Aus dem

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Predictable Context–Based Encoding of Observed Actions in Mirror Neurons of Macaque Premotor Area F5

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List of abbreviations

AIP	anterior intraparietal area
CNS	central nervous system
dPCA	demixed principal component analysis
DTI	diffusion tensor imaging
FEF	frontal eye field
fMRI	functional magnetic resonance imaging
GLM	generalized linear model
IPL	inferior parietal lobule
LB2	lower bank 2
LIP	lateral intraparietal area
lPFC	lateral prefrontal cortex area
MFC	medial frontal cortex
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
pAIP	posterior portion of the anterior intraparietal area
PCA	principal component analysis
PM	premotor cortex
PMd	dorsal premotor cortex
PMv	ventral premotor cortex
PTNs	pyramidal tract neurons
STPm	middle superior temporal polysensory area
STS	superior temporal sulcus
VIP	ventral parietal area
vlPF	ventrolateral prefrontal area

1. Introduction

The goal of the thesis

My thesis is an attempt to contribute to a better understanding of the role of the ventral premotor cortex as a major element of the so-called “mirror system" that has been implicated in a wide spectrum of functions that remain controversial despite three decades of meticulous research. My thesis was an attempt to critically examine one of the various hypotheses, namely the idea that this system might contribute to the subject´s ability to act, taking the behavior of others into account. In order to justify this hypothesis and certainly also in order to make it more comprehensible, we will first try to review what we know about the structure at stake, the ventral premotor cortex, also known as area F5.

1.1 The neuroanatomy of area F5 in macaque

1.1.1 Location and subregions of area F5

In 1870, Gustav Fritsch and Edvard Hitzig, two German physicians, discovered that direct current stimulation of specific regions of the cerebral cortex produces movement in dogs. Further, they demonstrated that a lesion of the movement-related region as determined by electrical stimulation produced palsy of the contralateral foreleg. Based on their findings, they concluded that this region on the convexity of the cerebrum was essential for the animal´s ability to move (Fritsch and Hitzig, 1870, Gross, 2007, Fritsch and Hitzig, 2009). Inspired by Fritsch and Hitzig’s study, David Ferrier in Great Britain used longer-duration biphasic electric stimulation to stimulate the cerebral cortex of dogs and other species, especially in monkeys, and demonstrated that the cerebral cortex offers a topographic "motor map" (Ferrier, 1873, Ferrier, 1875, Gross, 2007).

The present anatomical characterization of area F5 represents the endpoint of efforts to distinguish regional differences in the cerebral cortex that started with the work of Korbinian Brodmann early in the beginning of the twentieth century. Brodmann identified and numbered different areas of the cerebral cortex of both monkeys and humans as a result of observing characteristic subtle regional differences in cortical cytoarchitecture by staining neurons with the Nissl method. Two of the cortical regions distinguished by him were area 4 and area 6, distinguished in both monkeys and humans, the latter adjoining area 4 rostrally (Brodmann, 1909, Brodmann, 2007). Considering the work of Cécile and Oskar Vogt, who had shown that electrical stimulation of the cortex in front of the central sulcus evoked movement (Vogt and Vogt, 1907, Vogt and Vogt, 1926) – this corroborating the earlier findings of Fritschand Hitzig and Ferrier, Brodmann implicated the two areas as substrates of movement control, however, without being specific as to possible differentials contributions of the various regions to the movement. It was John F. Fulton who much later verified that lesions of area 6 in nonhuman primates produced disturbances in the execution of skilled movements that could be distinguished from those of area 4 and proposed a name – premotor cortex (PM) – conveying a functional role as an alternative to Brodmann´s purely descriptional designation as area 6. He showed that a lesion of the premotor cortex disrupted the coordination of highly integrated voluntary movements, caused the appearance of spasticity of the extremities with increased deep tendon reflexes, vasomotor disturbances and forced grasping, and other signs (Fulton, 1935). The Nissl technique suggested that the cytoarchitecture of area 6 is homogenous. Yet, the results of a novel approach to staining for cytochrome oxidase, a marker of cellular metabolic activity (Wong-Riley and Carroll, 1984, Wong-Riley, 1989), required a reconsideration of this view. More specifically, based on subtle differences in laminar patterns revealed by this staining method, the posterior part of the frontal lobe of macaque monkeys could be parcellated into 5 areas (F1-F5), with 4 of them (F2-F5) comprising Brodmann´s area 6 and F1 corresponding to area 4 adjoining the central sulcus. F4 and F5 located in the inferior part of area 6 are often collectively referred to as ventral premotor cortex (PMv) (Matelli et al., 1985, Rizzolatti et al., 1998). A further breakdown of F5 was suggested by Nelissen and coworkers in 2005, based on a combination of fine-grained cytoarchitectonic differences, revealed by SMI-32 immunocytochemistry, based on an antibody against a specific neurofilament-antigen and functional data, obtained by functional magnetic resonance imaging (fMRI) in rhesus monkeys. According to this work, F5 can be divided into three sectors: F5c, which lies on the convexity adjacent to the inferior arcuate sulcus, F5a which is the anterior sector located on the lower posterior bank of the arcuate sulcus and F5p which corresponds to the posterior sector located on the upper posterior bank of the arcuate sulcus (Nelissen et al., 2005). This breakdown of F5 into the aforementioned three sectors was confirmed by Belmalih and his coworkers in an immunohistological study of the monkey brain resorting to immunohistochemical staining for SMI-32 and calbindin (Belmalih et al., 2009). It is worth noting that the three sectors do not have clearly visible boundaries on the surface of the cortex. Moreover, T1- and T2-weighted magnetic resonance imaging (MRI) anatomical scans of the monkey brain have not been able to distinguish the three sectors, e.g. based on characteristic landmarks or signal differences (Figure 1) (Rizzolatti et al., 2014). Hence, the anatomical differentiation of these three sectors requires a post-mortem immunohistochemical investigation. It is at best possible to approximate them based on their location relative to the arcuate sulcus whose fundus marks the rostral boundary of F5. This sulcus is easily identifiable after the craniotomy, needed for the placement of microelectrodes, even when covered by dura. Functional data based on electrophysiological recordings suggest that F5a and F5c might play a role in memory-related processing as well as in decision-making based on sensory information discrimination (Kurata, 2018, Caprara and Janssen, 2021). This work has argued that information from F5a and F5c is transmitted to F5p where it contributes to eye-hand coordination.

