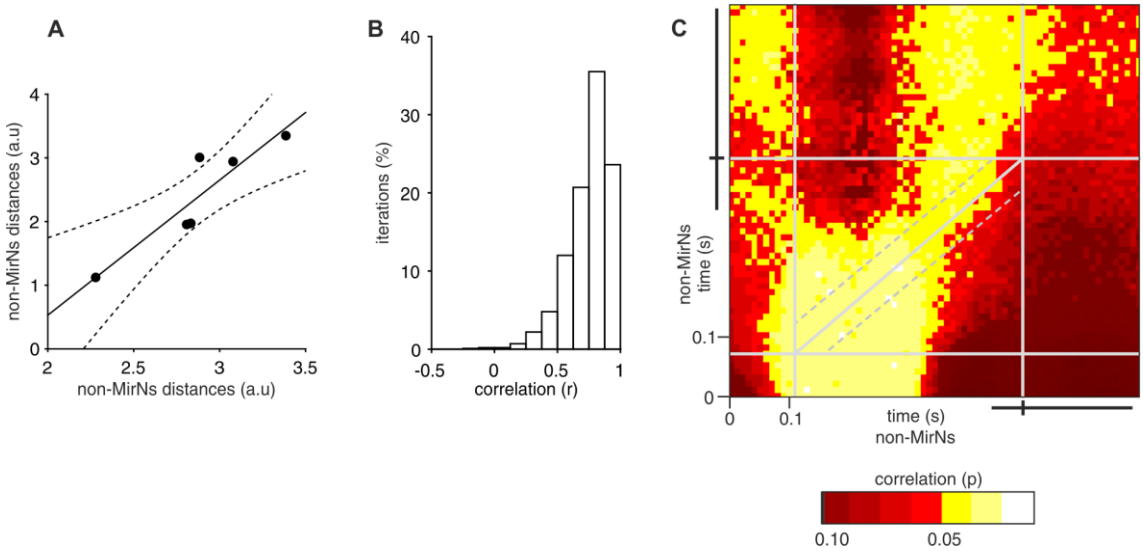

Figure 4.18 Representational similarity between neural activities of F5 non-MirNs from two different datasets. (A) Plot of the normalized action distances of the execution burst activity of two populations of non-MirNs [present study (abscissa) and 26 neurons from Raos et al 2006 (ordinate), tested with similar grips]. (B) Distribution of the representational correlation coefficients between the execution burst activities of the same populations. (C) Map of representational similarity across time between execution activities of the same populations. Abscissa and ordinate as in (A). Conventions as in Figure 4.11.

4.2 Mirror neurons in dorsal premotor cortex

4.2.1 Neuronal properties of MirNs and non-MirNs in PMd

Single-unit activity was recorded from the dorsal premotor cortex in two hemispheres of two monkeys that were trained initially to grasp objects with specific grips (execution task), and subsequently to observe the experimenter performing the same object-directed reaching-to-grasp actions (observation task). The same monkeys were used in the study of mirror neurons in the ventral premotor area F5. The location of the recorded neurons is illustrated in Figure 4.19. It is evident that the cluster of penetrations presumably corresponding to PMd is well distinct from that of F5 (Figure 3.1). A total of

218 task-related PMd neurons were recorded. Neurons from both monkeys displayed similar properties and thus data from both monkeys were combined..

Statistical analysis demonstrated that 140 neurons responded to both tasks whereas the remaining 78 were active only in the execution task. The differential response to the two tasks employed was used as a criterion for the classification of the recorded neurons. To quantify the difference between discharges for execution and discharges in response to observation we calculated the task amplitude index (tAI) for each neuron of the population. Similarly to the F5 neurons, the distribution of the tAIs of all the PMd neurons (Figure 4.20) was not unimodal (Hartigan's Dip Test, null hypothesis of unimodality rejected, p=0), thus indicating that the cells we found belong to two different groups (140 MirNs and 78 non-MirNs).

Single-neuron and population activity was selective for actions during both execution (MirNs and non-MirNs) and observation (MirNs, Figure 4.21, Figure 4.22 and Figure 4.23). MirNs and non-MirNs displayed action selectivity during execution which, for the majority of the neurons, was already present at movement onset (Figure 4.24, A and B). The selectivity of MirNs was similar during observation and execution. However, the selectivity onset during observation occurred later (about 150ms after movement onset) than that during execution (Figure 4.24, A and B). Activity bursts of MirNs and non-MirNs began and reached their maximum at the same time during execution. Nevertheless, the above temporal characteristics of MirN activity occurred later in observation than in execution (Figure 4.24, C to E).

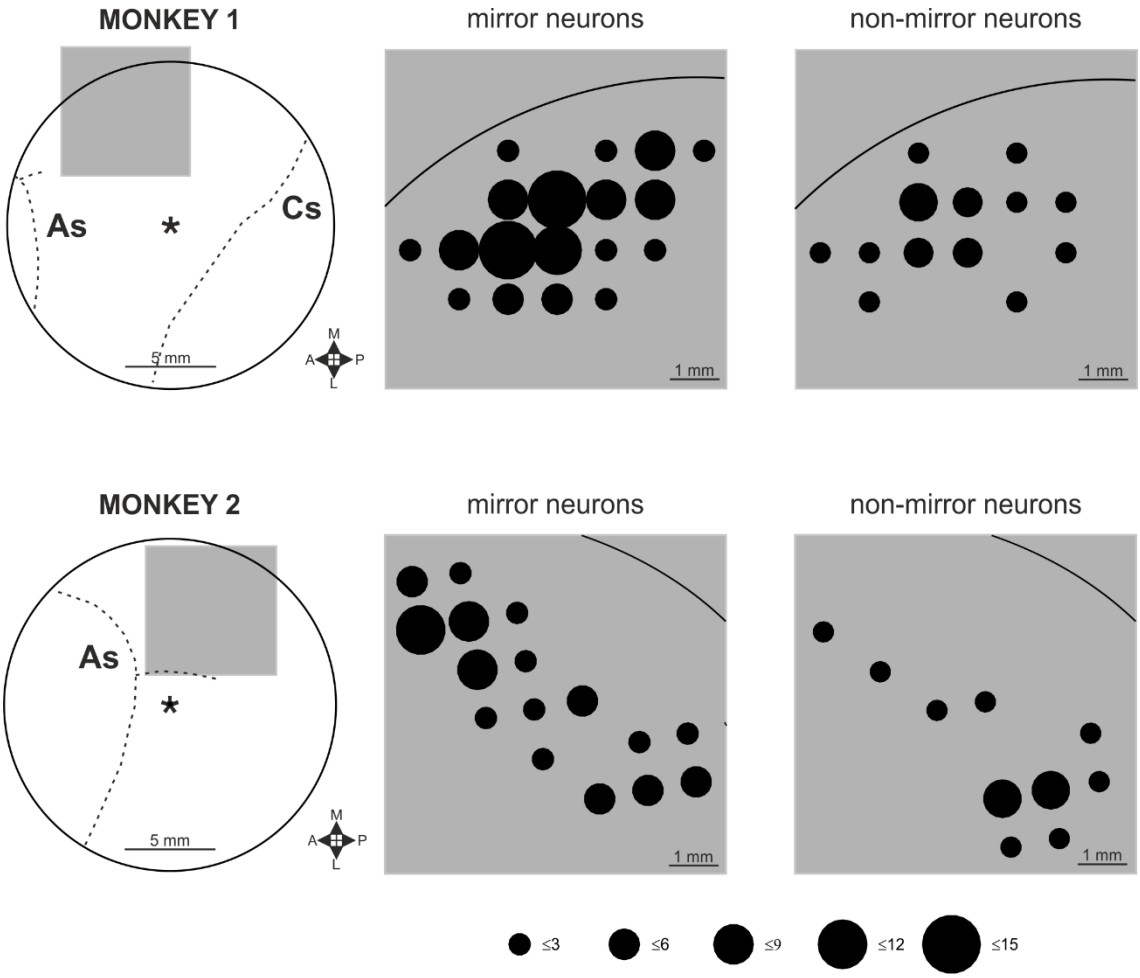

Figure 4.19 Location of recorded neurons in the PMd. Map of the cortical area exposed under each recording chamber. Conventions as in Figure 4.1.

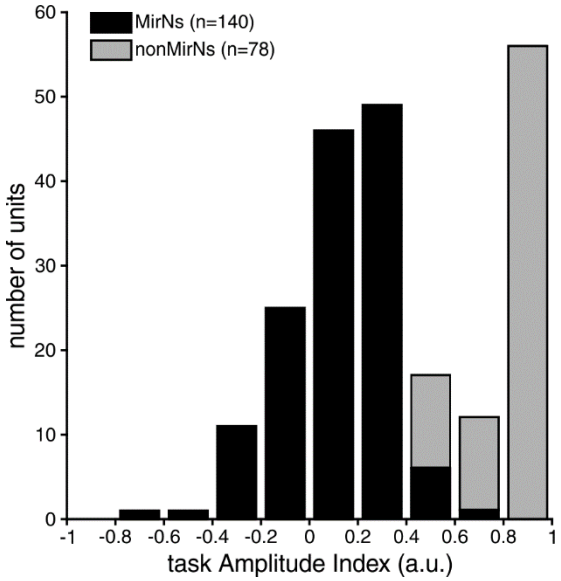

Figure 4.20 Amplitude difference between execution and observation responses in the PMd. Histogram of the task amplitude index of the recorded neurons.

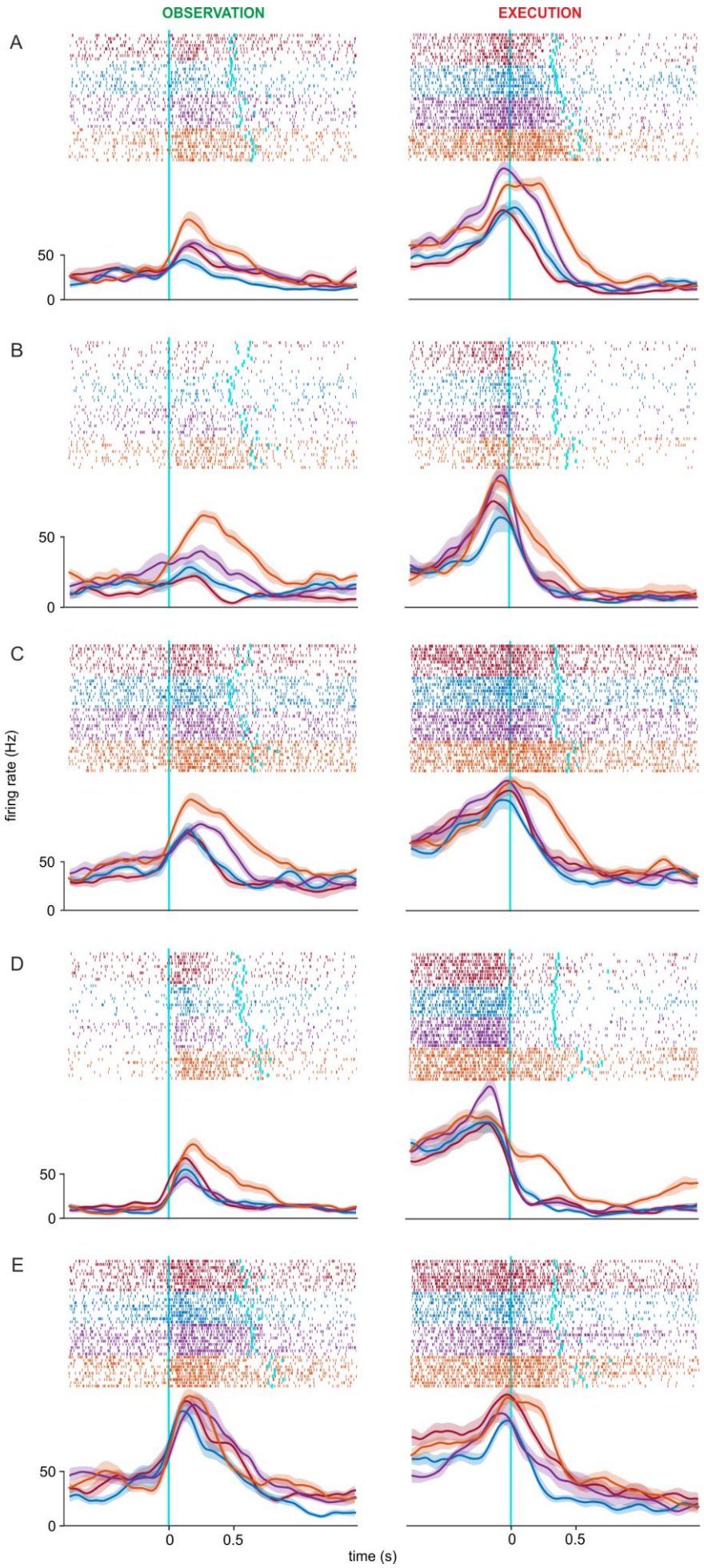
OBSERVATION
EXECUTION
A
B
C
D
E
50
0
firing rate (Hz)
0
0.5
time (s)

Figure 4.21 Examples of PMd mirror neurons. Activity of five PMd MirNs (A to E) during observation and execution presented in spike rasters and firing rates, both aligned at movement onset. All conventions as in Figure 4.3.

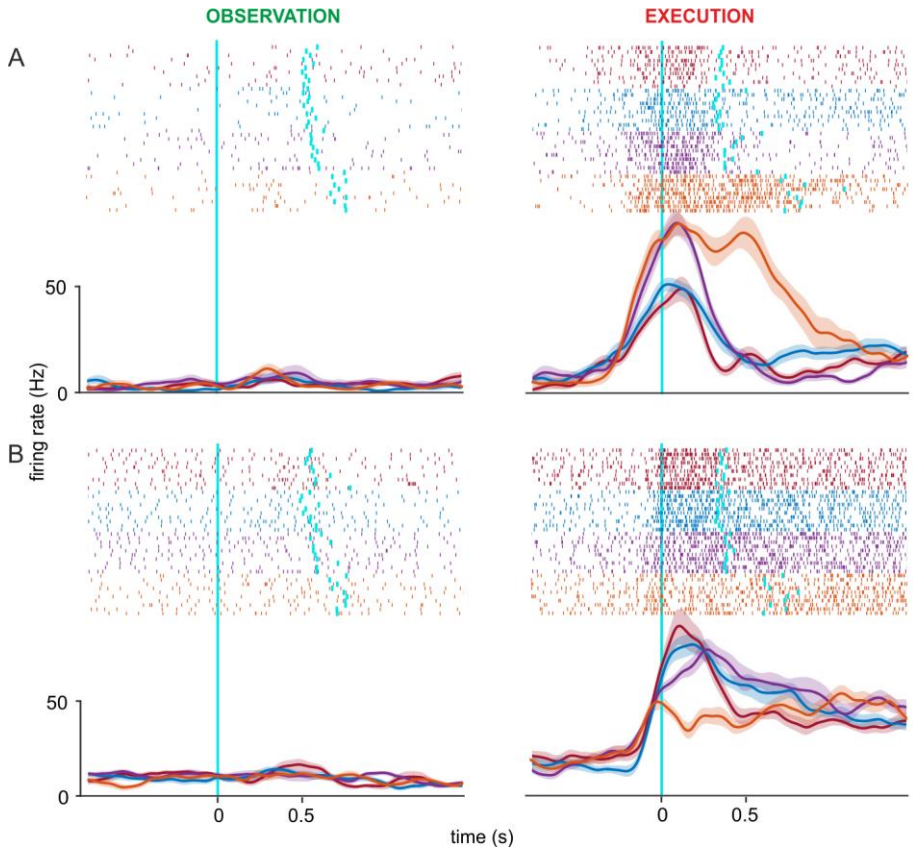

Figure 4.22 Examples of PMd non-mirror neurons. Activity of two PMd non-MirNs during observation and execution Neuron in B was recorded simultaneously with the neuron in Figure 4.31E. All conventions as in Figure 4.3.

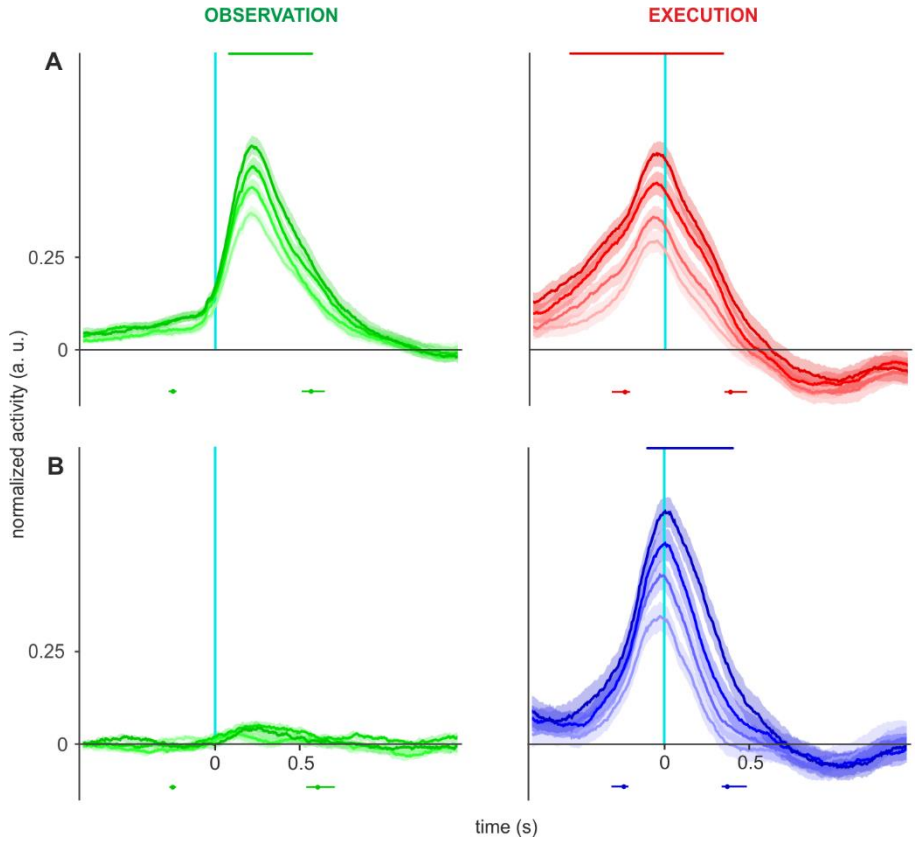

Figure 4.23 Population activity of PMd neurons during action observation and execution. Ranked net-normalized population activity of mirror (A) and non-mirror (B) neurons during observation (left) and execution (right), aligned at movement onset (cyan vertical line). Ranking of transitive actions is indicated by colored shading (preferred to non-preferred, dark to light). Colored marks and horizontal lines below the population activity denote the median and the 25th to 75th percentile times of the behavioral events (from left to right: go cue, movement end). Colored horizontal lines at the top of each panel denote the period displaying statistically significant selectivity among transitive actions.

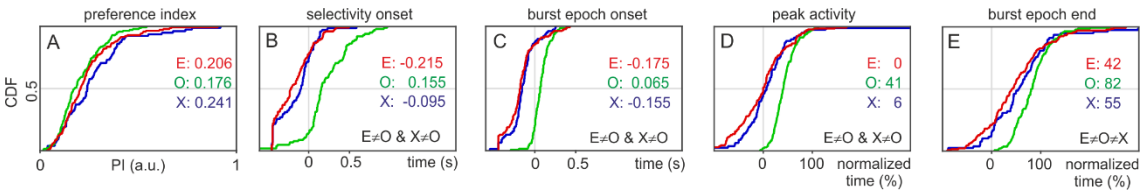

Figure 4.24 Activity characteristics of MirNs and non- MirNs in PMd. (A) Preference index (arbitrary units, a.u.). (B) Onset of action selectivity measured from the movement onset. (C) Start of burst epoch measured from the movement onset. (D) Time of peak activity expressed as percentage of movement duration. (E) End of burst epoch expressed as percentage of movement duration. In all panels, curves represent the empirical cumulative distribution function of MirN activity characteristics during execution (red) and observation (green) as well as non-MirN activity characteristics during execution (blue). Distribution medians and statistical comparisons (Kruskal-Wallis followed by Bonferroni, $p<0.05$) are reported in each panel (E: MirNs execution, O: MirNs observation, X: non-MirNs execution).

4.2.2 Representational similarity between action kinematics and activity of MirNs in the PMd

Prompted by the results in area F5, we used RDMs to investigate the representational similarity between neural activity in PMd and action kinematics. The representation of actions in the full kinematic space around the middle third of the movement was correlated with the burst activity of PMd MirNs during observation ($r=0.885$, $p=0.028$; Figure 4.25 A and B). As illustrated at the two-dimensional map of representational similarity across time, the points displaying statistically significant correlations between neural and kinematic spaces are gathered on and around the diagonal (Figure 4.25 C).

The neural RDMs were significantly correlated with the RDMs of each kinematic feature examined individually, as indicated by the proportion of time points with significant correlations ($p<0.05$) in a reference period (wrist position: 7%, wrist speed: 7.2%, aperture: 13%, aperture rate of change 18%, four dimensional kinematic space 25%). Similarly to F5, statistical comparison revealed that the proportions obtained by the features describing the hand preshaping were significantly higher than those obtained by the features describing the hand transport, and that the proportion achieved at the four dimensional kinematic space was the highest (Fisher's exact test, $p<0.01$).

To examine the effect of the neuronal population size on the representational similarity between the kinematics and the responses during observation, neuronal populations of different sizes ($n<=140$) were created by random selection with replacement. The representational similarity analysis was performed for each population size and the mean r of a reference period was estimated. This analysis revealed that ninety five neurons randomly selected from the population were sufficient to obtain significant correlations with coefficients as high as those achieved by the entire population (Figure 4.26) thus excluding the possibility that the resulting correlations were affected by certain individual neurons.

As in F5, we assessed the representational similarity between the proximal kinematics of the monkey’s actions and the activity of PMd MirNs during execution. The burst activity of MirNs during execution was significantly correlated with the kinematics around the middle third of the movement ($r=0.865$, $p=0.048$; Figure 4.27 A and B). In the temporally detailed map, the points displaying statistically significant correlations

gathered around the middle third of the movement in the kinematics representation, as in observation (Figure 4.27 C, Figure 4.25 C).