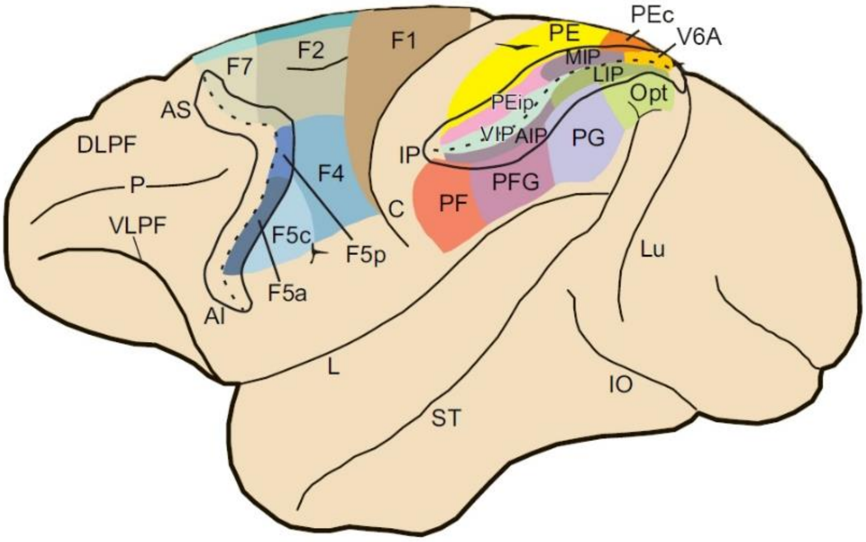

Figure 1 Location and sectors of F5 Adapted from **(Rizzolatti et al., 2014).** AI, inferior arcuate sulcus; AS, superior arcuate sulcus; C, central sulcus; DLPF, dorsolateral prefrontal cortex; IO, inferior occipital sulcus; IP, intraparietal sulcus; L, lateral fissure; Lu, lunate sulcus; Opt, part of caudal interior parietal convexity, caudal to PG; P, principal sulcus; PE, rostral superior parietal lobule; PEc, apex of the superior parietal lobule; PEip, the intraparietal sector of area PE; PF, part of rostral inferior parietal convexity; PFG, part of rostral inferior parietal convexity, caudal to PF; PG, part of caudal inferior parietal convexity, caudal to PFG; ST, superior temporal sulcus; VLPF, ventrolateral prefrontal cortex.

1.1.2 Types of neurons in area F5

Area F5 in the macaque is thought to be involved in the planning and control of hand movements and guided by sensory information. Neurons in area F5 can be separated into distinct groups, considering the different significance of sensory and movement-related influences. F5 motor neurons are characterized by movement-related activity, lacking sensory influences. They are typically activated by hand or mouth movements (Rizzolatti et al., 1981, Kurata and Tanji, 1986, Rizzolatti et al., 1988). Visual neurons are confined to the observation of an object-directed action (Rizzolatti et al., 1988). Neurons whose visual response can be more specifically associated with the vision of the object are referred to as canonical neurons (Murata et al., 1997, Rajmohan and Mohandas, 2007). Mirror neurons combine visual and motor-related influences. They discharge both during the observation of an object-directed action and the execution of a similar action by the observer (Di Pellegrino et al., 1992, Rizzolatti et al., 1996, Gallese et al., 1996). Mirror neurons, the central interest of my research, will be described in more detail later. Many F5 neurons contribute to the pyramidal tract (Dum and Strick, 1991). These F5 neurons - pyramidal tract neurons (PTNs) – not only exhibit movement-related signals (Shimazu et al., 2004, Lemon, 2008) but surprisingly also mirror neuron properties (Kraskov et al., 2009). Not directly related to the categorization of neurons along a sensory-to-motor axis are the well-established roles of F5 neurons in decision-making processes (Romo et al., 2004) and in working memory (Pardo-Vazquez et al., 2009).

1.1.3 The connections of area F5

To investigate the connections of area F5 of the macaque, mainly three methods have been used over the years: the study of the transport of retrograde/anterograde axonal tracers, MRI and simultaneous electrophysiological recordings across multiple cortex areas.

Tracer Studies

Axonal tracers come in two variants: retrograde tracers are taken up by synaptic endings of neurons and then transported back to the soma whereas anterograde tracers are taken up by the cell body and then transported anterogradely to the axonal terminals contributing to synaptic contacts. In both cases, the tracers are injected at locations whose connections are at question – hence this well-established approach to the study of connections in the central nervous system (CNS) (Borra et al., 2010) is in any case invasive. The most recent addition to the collection of tracers used are virus-based tracers, transported antero- or retrogradely and able to cross synapses, allowing the labeling of multisynaptic pathways in the brain (Xu et al., 2020). In Kiyoshi Kurata's review of tracer studies on the connections of area F5, he summarised 18 studies on the connectivity of macaque area F5, distinguishing three groups of connections: corticospinal connections, corticocortical connections, and thalamic nuclei connections (Table 1) (Kurata, 2018).

Table 1 Connections of macaque area F5 as revealed by axonal tracer studies

Groups	Connected areas
Corticospinal	C2-T1, L6-S1
Corticocortical	Frontal cortex: M1(F1), F2, F3, F4, F6, F7, 12, 24, 44, 46, DO, PrCO, GrFO
	Parietal cortex: PF, SII, VIP, AIP, PFG, PE, PG
Thalamic nuclei	X, VLc, VPLo, VLo, Cl, MD

MRI

fMRI uses changes in blood oxygenation and blood volume (the BOLD signal) as a proxy of neuronal activity (Glover, 2011). fMRI has been used to reveal the location of "mirroring activity" in the human brain, characterized by brain activity evoked by action execution and observation. As will be discussed in detail further below, mirroring activity is one of the key hallmarks of monkey area F5 (Fiave et al., 2018). It became quickly clear that "mirroring" related BOLD activity is not confined to the human premotor cortex but found in an extended network of areas that involves other parts of the parietal cortex (Iacoboni et al., 1999). Correlated changes of the BOLD signal in this mirroring or "mirror neuron" network have been used to draw conclusions on connections (Caspers et al., 2010). More recently, diffusion tensor imaging (DTI), a magnetic resonance imaging technique that detects differences in water diffusion as a proxy of fiber orientation in order to unravel fiber tracts connecting brain areas has been added to the portfolio of noninvasive tools for the study of connections (Hamzei et al., 2016). While widely used in studies trying to identify a human "mirror neuron network", the application of MRI-based approaches in the study of the mirror network in monkeys has been comparatively rare as they are unable to deliver the degree of resolution offered by invasive tract-tracing methods. Another reason that has limited the usage of MRI in studies of monkey area F5 is the constraining and noisy scanner environment that severely restricts the study of behaving monkeys, behavior that is essential for attempts to identify the functional role of this area and its network partners. One of the few early efforts to use fMRI in an attempt to further the understanding of monkey area F5 is the work of Nelissen and his coworkers, mentioned further up. This study identified cortical areas in macaque monkeys activated by action observation. The authors identified four frontal areas involved in action observation: F5 and areas 45A, 45B, and 46 that responded to the observation of a hand grasping objects (Nelissen et al., 2005). A subsequent fMRI study by Nelissen and his coworkers expanded the cortical territory involved in action observation by describing elongated regions in both the lower and upper banks of the superior temporal sulcus (STS), the inferior parietal lobule area PFG, and the anterior intraparietal area (AIP) to be activated when the macaque monkey observed a video clip showing grasping actions. In this study, the correlation of fMRI data with anatomic data based on axonal tracer studies suggested that two distinct functional pathways might convey visual action information from the STS to F5: (1) a middle superior temporal polysensory area (STPm)- PFG–F5c pathway, which might convey visual information that is involved in understanding the agent's intentions and (2) a lower bank 2 (LB2) –AIP–F5a/p pathway, which might be more concerned with the object of the action and might aid in understanding goal-directed motor acts (Nelissen et al., 2011). On the other hand, another monkey fMRI study carried out by the same group found that area F5, somatosensory cortex (SI, SII, area 5), AIP, PFG, and F1 are activated when the macaque performed reaching and grasping movements (Nelissen and Vanduffel, 2011). In a recent study, Nelissen's group combined the tasks of the two aforementioned fMRI studies (i.e. observation tasks (Nelissen et al., 2011) and execution tasks (Nelissen and Vanduffel, 2011)), which means that the monkeys needed to perform motor acts and in separate blocks of trials they had to observe video clips of humans performing similar actions. The authors found in both observation and execution tasks activity in (1) F5, AIP, PFG, the ventrolateral prefrontal area (vlPF) and SII in the contralateral hemisphere of monkeys and (2) F5, vlPF and SII in the ipsilateral hemisphere of animals (Fiave et al., 2018).