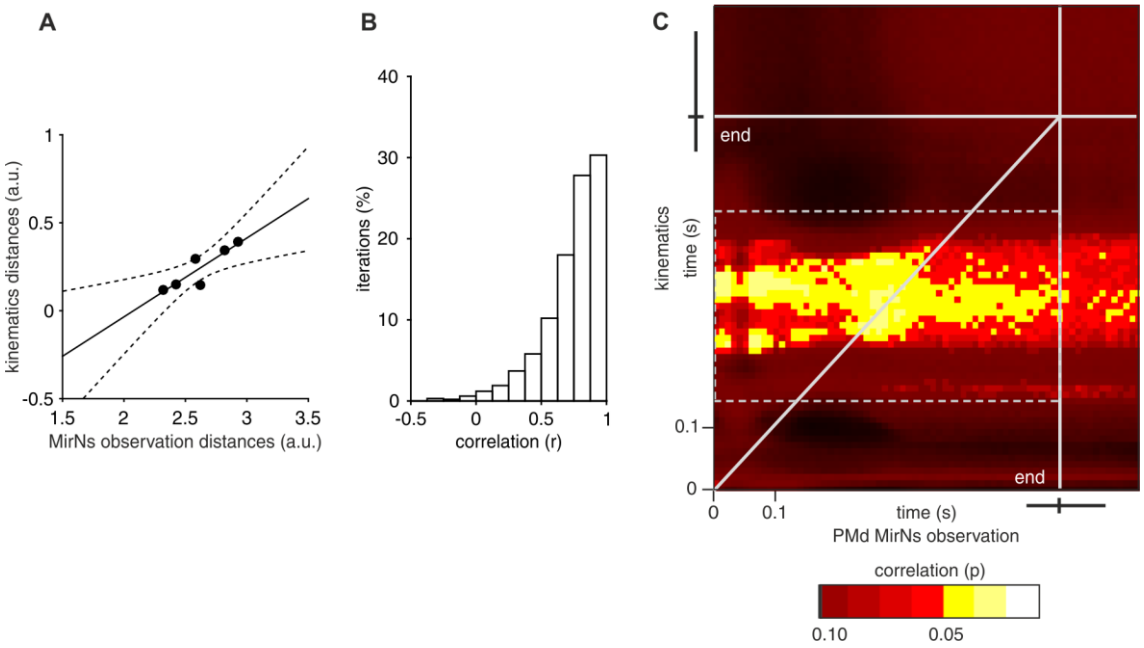

Figure 4.25 Representational similarity between action kinematics and activity of PMd MirNs during observation. (A) Plot of the normalized action distances of the kinematic features (ordinate) versus the normalized action distances of the observation burst activity of MirNs (abscissa). (B) Distribution of the representational correlation coefficients between the combination of the kinematic features and observation burst activity of MirNs. (C) Map of representational similarity across time between the combination of the kinematic features and observation activity of MirNs. Conventions as in Figure 4.11.

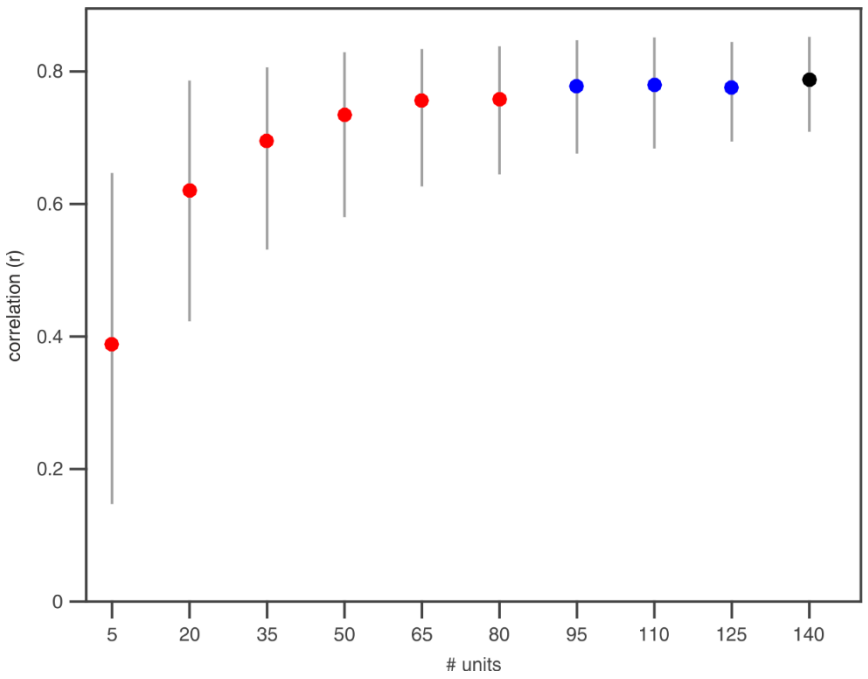

Figure 4.26 Effect of the neuronal population size on the representational similarity between kinematics and neural activity of PMd MirNs during observation.. Distributions of population sizes with less than 140 neurons were compared with the distribution of the full set of 140 neurons. Other conventions as in Figure 4.12.

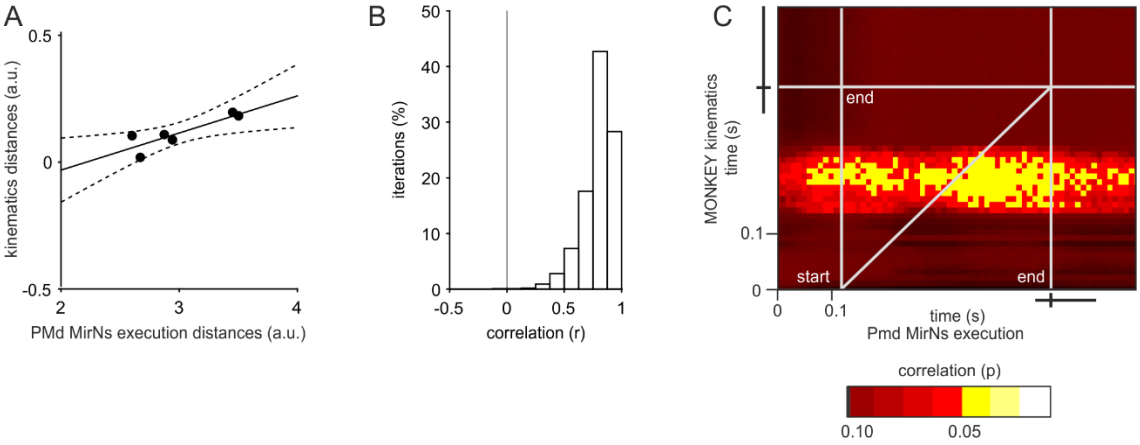

Figure 4.27 Representational similarity between action kinematics and activity of PMd MirNs during execution. (A) Plot of the normalized action distances of the kinematic features (ordinate) versus the normalized action distances of the execution burst activity of MirNs (abscissa). (B) Distribution of the representational correlation coefficients between the combination of the kinematic features and burst activity of MirNs during execution. (C) Map of representational similarity across time between the combination of the kinematic features and execution activity of MirNs. Conventions as in Figure 4.11.

4.2.3 Representational similarity between execution and observation activities of PMd MirNs

To investigate representational similarity of execution and observation activity of PMd MirNs, we compared the representations of the two tasks either at the single neuron or the population level. At the single neuron level, only 3% of PMd MirNs displayed representational similarity between execution and observation activities (Figure 4.28). At the population level, the burst activity RMDs of PMd MirNs during execution and observation were significantly correlated (r=0.950, p=0.020; Figure 4.29 A and B). In the temporally detailed two-dimensional map of correlations, the period of observation activity that displayed selectivity was correlated with a long period of execution activity that started before the movement onset and extended through the whole movement epoch (Figure 4.29 C).

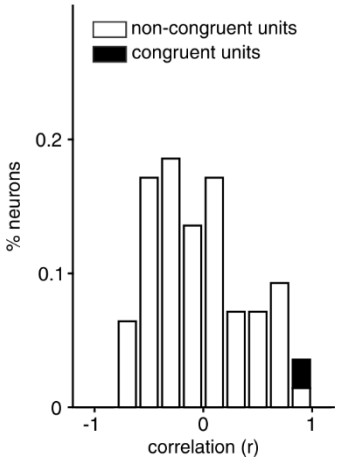

Figure 4.28 Distribution of the representational correlation coefficients as calculated for the burst activity of individual MirNs of PMd. The proportions of congruent units ($p<0.05$) are depicted with black colored bars.

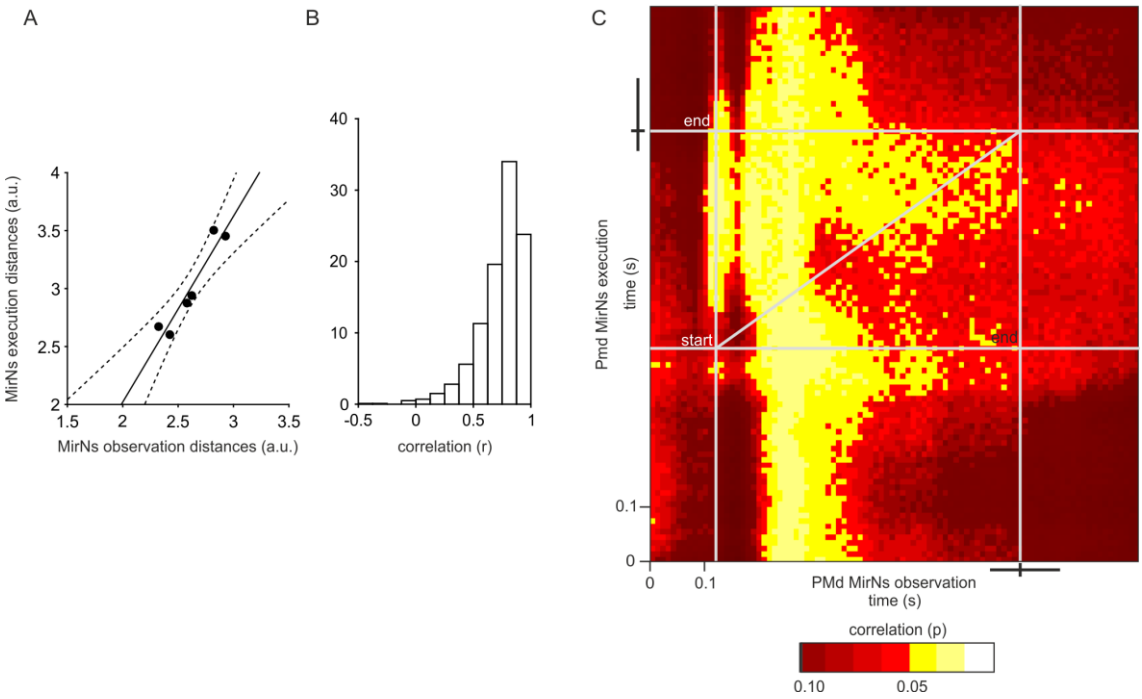

Figure 4.29 Representational similarity between execution and observation activities of PMd MirNs. (A) Plot of the normalized action distances of the execution burst activity of MirNs (ordinate) versus the normalized action distances of the observation burst activity of MirNs (abscissa). (B) Distribution of the representational correlation coefficients between observation and execution burst activity of MirNs. (C) Map of representational similarity across time between observation and execution activity of MirNs. Conventions as in Figure 4.11.

4.2.4 Representational similarity between the activities of MirNs and non-MirNs of PMd during execution

The RDMs of MirNs and non-MirNs in PMd during execution where correlated when activity during the burst epoch was considered (r=0.962, p=0.050; Figure 4.30 A and B). In the temporally detailed map of representational similarity, the activity on non-MirNs before the movement onset was correlated with the activity of MirNs at a period that started hundreds of milliseconds before the movement onset and extended in the first 150ms of the movement (Figure 4.30 C).

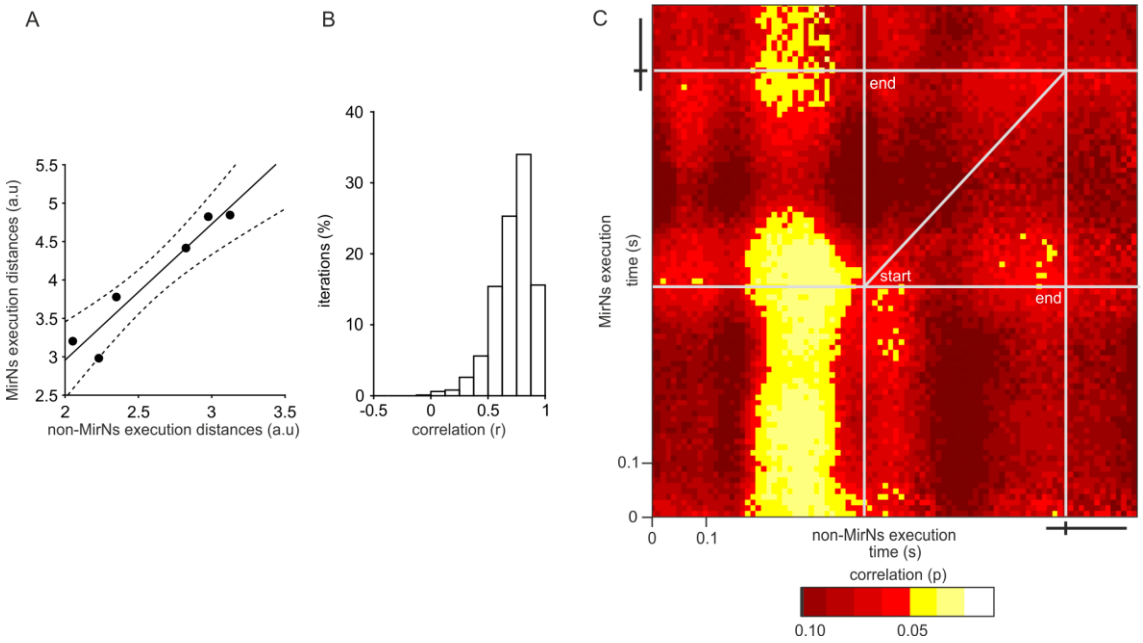

Figure 4.30 Representational similarity between the activities of MirNs and non-MirNs of PMd during execution. (A) Plot of the normalized action distances of the execution burst activity of MirNs (ordinate) versus the normalized action distances of the execution burst activity of non-MirNs (abscissa). (B) Distribution of the representational correlation coefficients between execution burst activity of MirNs and non-MirNs (C) Map of representational similarity across time between execution activity of MirNs and non-MirNs. Conventions as in Figure 4.11.

4.2.5 Response of PMd MirNs during the observation of intransitive actions

We examined the response of a subset of MirNs (n = 61) to the observation of both transitive and intransitive actions (examples are shown in Figure 4.31). We found that all tested MirNs responded to the observation of both transitive and intransitive actions. The baseline and burst activity of all MirNs studied during observation of the four transitive and the one intransitive actions is illustrated in Figure 4.32 and their normalized rates are illustrated in Figure 4.33. Observation of both transitive and

intransitive actions elicited similar response patterns (Figure 4.34 and Figure 4.35). The onsets and endings of the burst activity, as well as the time of maximum discharge occurrence for transitive actions coincided with those of the intransitive ones (Figure 4.35, A to C). The amplitude of the response to the observation of the intransitive action was lower than that of the response to the preferred transitive action but higher than that of the response to the non-preferred transitive action (Figure 4.35, D). The onset of action selectivity between transitive actions coincided with the onset of selectivity between transitive and intransitive actions (Figure 4.35, E).

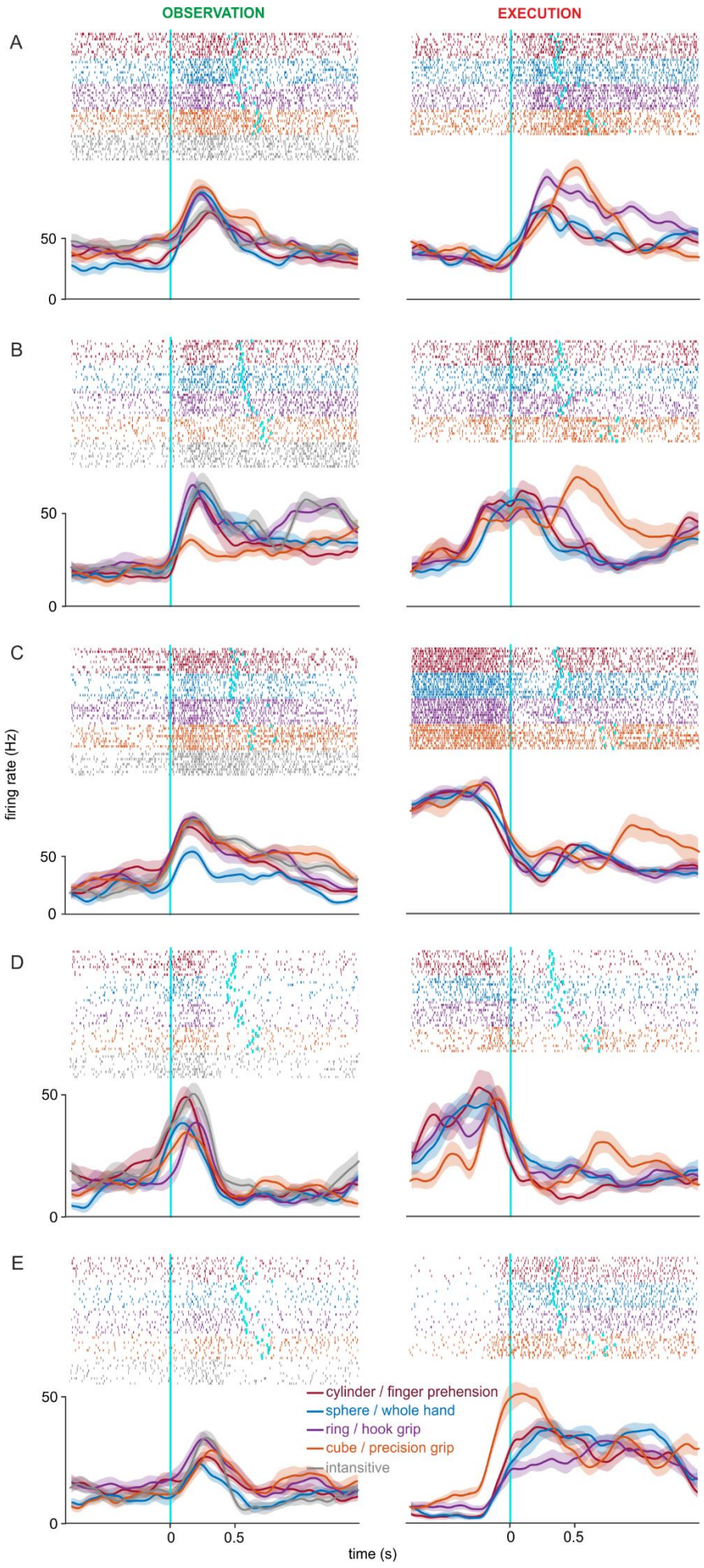
OBSERVATION
EXECUTION
A
B
C
D
E
50
0
firing rate (Hz)
cylinder / finger prehension
sphere / whole hand
ring / hook grip
cube / precision grip
intansitive
0
0.5
time (s)

Figure 4.31 Examples of PMd mirror neurons. Activity of five PMd mirror neurons (A to E) during observation (left) and execution (right) presented in spike rasters and firing rates, both aligned at movement onset (cyan vertical line). All conventions as in Figure 4.3.

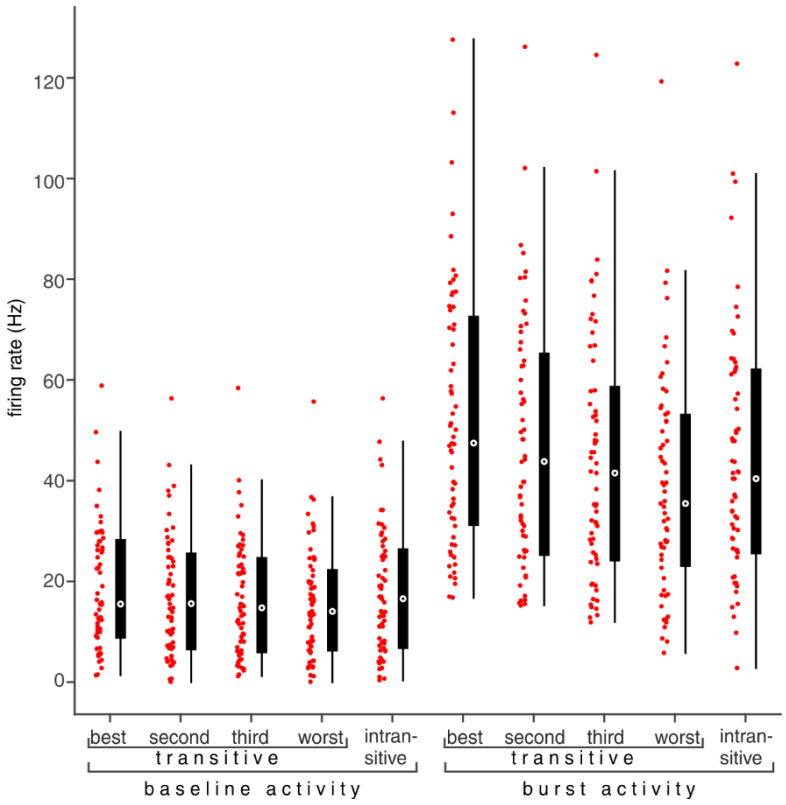

Figure 4.32 Baseline and burst activity of PMd MirNs during observation of actions. Box plots showing baseline and burst activity of MirNs during observation of four transitive and one intransitive actions. All conventions as in Figure 4.7.

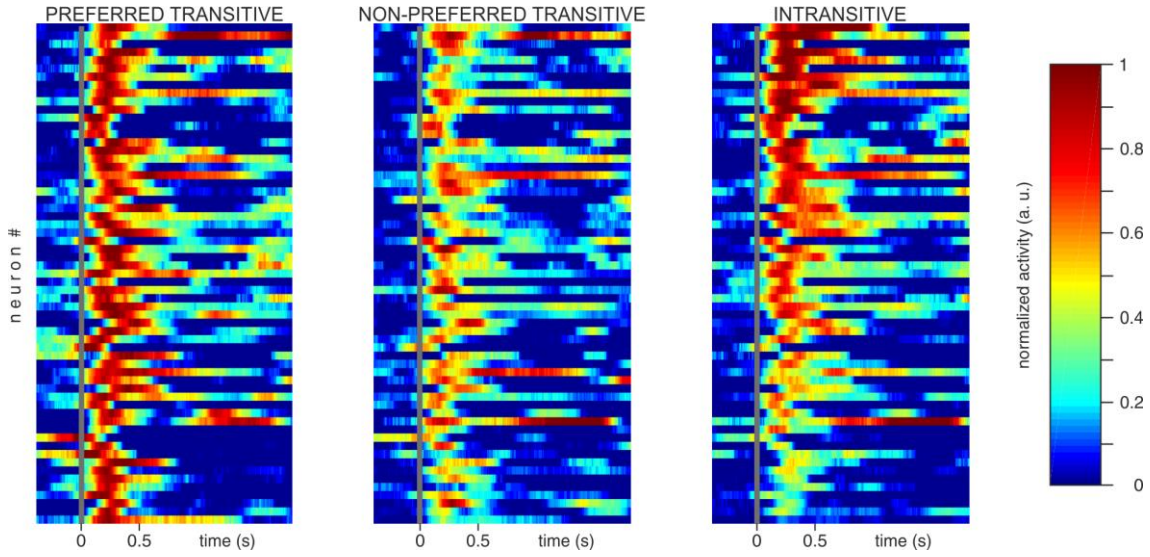

Figure 4.33 Net normalized activity of PMd MirNs during observation of actions. Time course of the net normalized activity of MirNs during observation of transitive (preferred, non-preferred) and intransitive actions aligned at movement onset (grey vertical line). Conventions as in Figure 4.8.