We may add that not only agent–object interactions, no matter if executed or observed, but also the observation of social interactions between other individuals (monkey-monkey) as well as physical interactions implying a causal relationship (object-object) can activate area F5 and the posterior portion of the anterior intraparietal area (pAIP) (Sliwa and Freiwald, 2017).

Simultaneous electrophysiological recordings across multiple cortical areas

Functional signals revealed by fMRI provide some insight into patterns of interactions, yet the limited spatial and in particular, the limited temporal resolution prevent zooming in into more fine-grained interactions of area F5 with other parts of the brain. Much more information is promised by simultaneously recording neuronal activity across cortical areas in order to unravel the functional significance of connections demonstrated by axonal tracer studies. This is the idea that was implemented by Schaffelhofer and Scherberger (2016) who simultaneously recorded neurons from AIP, F5, and M1, areas implemented as key areas of a cortical grasping network, considering the results of electrophysiological studies of area F5 mentioned before and related work on areas M1 and AIP (Castiello, 2005, Davare et al., 2011). In the experiments by Schaffelhofer and Scherberger the monkeys saw different objects, requiring different grips that they were asked to perform. The results obtained suggested a flow of information on the visual properties of an object and its affordances in AIP to information relevant to movement execution in M1 with area F5 representing an intermediary stage that could be loosely related to movement preparation. In a follow-up publication, the same research group showed that neurons in all three areas encoded the actual grasp force during grasp execution similarly whereas the intended grasp force was most strongly represented in area F5 (Intveld et al., 2018).

Simultaneous recordings were also carried out from areas AIP, F5 and F6 (Ferroni et al., 2021). In this experiment, monkeys observed an experimenter performing a Go/No-Go grasping task or, alternatively, performing the task themselves. Observation-related discharge was found in all three areas. When analyzing the direction of information exchange between these areas, the authors revealed a clear dependence on the task phase:

(1) In the “instruction cue” phase and “go signal” phase respectively, information flowed from area F6 to F5 and then to AIP. (2) In the “object presentation” phase, the flow was from area AIP to F5 and then to F6 in accordance with the conclusion of the aforementioned study by Schaffelhofer and Scherberger (2016). (3) Finally, in the action observation phase, the starting point of information was F5, and then it was passed to F6 and AIP respectively.

Simultaneous recordings from areas F5 and M1 were also carried out by Mazurek et al. (2018). These authors focused on mirror neurons, found earlier also in M1 (Tkach et al., 2007, Dushanova and Donoghue, 2010, Vigneswaran et al., 2013), in an attempt to unravel functional differences. According to Mazurek et al., F5 mirror neurons exhibited much more similar observation and self-action-related discharge profiles than mirror neurons in M1. Interestingly, PTNs in M1 may also exhibit the characteristic features of mirror neurons (Vigneswaran et al., 2013). As these neurons contact spinal cord motoneurons, a one-to-one transformation of observation-related discharge onto execution-related responses would render the agent a slave of others´ actions, forced to continuous imitation. Hence, the fact that the observation-related discharge of these PTN neurons differs profoundly from the execution-related discharge, in many cases exhibiting an inhibitory signature rather than the excitatory profile of the execution-related discharge, clearly argues against a role in imitation (Vigneswaran et al., 2013). As said earlier, PTNs are not confined to M1 but may also occur in the premotor cortex including area F5 where they may exhibit the mirror neuron property (Kraskov et al., 2009). But also these F5 PTN mirror neurons often present inhibitory discharge profiles to action observation associated with excitatory profiles for execution.

Another cortical region containing neurons that combine sensitivities to the observation or monitoring of actions carried out by the other and actions executed by the observer (i.e., self vs. other actions) is the medial prefrontal cortex (mPFC). A recent electrophysiological study explored the flow of information between F5 and the mPFC (Ninomiya et al., 2020). These authors found increased delta-band coherence between the two areas, both during action observation and execution. Resorting to a cutting-edge double viral vector infection inactivation technique (Kinoshita et al., 2012, Kinoshita et al., 2019), causing a selective blockade of the F5 to mPMC projection, this group established that this manipulation compromised the monkey´s ability to process observed, yet not executed actions.

Electrophysiological studies trying to shed light on the interdependence of areas in the motor cortex have not only been performed in the same brain hemisphere but also across hemispheres. In a recent study by Moreau-Debord et al. (2021) on the mechanisms of recovery from motor cortex lesions, the authors asked if experimental lesions of M1 might entail adjustments in the contralateral hemisphere. To this end, they reversibly inactivated ipsilateral M1 with muscimol while recording from PMv in both hemispheres in association with grasping movements of both the affected and the unaffected arm. They found substantial changes in firing rates in PMv on both sides that varied with the movement phase. If and how this reorganization could drive the recovery from longer-lasting lesions needs to be explored.

1.2 A closer look at F5 mirror neurons

F5 mirror neurons were discovered in area F5 of Macaca nemestrina monkeys in a seminal study carried out at the University of Parma in 1992 (Di Pellegrino et al., 1992). The authors – Giacomo Rizzolatti and his coworkers had trained the experimental monkeys to retrieve objects of various sizes and shapes offered in different spatial positions at different points in time after the presentation of an object. The goal of the planned experiments had been the investigation of neuronal signals guiding the object grasp, exhibited by F5 neurons, introduced further up as motor neurons. However, they found not only motor neurons but serendipitously also neurons in F5 that could be activated not only when the monkey retrieved objects but also when the monkey passively observed the experimenter carrying out a similar object-directed action. In addition, they also found that this activity remained absent when the demonstrator simulated the grasping action, yet the object was absent. Moreover, the spatial position and the distance between the object and the monkey influenced the intensity of neuronal responses. In the publication that reported this seminal finding, the authors suggested that these neurons might be the neural substrate of action understanding and in follow-up studies they introduced the term - “mirror neuron” to refer to this conspicuous type of neuron (Rizzolatti et al., 1996, Gallese et al., 1996) characterized by a discharge modulation evoked by both, an individual carrying out a grasping action and the same individual seeing another (the "demonstrator") performing a similar motor act.

It is worth noting that in the initial study and subsequent studies of neuronal activity in F5, the experimental animals watched the demonstrator carrying out a motor act without any involvement in the other´s motor act, thus in a fully passive and largely uncontrolled manner. Only later, eye trackers were used to ensure that the experimental animals would at least pay attention to the demonstration. However, ensuring the vision of the demonstrated motor act by imposing an eye position control window aligned with it does not necessarily require processing of the semantic content of the observed motor act needed to guide the observer's own behavior.

The discovery of mirror neurons in F5 of the macaque cortex and their further investigation prompted a wealth of research that explored the possible existence of mirror neurons in other parts of the brain of macaque monkeys and soon also other species including humans. In fact, before long, neurons with mirroring properties in the macaque cortex were found in the inferior parietal lobule (IPL) (Fogassi et al., 2005), M1 (Tkach et al., 2007, Dushanova and Donoghue, 2010, Vigneswaran et al., 2013), F2 – also referred to as dorsal premotor cortex (PMd) (Tkach et al., 2007, Papadourakis and Raos, 2019), lateral intraparietal area (LIP) (Shepherd et al., 2009), ventral parietal area (VIP) (Ishida et al., 2010), medial frontal cortex (MFC) (Yoshida et al., 2011), AIP (Pani et al., 2014), lateral prefrontal cortex area (lPFC) (Falcone et al., 2016), area F6 – also referred as the pre-supplementary motor cortex (Livi et al., 2019) –, medial parietal cortex V6A (Breveglieri et al., 2019) and S1 (Atique and Francis, 2021). The finding that neurons in all these places may combine sensitivity to observed and executed actions much like F5 mirror neurons should not obscure the fact that on closer examination such neurons may be characterized by important differences, depending on the region in which they reside. For instance, mirror neurons in M1 differ from those in F5 by exhibiting much less similar observation and self-action-related discharge profiles (Mazurek et al., 2018, Jerjian et al., 2020).