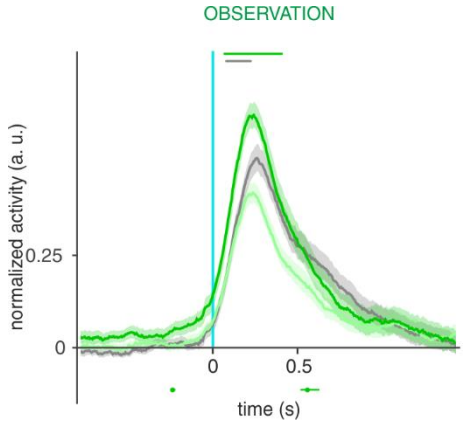

Figure 4.34 Population activity of PMd neurons during action observation of transitive and intransitive actions. Net-normalized population activity of PMd MirNs (n = 61) during observation, aligned at movement onset (cyan vertical line). Preferred and non-preferred transitive actions are indicated by colored lines and bands (dark and light color respectively). The grey line and band indicate the activity during observation of the intransitive action. Other conventions as in Figure 4.5.

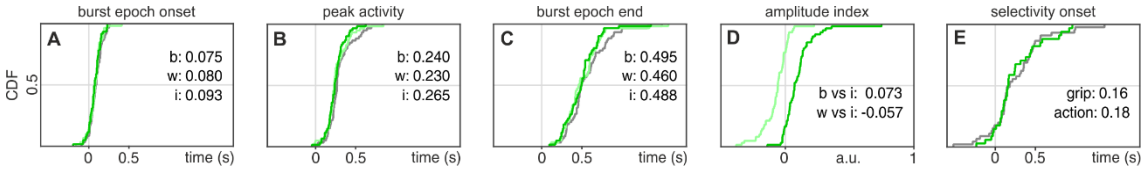

Figure 4.35 Activity characteristics of PMd MirNs during observation of transitive and intransitive actions. (A) Start of burst epoch. (B) Time of peak activity. (C) End of burst epoch. Characteristics in (A) to (C) are measured from movement onset and curves represent cumulative distribution functions of activity characteristics during observation of preferred (dark green) and non-preferred (light green) transitive actions as well as during observation of the intransitive action (grey). (D) Amplitude index between the preferred transitive and the intransitive action (dark green) and between the non-preferred transitive and the intransitive action (light green) (E) Onset of action selectivity between transitive actions (green) and between transitive and intransitive actions (grey) measured from the movement onset. Distribution medians are reported in each panel (b: preferred transitive, w: non preferred transitive, i: intransitive action, a.u.: arbitrary units).

4.3 Comparison of neuronal properties and action representation between F5 and PMd

In this study, we recorded 192 neurons from area F5 and 218 neurons from the PMd of the macaque brain during the execution and observation of a reach to grasp task.

This dataset allows the interareal comparison of the functional properties of the recorded neuronal populations.

4.3.1 Neuronal properties

During observation, MirNs of the dorsal and ventral premotor cortex displayed similar levels of grip selectivity (Figure 4.36, A). However, the grip selectivity emerged earlier in PMd than in F5 (Figure 4.36, B). This was also reflected in the temporal landmarks of the burst epoch: the start and end of the burst epoch as well as the time of peak activity of MirNs during observation occurred earlier in PMd than in F5 (Figure 4.36, C to E). The comparison of the MirNs responses during execution in the two areas revealed analogous similarities and differences between them. Specifically, the grip selectivity of PMd MirNs was similar to that of F5 MirNs (Figure 4.36, F). The selectivity onset, the onset and end of the burst epoch and the time of peak activity occured earlier in PMd compared to F5 (Figure 4.36, G to J). Non-MirNs of area F5 were more selective than non-MirNs of area PMd (Figure 4.36, K). As in the case of MirNs, all the temporal landmarks occurred earlier in PMd than in F5 (Figure 4.36, L to O).

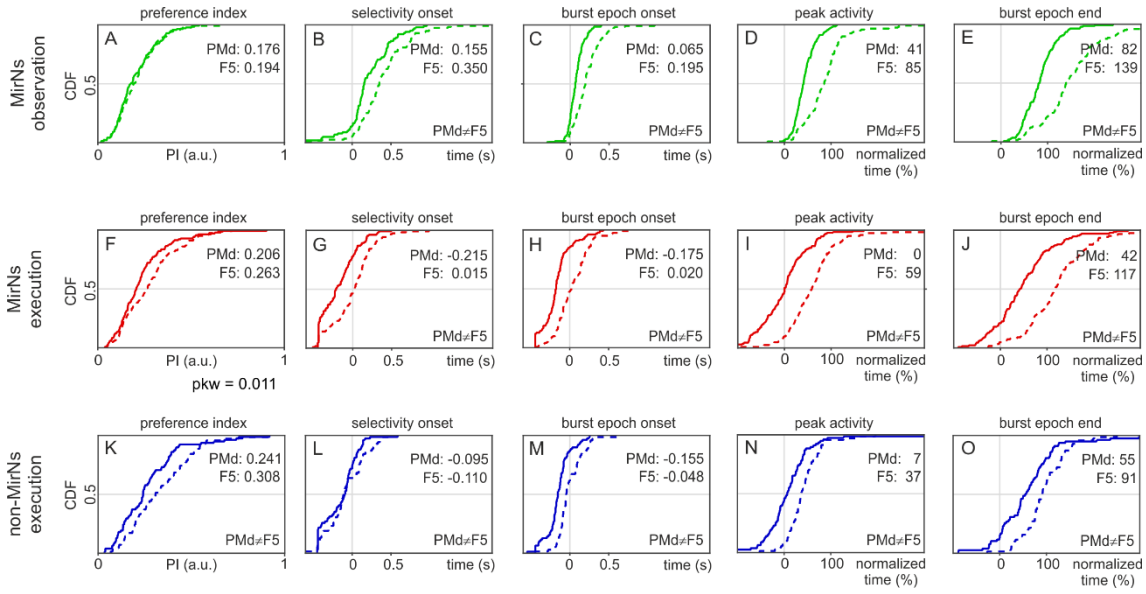

Figure 4.36 Comparison of activity characteristics of homologous populations in PMd and F5. (A, F and K) Preference index (arbitrary units, a.u.). (B, G and L) Onset of action selectivity effects measured from movement onset. (C, H and M) Start of burst epoch measured from movement onset. (D, I and N) Time of peak activity expressed as percentage of movement duration. (E, J and O) End of burst epoch expressed as percentage of movement duration. In panels A to E, curves

represent the empirical cumulative distribution function of PMd (green solid line) and F5 (green dashed line) MirN activity characteristics during observation. In panels F to J, curves represent the empirical cumulative distribution function of PMd (red solid line) and F5 (red dashed line) MirN activity characteristics during execution. In panels K to O, curves represent the empirical cumulative distribution function of PMd (blue solid line) and F5 (blue dashed line) non-MirN activity characteristics during execution. Distribution medians and statistical comparisons (Kruskal-Wallis followed by Bonferroni, $p<0.05$) between the two areas (PMd and F5) are reported in each panel.

To explore whether the temporal differences of firing between the two areas are affected by different behaviors either observed or executed we compared the durations of the movements performed either by the experimenter or the monkeys in the observation and execution conditions, respectively. The durations of the experimenter's movements performed during the acquisition of the neuronal activity in PMd did not differ from those during the recording in F5 (Table 2). During the PMd recordings the monkeys executed the movements slightly faster (3 to 27 ms) than during F5 recording (Table 2). Therefore, it is unlikely that the lag of F5 in relation to PMd during both observation and execution is due to temporal differences in behavior.

Table 2 Duration of movements (in ms) executed by the experimenter and the monkey.

	observation task (experimenter)				execution task (monkey)			
	cylinder	sphere	ring	cube	cylinder	sphere	ring	cube
F2	535±42[1]	507±46	584±47	700±57	357±25	357±25	404±33	674±107
F5	531±50	499±48	589±54	707±56	366±23	359±24	415±40	701±86
t-value	0.744	1.261	-0.677	-1.037	-3.071	-0.930	-2.390	-2.223
p value	0.457	0.208	0.499	0.300	0.002	0.353	0.018	0.027

[1] standard deviation

To quantify the difference of the magnitude of the discharge between execution and observation we calculated a task amplitude index (tAI) for each neuron. In both cortical areas, the response during execution was higher than that during observation ($\tilde{x}_{tAI\text{-}PMd}=0.10$, $\tilde{x}_{tAI\text{-}F5}=0.15$). These values of tAI reflect absolute amplitude differences between execution and observation of 20% and 35%, respectively. The distributions of amplitude indices of the two areas were not statistically different (($p = 0.056$; Figure 4.37).

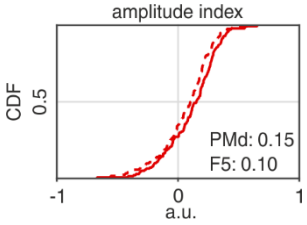

Figure 4.37 Amplitude index between the execution and observation activity of MirNs in PMd and F5. Solid line represents the empirical cumulative distribution function for the PMd neurons and the dashed line the empirical cumulative distribution function for the F5 neurons. The distribution medians are reported in the panel. The two distributions were not significantly different (Kruskal-Wallis, $p>0.05$).

4.3.2 Representational similarity between homologous populations in PMd and F5

We described MirNs in PMd that display similar levels of grip selectivity to MirNs in area F5. Moreover, MirNs in the PMd start discharging earlier than those of F5 both during execution and observation. To explore the action encoding of the different populations, we performed representational similarity analysis in the burst epoch rates and in a sliding bin that covered pre-movement, movement and hold epochs.

The burst activity RMDs of PMd and F5 MirNs during observation were significantly correlated ($r=0.951$, $p=0.039$; Figure 4.38 A and B). In the temporally detailed two-dimensional map of correlations, the period at which the observation activities of the two areas were significantly correlated extended from the middle of the movement to the beginning of the object holding epoch (Figure 4.38 C). Action encoding in area F5 followed the action encoding in PMd, as indicated by the scatter of significant time points below the diagonal. During execution, the burst activity RMDs of PMd and F5 MirNs were also significantly correlated ($r=0.762$, $p=0.048$; Figure 4.39 A and B). The detailed two-dimensional map of correlations revealed similar action encoding in the pre-movement period, a result compatible with the early selectivity onsets of both populations (Figure 4.39 C). The RDMs of PMd and F5 non-MirNs during execution where not correlated when the activity during the burst epoch was considered ($r=0.684$, $p=0.104$; Figure 4.40 A and B). At the temporally detailed map of representational similarity, points with statistically significant correlations were concentrated into a period around the second half of the movement (Figure 4.40 C).

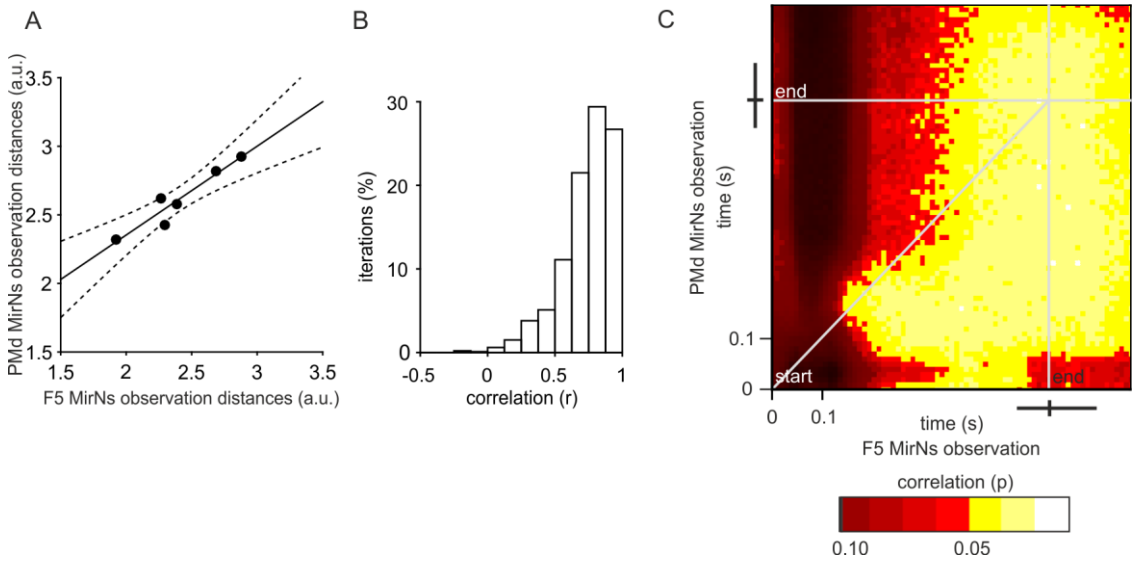

Figure 4.38 Representational similarity between observation activities of PMd and F5 MirNs. (A) Plot of the normalized action distances of the observation burst activity of PMd MirNs (ordinate) versus the normalized action distances of the observation burst activity of F5 MirNs (abscissa). (B) Distribution of the representational correlation coefficients between burst activity of PMd and F5 MirNs during observation (C) Map of representational similarity across time between activity of PMd and F5 MirNs during observation. Conventions as in Figure 4.11.

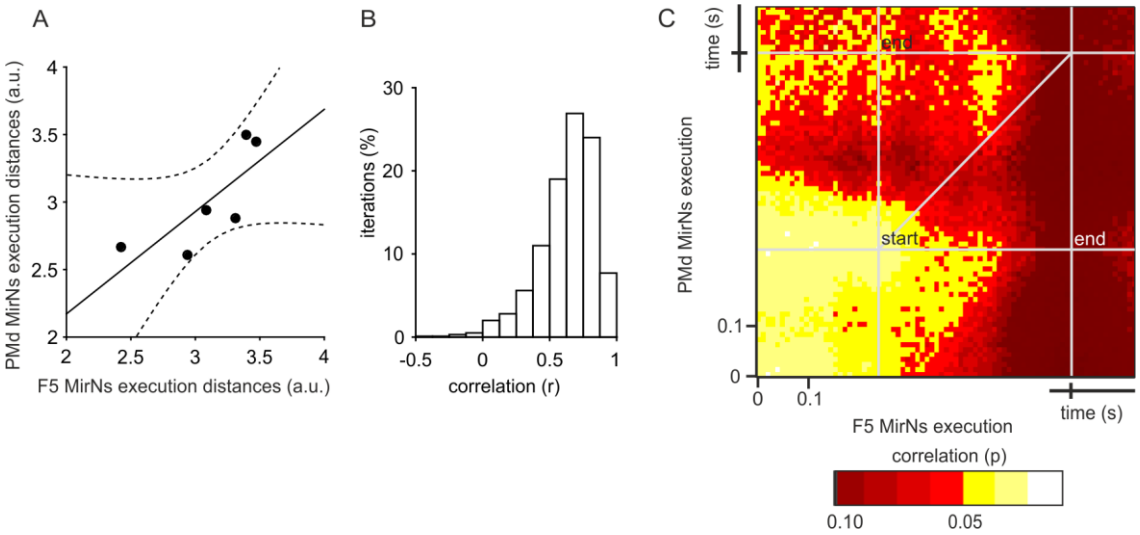

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Figure 4.39 Representational similarity between execution activities of PMd and F5 MirNs. (A) Plot of the normalized action distances of the execution burst activity of PMd MirNs (ordinate) versus the normalized action distances of the execution burst activity of F5 MirNs (abscissa). (B) Distribution of the representational correlation coefficients between burst activity of PMd and F5 MirNs during execution (C) Map of representational similarity across time between activity of PMd and F5 MirNs during execution. Conventions as in Figure 4.11.

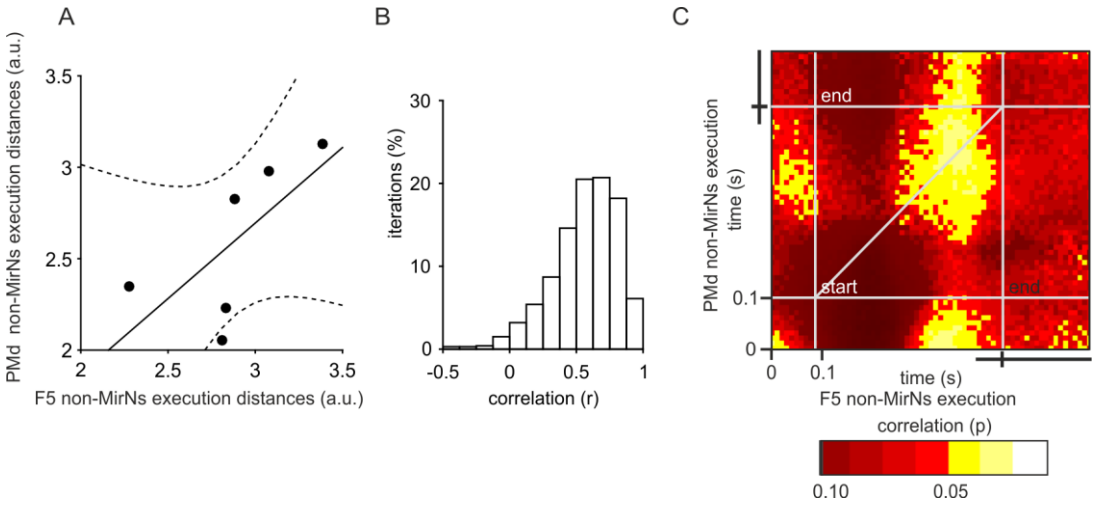

Figure 4.40 Representational similarity between execution activities of PMd and F5 non-MirNs. (A) Plot of the normalized action distances of the execution burst activity of PMd non-MirNs (ordinate) versus the normalized action distances of the execution burst activity of F5 non-MirNs (abscissa). (B) Distribution of the representational correlation coefficients between burst activity of PMd and F5 non-MirNs during execution (C) Map of representational similarity across time between activity of PMd and F5 non-MirNs during execution. Conventions as in Figure 4.11.

4.4 Additional controls

4.4.1 Effect of individual neurons on the representational similarity

Representational similarities presented in this study are the outcome of computations done on the population level. The distances used for the construction of RDMs (Eq 1) is the sum of the contribution of each individual neuron comprising the population. Some neurons may contribute large amounts to the sum to the sum and thus may disproportionately affect the result. To verify that the observed significant correlations in the time detailed maps illustrate a consistent result and not an artifact caused by small number of individual neurons, we used a bootstrap procedure. The time detailed maps of representational similarity were constructed 100 times, each time using a random resampling (with replacement) of the original neuronal populations. Figure 4.41 reports the resulting maps for the representational similarity maps between F5 observation and human kinematics (Figure 4.41A), PMd observation and human kinematics (Figure 4.41B) and observation activities of F5 and PMd (Figure 4.41C). It is evident that periods of significant correlations were consistent across different populations of neurons, and these periods correspond to the ones highlighted in the original maps.

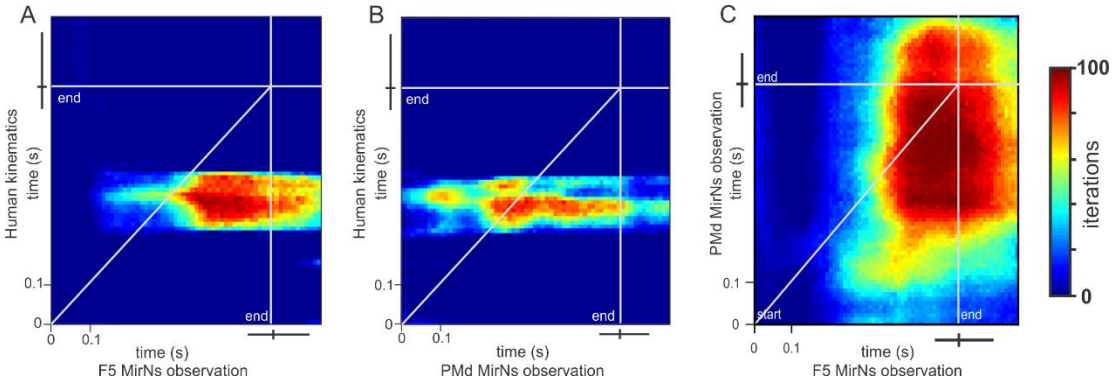

Figure 4.41 Bootstrapped representational similarity maps of key comparisons. Map of representational similarity across time between (A) the combination of the kinematic features and observation activity of F5, (B) the combination of the kinematic features and observation activity of PMd and (C) the activity of F5 and PMd MirNs during observation. Color indicates the number of times each comparison was significant ($p<0.05$). Other conventions as in Figure 4.11

4.4.2 Gaze-related modulation of activity in dorsal and ventral premotor cortex.

To investigate the effect of orbital eye position on the neuronal activity we performed planar regression analysis (Boussaoud et al. 1998; Cisek and Kalaska 2002) in which the neuronal activity during fixation periods was expressed as a function of the horizontal and vertical components of gaze direction. The strength of the modulation was assessed by the coefficient of determination (r^2) of the regression. The results of this analysis are summarized in Table 3. It is evident that more than half of the neurons in each area, condition and epoch do not display any statistically significant modulation of the activity by the gaze. Furthermore, for 75% of the rest of the cells the statistically significant gaze effect accounted for <15% of the observed response variance. Thus, the gaze related modulation in the present dataset is weak and is unlikely to account for the selective activity reported during both execution and observation. Analogous results were obtained also by Cisek and Kalaska (Cisek and Kalaska 2004) who investigated the strength of gaze related modulation of PMd neurons similar to ours that discharged both when a monkey executed a conditioned task and when the monkey observed visual stimuli associated with the performance of the same task either replayed or executed by the experimenter.

Recent studies reported that about 50% of the F5-MirNs were gaze–dependent (i.e. their discharge was stronger when the monkey looked at the action than when it did

not look at it). In addition, the response of these gaze-dependent MirNs was more intense and started earlier when the fixation onset occurred before than after hand–target contact (Maranesi et al. 2013). Thus, to verify the animals' engagement in the task, an eye position window of 9.5° in diameter, centered at the object, was used to count the trials at which monkey's gaze was within this window before the contact of the hand with the object. We found that this was the case in the vast majority of the trials (98.4% for PMd and 96.2% for F5). This high percentage indicates that our monkeys reliably gazed at the reference window before the end of the observed movement and guarantees that action observation evoked the optimum response of the gaze dependent MirNs.

Table 3: Impact of gaze position on the neuronal discharge

AREA	CONDITION	EPOCH	r^2					significant regressions (p<0.01)	
			min.	1st quartile	**median**	3rd quartile	max.	#	%
PMd	observation	800 ms prior to move	0.016	0.037	**0.052**	0.076	0.195	34	28
		movement	0.000	0.007	**0.018**	0.041	0.130	31	25
		hold	0.010	0.039	**0.049**	0.066	0.190	48	39
	execution	800 ms prior to move	0.021	0.039	**0.048**	0.066	0.214	46	38
		movement	0.039	0.063	**0.096**	0.137	0.328	48	39
		hold	0.029	0.048	**0.069**	0.096	0.230	53	43
F5	observation	800 ms prior to move	0.029	0.034	**0.036**	0.056	0.168	11	9
		movement	0.028	0.039	**0.057**	0.071	0.130	20	16
		hold	0.027	0.036	**0.054**	0.079	0.181	44	36
	execution	800 ms prior to move	0.022	0.036	**0.048**	0.071	0.276	41	34
		movement	0.051	0.072	**0.093**	0.126	0.311	31	25
		hold	0.022	0.049	**0.070**	0.100	0.351	47	39

4.5 Summary of results

- Mirror neurons in area F5 respond to the observation of both transitive and intransitive actions.
- The discharge of MirNs in area F5 is correlated with the action kinematics.
- Mirror and non-mirror neurons of area F5 represent actions differently during execution.
- Mirror neurons exist in the PMd.
- Mirror neurons in the PMd respond to the observation of both transitive and intransitive actions.