Neurons with mirroring properties have not only been identified in electrophysiological recording studies of the macaque cortex but have also been found in quite a few other species such as songbirds (Prather et al., 2008), marmosets (Suzuki et al., 2015), bats

(Omer et al., 2018) and rats (Carrillo et al., 2019, Viaro et al., 2021). Trying to relate these neurons to mirror neurons found in the macaque brain and in particular to those in F5 is far from being straightforward as the brain regions implied in the various species are not easily comparable and because of differences in the modalities studied (e.g. mirroring in the auditory domain in the case of songbirds in contrast to an emphasis on vision in studies of nonhuman primates) or the motor acts studied (e.g. vocalization in the case of songbirds vs. hand movements in macaques). As to humans, the first attempt to identify cortical areas possibly accommodating mirroring activity resorted to an fMRI approach. In this experiment, subjects were asked to observe a finger movement carried out by a demonstrator in order to then imitate it (Iacoboni et al., 1999). Two parts of the brain showed significant BOLD activity in conjunction with the imitation required, the left frontal operculum and the right anterior parietal lobule. Although the authors discussed their results within the framework of previous studies of F5 mirror neurons, any attempts to compare the BOLD patterns found with data on mirroring in monkeys are disqualified by the absence of evidence for imitation in the latter. Moreover, the imitation task used by Iacoboni and colleagues studied an intransitive action, i.e. an action that was not object-directed in contrast to the study of object-directed motor acts in monkeys. In any case, this study was the starting point of a flood of studies that have tried to find support for the existence of a human "mirror neurons system" resorting to fMRI (Bonini et al., 2022). The merits of this work notwithstanding, arguably much more important have been the few attempts to demonstrate the existence of actual mirror neurons in electrophysiological recordings from the human brain. As recordings are inevitably invasive they are only acceptable if carried out in order to serve a clinical need in the very best interest of the patient. This was the case in the seminal study in which the researchers recorded single neurons from patients with pharmacologically intractable epilepsy, requiring an electrophysiological identification of the origin of their seizures with depth electrodes (Mukamel et al., 2010). In this study the authors found that a significant proportion of neurons encountered in the medial frontal cortex and medial temporal cortex were activated by both action execution and action observation, therefore certifying them as mirror neurons.

1.3 Towards the function of F5 mirror neurons

Since 1992, a large number of articles on mirror neurons in animals and humans have been published, and they concur that mirror neurons play some role in processing information on goal-directed actions (Glenberg, 2011). Yet, beyond this very general characterization, working out a more specific function has turned out to be difficult and the resulting concepts have remained controversial. In fact, one may wonder if there is one unique function, given the fact that mirror neurons are found not only in F5 but many other parts of the brain and, moreover, in quite diverse species, suggesting that mirror neurons may actually be functionally heterogeneous (Bonini et al., 2022, Heyes and Catmur, 2022). Here, we will focus on mirror neurons in F5 of the macaque and review the evidence for 2 potential functions of relevance for my study, whose goal was basically to help decide between these two functional concepts.

1.3.1 Action understanding

Already in their initial study, Rizzolatti and his coworkers suggested that mirror neurons might be involved in action understanding by writing that (action) "*...retrieval can also occur in response to the meaning of the gestures made by other individuals*" (Di Pellegrino et al., 1992). Findings that paved the ground for an elaboration of the action observation function of mirror neurons were obtained in subsequent studies. In experiments by Gallese et al. (1996), the group compared the impact of different grip types and motor acts of the human demonstrator and the experimental monkey respectively. For instance, the demonstrator could grasp an object using a precision grip, multi-finger prehension or whole-hand prehension in order to hold the object, manipulate it or place it elsewhere using various grip types as required by the size, shape and the object. And similarly, depending on the object, the self-action of the experimental monkey could require a precision grip, multi-finger prehension or whole-hand prehension. Relying on this richer set of motor acts, F5 mirror neurons could then be further subdivided into three subgroups, considering the degree of congruence between their observation and execution-related discharge modulation. Out of a group of 92 mirror neurons, only a minority of 31.5% were found to be 'strictly congruent' mirror neurons, characterized by a strict correspondence of the preferred motor act during observation and self-action. However, two-thirds lacked this correspondence, the 'non-congruent' mirror neurons (7.6%), not presenting any relationship between observation and self-action related responses at all and a much larger group, the 'broadly congruent' mirror neurons (60.9%) exhibiting at least some similarity, i.e. at least one action type could activate neuronal responses in both action observation and action execution. For instance, such a mirror neuron might prefer the execution of a precision grip and also respond to the observation of a precision grip, yet also indiscriminately respond to the observation of other grip types. The results from a second study of the same group that used very similar paradigms yielded comparable results (Rizzolatti et al., 1996). These studies that had established the existence of at least a fraction of mirror neurons exhibiting strict congruence of their observation and self-action preferences, allowed this group to draft the idea that F5 mirror neurons might accommodate action understanding by mapping information on observed actions onto the observer´s motor repertoire – the "direct-matching hypothesis" or in the authors own words: "*We understand actions when we map the visual representation of the observed action onto our motor representation of the same action*" (Rizzolatti et al., 2001). The idea was basically that the observer's motor system "resonated" when a specific type of action was observed.