- The discharge of MirNs in PMd is correlated with the action kinematics.
- The discharge of PMd MirNs starts earlier than the discharge of F5 MirNs during both observation and execution.
- PMd and F5 MirNs represent actions similarly in both execution and observation.

5 DISCUSSION

5.1 Mirror neurons in area F5

One of the few claims regarding MirNs that has not been revised over the years is the claim that they require an interaction between effector and object in order to be triggered (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996; Rizzolatti et al. 2001; Rizzolatti and Craighero 2004; Rizzolatti and Sinigaglia 2010; Rizzolatti et al. 2014). In contrast to this claim, we demonstrate that MirNs are activated by the observation of both transitive and intransitive actions. Moreover, our study is the first to show that MirNs represent kinematic features of the transport and preshaping of the hand during observation of actions. Finally, in contrast to previous notions (Rizzolatti et al. 2014), we demonstrate that the motor properties of MirNs and non-MirNs of area F5 are not similar. Therefore the claim that MirNs encode the goal of the observed action, because non-MirNs do so (Umilta et al. 2008; Rizzolatti et al. 2014), is unsubstantiated.

5.1.1 Methodological considerations

Already in the early studies it was evident that the discharge of MirNs can be influenced also by factors other than the mere action observation. Among these factors are the hand used by the experimenter and the direction of the observed reaching-to-grasp action (Gallese et al. 1996). Recent studies revealed additional factors that modulate the discharge of MirNs. Caggiano et al. (Caggiano et al. 2009) found that the location, relative to the observer, at which the motor act takes place influences the discharge of MirNs. The perspective from which the motor acts of others are observed is another factor that modulates the discharge of the majority (74%) of MirNs (Caggiano et al. 2011). Many MirNs are also sensitive to the importance that the observed action has for the monkey (Caggiano et al. 2012). In our study, during observation, the actions were executed by the experimenter with its right hand, at the monkey's extrapersonal space, always at the same distance from the animal, with the same direction (from right to left) and a 45^{o} perspective. Moreover, the monkey received the same amount of water as reward at the end of each

trial. By keeping constant all the factors found to modulate MirNs' response we eliminated the possibility that factors other than the characteristics of the observed action contributed to MirNs' differential discharge. In our study the observed actions were performed at the extrapersonal instead of the peripersonal space. This arrangement provided the animal with a better view of the actions as they unfold and prevented monkey's own movements that might had occurred if the observed actions were executed in the monkey's reaching distance. Consequently, the observation elicited discharge was not contaminated with movement execution related activity. Finally, in the present study no cues were visible by the monkey during action observation. This way any influence of the presence of a contextual cue on the timing and the intensity of the activity during observation has been eliminated (Papadourakis and Raos 2013).

As is the case in the majority of electrophysiological studies of MirNs (Gallese et al. 1996; Umilta et al. 2001; Caggiano et al. 2009; Kraskov et al. 2009; Caggiano et al. 2012), a free-gazing observation condition has been adopted in our study. Animals' eye movements were recorded during the observation task only to ensure that the monkeys observed the presented action without any intention to pose restrictions on animals' oculomotor behavior. However, it has been reported recently that the discharge of half of the recorded MirNs was stronger when the monkey looked at the action than when it did not look at it. Moreover, the discharge of these gaze-dependent neurons was stronger and started earlier when fixation onset occurred before hand–target contact compared with when fixation onset occurred after hand–target contact (Maranesi et al. 2013). The consistency of the oculomotor behavior of our monkeys, as indicated by the high percentage of trials at which the gaze was within the reference window before the end of the observed movement, guarantees that the optimum response of the gaze dependent MirNs was evoked by action observation. Moreover, the gaze related modulation in the present dataset is weak and is unlikely to account for the selective activity reported during both execution and observation. Analogous results were obtained also by Cisek and Kalaska (Cisek and Kalaska 2004) who investigated the strength of gaze related modulation of PMd neurons similar to ours that discharged both when a monkey executed a conditioned task and when the monkey observed visual stimuli associated with the performance of the same task either replayed or executed by the experimenter

In our behavioral paradigm, the monkeys observed real natural actions instead of filmed ones because the former actions evoke stronger responses than the later (Caggiano et al. 2011). Inevitably, this choice lost us the opportunity to study characteristics of MirNs that are not possible using naturalistic stimulation. For example, the use of filmed actions would allow us to have transitive and intransitive movements with identical kinematics. This set up would allow us to quantify the contribution of the object presence and hand-object interaction at the discharge of single neurons. The choice of real hand actions has also the disadvantage of introducing variability in the kinematics across the sessions. This variability however was rather limited because the observed actions were well-practiced movements executed by the experimenter on a daily basis for months.

5.1.2 MirNs respond to the observation of intransitive actions

A previous study stated that pyramidal tract neurons in macaque ventral premotor cortex are modulated by the observation of intransitive hand actions, without providing any relevant data but the percentage of neurons endowed with this property (Kraskov et al. 2009). Our study confirms and extends these findings by providing a detailed description of the temporal profile and the intensity of MirNs' responses to the observation of both transitive and intransitive hand actions. Our findings are also compatible with the reports that MirNs in the inferior parietal areas AIP and PFG, which are reciprocally connected with area F5 (Petrides and Pandya 1984; Matelli et al. 1986; Cavada and Goldman-Rakic 1989; Luppino et al. 1999; Rozzi et al. 2006; Borra et al. 2008), respond to the visual presentation of an intransitive hand action (Maeda et al. 2015) or during the passive observation of movements of a simple shape in the visual field (Pani et al. 2014).

Our study resolves a contradiction in earlier reports concerning human and monkey MirN data. Observation of intransitive/meaningless movements activated the MirN system in humans (Fadiga et al. 1995; Iacoboni et al. 1999; Nishitani and Hari 2000; Buccino et al. 2001; Maeda et al. 2002; Grezes et al. 2003; Johnson-Frey et al. 2003; Patuzzo et al. 2003; Wheaton et al. 2004; Caspers et al. 2010), whereas did not activate MirNs of monkeys (Rizzolatti et al. 2014). In contrast to the last report, we had previously demonstrated by the quantitative ^{14}C-deoxyglucose method that intransitive movements

activate area F5 in monkeys, and that the intensity of this activation is half as strong as that induced by observation of a transitive action (Raos et al. 2007; Raos et al. 2014). In the present study we conclusively show that MirNs in the monkey brain respond to the observation of both transitive and intransitive actions and that the intensity of the MirNs' response to the observation of an intransitive action is lower than the response to the observation of the preferred transitive action. Consequently, there is no longer any reason to think that the human and monkey MirN systems respond differently to the observation of intransitive actions.

Contrary to our finding that the observation of intransitive actions activates MirNs, earlier studies did not report such responses (Umilta et al. 2001). What could be the reason for this discrepancy? It has been proposed that the training of the monkey in the execution task and its interaction with the experimenter might have augmented monkey's ability to build associations between executed and observed actions and this led to the high proportion (73%) of pyramidal tract MirNs responding to the observation of intransitive hand actions (Kraskov et al. 2009). A similar proposal was also advanced to explain the responses of MirNs to the observation of actions made with tools (Ferrari et al. 2005). Although the monkeys, either in ours or in Kraskov's study, were not trained to perform the intransitive actions, the impact of the frequent view of these actions during the experimental sessions on the neuronal response cannot be excluded.

5.1.3 Modulation of MirNs' responses by the kinematics of the observed actions

The hand motion, which can be described by kinematics parameters, is the common characteristic between transitive and intransitive actions and suffices to trigger the response of MirNs during observation. The strong representational similarity between the kinematics and the neural responses revealed in our study, suggests that MirNs in area F5 may provide a kinematics based representation of actions. The kinematic differences between transitive and intransitive actions (Goodale et al. 1994; Laimgruber et al. 2005; Fukui and Inui 2013) may contribute to the differential amplitude of the response to the observation of transitive and intransitive actions. Our finding is also consistent with the selective activation of the action observation network in humans observing actions that obey the kinematic laws of biological movements (Dayan et al. 2007; Press et al. 2011;

Agosta et al. 2016). Moreover, it is in agreement with the prediction of different families of models that MirNs are sensitive to the speed of the observed action (Demiris and Khadhouri 2006; Bonaiuto et al. 2007; Demiris et al. 2014). The sensitivity of MirNs to low–level features of the observed actions has also been proposed by other researchers (Cook and Bird 2013; Cook et al. 2014).

The kinematics of actions can be affected by various factors such as the quality of the object on which the action is directed (Jakobson and Goodale 1991; Paulignan et al. 1991; Castiello et al. 1993; Churchill et al. 2000; Gentilucci 2002; Schettino et al. 2003; Winges et al. 2003; Rand et al. 2007) or the lack of visual information for the movement guidance (Jakobson and Goodale 1991; Paulignan et al. 1991; Castiello et al. 1993; Churchill et al. 2000; Gentilucci 2002; Schettino et al. 2003; Winges et al. 2003; Rand et al. 2007). Moreover, the details of the kinematics available to the observer depend on the hand used by the actor or on the direction of the action as well as on the observer's view point. We suggest that the reported modulation of MirNs by factors such as the type of grasped object (Caggiano et al. 2012), used hand (Gallese et al. 1996), action direction (Gallese et al. 1996), viewpoint (Caggiano et al. 2011) and actor's gaze direction (Coude et al. 2016) may not be due to these factors per se. Based on our finding that the kinematics of the observed actions are represented in the discharge of MirNs, we propose that the alteration of the kinematics or of their availability to the observer induced by these factors may account for the reported differential responses of MirNs.

Umilta et al. (Umilta et al. 2001) reported that about half of the recorded MirNs fire during the observation of a transitive hand action even when the part of the action containing the interaction of the hand with the object is not available to the monkey because it takes place behind an occluder. The response in this "hidden" condition is obtained only if the monkey knows that an object exists behind the occluder. In the light of our finding it could be hypothesized that the view of the initial part of the action is sufficient to trigger the kinematic representation of the observed action and this results in similar observation elicited responses during the "early movement" epoch. The occlusion of the action kinematics during the "late movement" epoch may be responsible for the lower discharge obtained in the hidden condition as compared to the condition with the unconstrained view of the action.

Fogassi et al. (Fogassi et al. 2005) trained monkeys to perform and observe two actions: grasping a piece of food and putting it into the mouth (grasp-to-eat), or grasping a metallic solid and putting it into a container (grasp-to-place). They found that the discharge of many parietal MirNs during the execution or observation of the first part of the above actions (grasping) is influenced by the subsequent act (eating or placing). It has been proposed that the activation of these MirNs reflects 'intention understanding' in monkeys. The authors mention that the activation of MirNs may be modulated by factors such as the type of the object (food or metallic solid) or the context in which the action occurs (presence or absence of the container). On the other hand, the differences in movement kinematics were not considered to contribute to the neuronal selectivity. The authors recorded the kinematics of the monkey's actions and reported them to be different across conditions. By qualitatively inspecting neuronal and kinematic differences, they argued that the motor discharge of the parietal MirNs was not linearly correlated with the monkey's wrist speed. Unfortunately, the kinematic profile of the experimenter's actions observed by the monkey was not reported at all, making it hard to draw any conclusions on the influence of the kinematics to the modulation of the neuronal discharge during observation.