Considering the results obtained in subsequent work, the notion that F5 mirror neurons might underpin action understanding has continued to develop with increasing attention attributed to a role in representing the other´s action goals and/ or intentions. The first major argument suggesting this notion was provided by Umiltà et al. (2001) who tested observation-related responses of F5 mirror neurons in 4 conditions. The reference was a standard condition with full visual access to the other´s object-directed action (full-vision condition), a hidden condition in which the final part of the action was hidden, a full-vision miming condition, in which the other mimicked an action in the absence of an object and finally a hidden miming condition, in which – similar to the hidden condition– the final part of the hand trajectory was hidden. Before the beginning of each trial, the monkey knew whether the object would be presented or not. Note that in the hidden condition, the action goal could be inferred as the object and the beginning of the object-directed action could be seen initially. Not so in the hidden mimicking condition as no object could be seen. The authors found that about half of the mirror neurons exhibited significant observation-related discharge also in the hidden condition. In view of these results, the authors concluded that mirror neurons encoded the goal of the other´s action rather than providing a detailed description of the movement. Further evidence in support of this interpretation has come from the observation that some F5 mirror neurons – dubbed "audio-visual" mirror neurons by the authors – discharge regardless of whether the action of breaking a peanut is seen and heard, just seen, or just heard (Kohler et al., 2002). Also interpretable within the framework of goal relatedness is the finding of "tool-responding" neurons in F5 by Ferrari et al. (2005) who found a subpopulation of mirror neurons that responded to the observation of actions performed by an experimenter using a tool (pliers or stick) to interact with objects. 90.9% (30/33) of these "tool-responding" mirror neurons showed a very high similarity in their discharge between the observation and the execution of actions involving tools. As these neurons did not respond to the observation of actions using natural effectors like the hand or the mouth, their classification as mirror neurons could be questioned. However, one might argue that these neurons are in fact typical mirror neurons, retuned to a new, quasi-biological effector, the prolongation of the hand by a tool accommodating the goal to take possession of an object and to modify its state. An alternative interpretation, discussed in a later study from the same laboratory is that the "tool-responding" neurons discovered by Ferrari et al. may not have been mirror neurons at all but mouth motor neurons. What was mistaken as observation-related discharge might in fact have been activity preceding the planning of mouth movements in expectation of a reward (Rochat et al., 2010). However, regardless of the identity of the "tool-responding" neurons studied by Ferrari et al., Umiltà et al. (2008) and Rochat et al. (2010) showed that most mirror neurons that respond to natural grasping hand actions will also discharge if the goal to grasp an object is achieved by using a tool. The importance of the action goal rather than movement kinematics is emphasized by the fact that these neurons were activated, no matter if normal pliers or reverse pliers – the latter requiring hand opening rather than closing for the grasp – were deployed. If it was indeed the action goal that matters, kinematically highly similar motor acts serving different goals should be associated with different mirror neuron responses. This was initially established not for F5 mirror neurons but for mirror neurons in area PFG of the IPL that exhibit differential responses to grasping motor acts. As reported by Fogassi et al. (2005), most parietal mirror neurons code the same act (grasping) differently according to the underlying intention of the action (grasping for placing versus grasping for eating). Subsequently, a similar paradigm was also used to study mirror neurons in area F5 and very similarly to mirror neurons in PFG, also mirror neurons in F5 were demonstrated to exhibit discharge profiles that differentiated according to the purpose of the grasping motor act (Bonini et al., 2010). These results from comparative work on mirror neurons in F5 and the IPL support the notion that F5 mirror neurons represent action goals or intentions rather than movement kinematics. Moreover, they suggest that the same concept seems to apply to parietal mirror neurons.