In an elegant experiment, Umilta and collaborators trained monkeys to grasp objects using tools that required different hand movement for the achievement of the action (Umilta et al. 2008; Rochat et al. 2010). They demonstrated that the representation in motor and mirror neurons is independent of joint or muscle-related details of movement. It has been speculated that the activity of motor and mirror neurons encodes the goal of a movement, regardless of how the goal is accomplished. However the goal of the movement is not the only extrinsic parameter that may be represented by the neuronal activity. Other extrinsic factors such as the direction of action in space (Kakei et al. 2001) or the motion of the end effector (fingers or tool) (Arbib et al. 2009) may also be represented. Both these latter factors can be described in kinematic terms that may be represented in the neuronal discharge as suggested in our study.

An argument used against the contribution of kinematics to the differential discharge of MirNs is the existence of equal proportions of neurons displaying opposite preferences (e.g. congruent and incongruent directions of gaze and hand actions) (Coude et al. 2016).

In this argument it is assumed that all neurons should exhibit the same direction of relationship between discharge and kinematics, i.e. a homogeneous group of neurons exists. Although appealing due to its simplicity, this assumption has never been verified experimentally. Another argument used to rule out the kinematic account is the timing at which the maximum differential discharge exists (Umilta et al. 2001; Coude et al. 2016). The rationale is that if the maximum differential discharge occurs at the contact of the hand with the object or later, when the hand does not move and its configuration is no longer changing, then the kinematics of the observed action cannot influence the discharge. In other words, it is assumed that the encoding of the kinematics of the observed movement by the discharge of premotor neurons should be synchronous, not affected by any transmission and processing delays. However, the response of premotor neurons is delayed in relation to the appearance of visual stimuli that trigger the neuronal activity and the average latency has been estimated to 127 ms (Lamme and Roelfsema 2000). In our study, 68% of the neurons exhibited their maximum differential discharge before the contact of the hand with the object. This percentage increased to 89% at 127 ms following this landmark. Thus, the response profile of the vast majority of the neurons is compatible with the processing of the kinematics. Moreover, the presence of differential discharge during the static phase of the action in the holding epoch has been also used to argue against the encoding of the kinematics by MirNs. Our analysis revealed that although the contribution of the object features to the regression model rose from 9.5 to 23.9 % as the hand changed from moving in the movement epoch to static in the holding epoch, the impact of the kinematics exceeded that of the object even when the hand was immobile.

Psychophysical studies have demonstrated that kinematics are influenced by the intention of the reaching-to-grasp action even when the object to-be-grasped is the same (Marteniuk et al. 1987; Ansuini et al. 2006; Ansuini et al. 2008). These results led to the proposal that the ability to perceive the intentions of others may be related to our competence to detect the kinematic dissimilarities between actions with different intentions (Ansuini et al. 2014). Although the evidence about the ability of the observers to use action kinematics in order to obtain intention information is contradictory (Manera et al. 2011; Sartori et al. 2011; Stapel et al. 2012; Naish et al. 2013), we cannot rule out the possibility that MirNs are involved in capturing some sort of high level cognitive

information concerning the observed actions by detecting subtle differences in the kinematics of actions with different intentions.

Models of the system of action observation, which take into account the fact that MirNs are active during both observation and execution, propose that MirNs may contribute to monitoring the progress of a self-action and in evaluating whether the performed action deviates from the intended one (Oztop and Arbib 2002; Oztop et al. 2005; Bonaiuto and Arbib 2010; Oztop et al. 2013). This function of MirNs would be consistent with our findings. The involvement of MirNs in action monitoring is further supported by the report that inactivation of area F5 on the convexity, where the majority of MirNs reside, results in a slowing down of the movement with no other cognitive or motor deficits (Fogassi et al. 2001). Moreover, the prediction of different families of models that MirNs are sensitive to the speed of the observed action is in agreement with our finding that action kinematics are encoded by MirNs (Demiris and Khadhouri 2006; Bonaiuto et al. 2007; Demiris et al. 2014).

All in all, these results dictate a re-evaluation of the function of MirNs. Our finding that MirNs start discharging shortly after the onset of the observed movement, combined with the finding that they encode movement kinematics, indicate that these neurons may be involved in the rapid detection and monitoring of others' actions as they unfold. In other words, action mirroring may also be performed at the level of the observed action kinematics.

5.2 Mirror neurons in PMd

The existence of mirror neurons in PMd documented in our study is in agreement with a neuroimaging study reporting that observation of others' actions activates dorsal premotor areas F2 and F7 of the macaque brain (Raos et al. 2007). It is also compatible with neurophysiological studies reporting that neurons in the dorsal premotor cortex discharge both when a monkey executes a conditioned task (moving a cursor to capture targets on a computer screen) and when the monkey observes the same task done by the experimenter (Cisek and Kalaska 2004; Tkach et al. 2007). Although these neurons displayed a response pattern similar to that of mirror neurons, they were not considered to be "mirror neurons" and PMd was not considered to be a node of the mirror neuron

circuit. Our study conclusively demonstrates that PMd contains neurons that fulfil the "mirror neuron" criteria as defined in the original studies (di Pellegrino et al. 1992; Gallese et al. 1996) .i.e. they fire both when the animal performs goal-directed actions and observes another agent performing similar actions.

5.2.1 Origin of visual responses of PMd

The presence of neurons that are influenced by the mere observation of a motor act in the PMd raises the question of the origin of their visual input. The first candidates for supplying this kind of information are the cortical areas containing MirNs, i.e. the ventral premotor area F5 (di Pellegrino et al. 1992; Gallese et al. 1996) and the areas of the rostral half of the inferior parietal lobule (Fogassi et al. 2005; Rozzi et al. 2008). Area F5 is heavily connected with the lateral part of F2, F2vr (Marconi et al. 2001). However, the fact that the responses of F5 neurons to action observation follow those of PMd neurons favors a direction of information flow from dorsal to ventral premotor cortex rather than the reverse. Therefore, area F5 is not a possible source of action-related visual input to PMd. On the other hand the possibility that the parietal cortical areas transmit this kind of information to PMd cannot be excluded, although the parietofrontal projection is weak (Rozzi et al. 2006).

F2vr is the only premotor area that receives input from an area specifically devoted to motion perception. The ventral part of area F7 (excluding the SEF) and area F2vr are both targets of the caudal part of the upper bank in the STS (uSTS). Specifically, F2vr is the target of projections from a relatively more rostral and ventral sector of the uSTS, close to the fundus of the sulcus, which presumably corresponds to area MST (Luppino et al. 2001). Area MST plays an important role in visual motion processing (Komatsu and Wurtz 1988) and is the target of projections from the motion-sensitive visual area MT/V5 (Maunsell and van Essen 1983; Ungerleider and Desimone 1986). Consequently, areas of the superior temporal lobe could supply motion related information directly to the dorsal premotor cortex. Interestingly, observation of others' actions activates components of the motion complex in STS, including the medial superior temporal area (MST), the fundus of superior temporal area (FST), and the middle temporal area (MT) (Kilintari et al. 2014).

Another possible, not mutually exclusive, hypothesis is that visual information is provided to the dorsal premotor cortex by superior parietal areas endowed with visual properties. F2vr receives its major visual input from areas V6A and MIP of the superior parietal lobe whereas ventral and caudal parts of F7 are targets of area PGm in the medial wall of the hemisphere and the ventral part of V6A (Cavada and Goldman-Rakic 1989; Johnson et al. 1996; Matelli et al. 1998; Shipp et al. 1998; Marconi et al. 2001) (Petrides and Pandya 1984). Motion-related visual information is conveyed to the posterior parietal areas from extrastriate areas of the occipital lobe, including V6, and from visual areas of the superior temporal sulcus (Galletti et al. 2001; Gamberini et al. 2009; Passarelli et al. 2011). It should be noted that the posterior parietal areas V6A, MIP, and PGm, suggested to be nodes of the circuit transmitting visual information to the dorsal premotor cortex, were found activated by observation of others' transitive and/or intransitive actions (Evangeliou et al. 2009; Raos et al. 2014).

5.2.2 Role of PMd MirNs in action observation and execution

A reasonable question would be, which is the role of MirNs in PMd. PMd is the premotor hub of the dorso-dorsal (Rizzolatti and Matelli 2003) or dorsomedial (Galletti et al. 2003) pathway of the dorsal visual stream (Ungerleider and Mishkin 1982; Goodale and Milner 1992; Milner and Goodale 1995) which originates from the superior parietal lobule. It is widely accepted that this pathway is implicated in the "on line" control of actions (Desmurget et al. 1999; Pisella et al. 2000; Galletti et al. 2003; Rizzolatti and Matelli 2003; Galletti and Fattori 2017).To guide forelimb movements visually, information about the location and configuration of the hand as well as about the location of the target is necessary. PMd neurons carrying such information have a central role in the visual control of movements. It was reported that neuronal activity in the PMd during planning a target-capturing task co-varied with the image motion rather than with the actual movement of the arm, in other words PMd was involved in processing visual information for the spatial guidance of the movement trajectory (Ochiai et al. 2002). Raos et al (Raos et al. 2004b) found that many reaching-to-grasp neurons were influenced by the visual input concerning the scene in which the action occurred and, in particular, by the vision of the hand approaching to and interacting with the object. In agreement with these findings, the ventral part of the dorsal premotor area F2 was activated exclusively

for visually-guided forelimb movements in neuroimaging studies (Gregoriou and Savaki 2003; Gregoriou et al. 2005; Raos and Savaki 2011). Archambault et al (Archambault et al. 2011) examined the hand trajectories of monkeys performing reaching movements either to stationary targets or to targets that were displaced to a new spatial location at the movement onset. In the former case the trajectories of the planned and the executed movements were the same whereas in the latter case the trajectory of the planned movement had to be updated to account for the changed location of the target. It was demonstrated that the same neurons were active during both direct and corrected reaches and that PMd neurons were recruited earlier than the parietal ones. This timing of the responses prompted the investigators to suggest that the role of the PMd is to detect the need for trajectory corrections while the parietal cortex is crucial for the update of the trajectory by providing an estimation of the kinematics of the new movement.

The participation of PMd in the online correction of movements is manifested by its transient inactivation and its lesion. Perturbation of PMd by transcranial magnetic stimulation during a visuomotor adaptation task disrupted the ability of the subjects to make online adjustments only when the vision of the hand was available but not when prevented (Lee and van Donkelaar 2006). Patients with premotor lesions needed more time to correct the trajectory of their movements to displaced targets than controls or patients with lesions to other cortical areas whereas did not display any deficit in accuracy (Buiatti et al. 2013).

The fact that the view of the continuously changing configuration of the hand as it approaches the object to be grasped triggers the discharge of MirNs renders these cells ideal for monitoring the progress of actions as they unfold. We propose that MirNs may be part of a physiological process detecting whether the ongoing response deviates from its goal. In the execution mode, this information may be used by the motor system of the actor to modulate the motor control signals in order to specify/formulate a corrected plan for the achievement of the goal, thus providing real-time visual guidance of movements. In the observation mode, it may allow the observer to appraise whether the observed movement will be successful or not in order to organize its own behavior.

The proposed function of MirNs complements rather than excludes the involvement of MirNs in action understanding. Visual control parameters sub-serving sensorimotor control have been used for the development of computational models that

could effectively infer mental states of others (Oztop et al. 2005). The involvement of MirNs in monitoring the success of a self-action was originally advanced by Bonaiuto and Arbib (Bonaiuto and Arbib 2010) who predicted that the discharge of MirNs during execution would be modulated by the view of the monkey's hand. Maranesi et al (Maranesi et al. 2015) confirmed this prediction showing that MirNs encode, in addition to the view of the other's hand, the visual feedback of monkey's own hand. The validity of our proposal could be verified in future experiments that will study whether, how and when the alteration of the view of one's own or other's hand influence the discharge of MirNs during execution and observation.

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