What has been said before corresponds to the state of knowledge that Rizzolatti and Sinigaglia summarized in influential reviews (Rizzolatti and Sinigaglia, 2010) in which they rephrased the role of F5 mirror neurons as major elements of a parieto-frontal network as the basis of a mirror mechanism that allows the observer to understand the other´s goals and/or intentions. This ability is based on the activation of "motor possibilities" in the observer´s own brain, a concept dubbed "*understanding actions from the inside*" by the authors. They attached great importance to emphasizing that understanding actions from the inside based on mirroring is not the only way to arrive at an understanding of the goals and/or intentions of the other´s actions. Obviously, mirroring as the basis of action understanding from the inside requires the identity of the motor repertoires of the agents involved. As an example that this identity is not necessarily available, they discuss the barking of a dog, whose goal may be easily inferred by an experienced dog owner, who will hardly resort to barking in order to express related needs.

In principle in line with the concept of action understanding from the inside are also subsequent experiments in which the Parma group tried to address the distinction between goals that may be inferred by the observation of an observed action and the ability to fathom the goal of an action, not approached, i.e. an intention that was not executed. In fact, based on experiments carried out by Bonini et al. (2014b) and Maranesi et al. (2014), a subgroup of mirror neurons may discharge to the observation of an "inaction" quite similar to the observation of an action to which other mirror neuron may be tuned, provided that the context allows the observer to infer the original action plan or intention that corresponded to the preferred action. In the study of Maranesi et al., a tone cue indicated whether the demonstrator would have to choose an action or inaction following the later occurrence of a go signal. It turned out that in this fully predictable context, 17.4% of action mirror neurons (driven only by the observation of an action) and 59.6% of inaction mirror neurons (driven both by the observation of an action and an inaction) started to discharge well before the go signal. Interestingly, the predictive discharge of inaction mirror neurons was found to be initially quite similar for inaction and action but started diverging at the action start with stronger discharge to the executed action compared to the observation of its suppression. According to the authors, these results suggest that F5 mirror neurons generate an internal representation that predicts the other´s actions and allows the differentiation of an intention that is executed and an intention that is withheld (Maranesi et al., 2014).

The aforementioned possibility of alternative routes to understanding others – not necessarily requiring the activation of the observer´s motor systems – and the need to dissociate action goals and covert or preparatory intentions tried to cope with some of the serious criticism that the action understanding concept had received over the years, a scientific debate that continues down to the present day (Hickok, 2009, Glenberg, 2011, Cook and Bird, 2013, Hickok, 2013, Cook et al., 2014, Kemmerer, 2021).

Arguably, much of the controversy is the result of the fuzziness of terms like action understanding, goal and intention, which are not always clearly differentiated and sometimes used differently. To remedy this deficiency as a starting point for constructive debate, Thompson et al. (2019) suggested dividing action understanding into three levels: (1) “action identification”, which according to the authors requires the consideration of the configural relationships between body parts as a prerequisite for the distinction of one observed action from another, e.g. the differentiation of a precision grip from a whole hand grip; (2) “goal identification”, the process of generalizing across different observed actions in order to identify the common goal of the family of differentiated actions such as the different ways of grasping a cup; (3) “intention identification”, the ability not only to generalize across a family of observed actions but the capacity to infer a hidden mental state that underpins the generation of goals. For instance, the intention to quench thirst may – depending on the context – prompt different actions such as trying to grasp a small glass containing a beverage or a larger one, assuming that a choice might be possible. Note that in this definition, an intention is basically equated with a mental state – in the aforementioned example to be thirsty and to wish to quench the thirst. Yet, in contrast to Thompson et al., one might argue that an intention corresponds to the generation of a covert goal, awaiting realization sometime later. In any case, considering the definition of these three levels of cognitive processing the authors concluded that mirror neurons or the brain areas containing such neurons are more likely to only encode the identification of actions. However, arguing that mirror neurons are confined to action analysis does not answer the question of whether action identification or understanding requires recourse to the observer´s motor repertoire rather than solely relying on visual analysis (the "visual hypothesis" of action understanding). According to Rizzolatti et al., the main weakness of the "visual hypothesis" is that it does not explain the process of "validating" the meaning of an observed action (Rizzolatti et al., 2001), in his concept easily achieved by resonance of the motor vocabulary. While this may be true on the conceptual level, the fundamental problem remains that the single-cell data on area F5 are not able to refute the "visual hypothesis". The fact that F5 mirror neurons discharge during self-action does not necessarily mean that the function of observation-related responses must be linked to motor preferences.

The many challenges notwithstanding, the hypothesis that F5 mirror neurons might serve a variant of action understanding keeps dominating the thinking about their functional role (Bonini et al., 2022). However, this does not mean that no alternative conceptual frameworks would have been considered. One of these frameworks is the response selection hypothesis.

1.3.2 Response selection

Already in their initial study, Rizzolatti and his coworkers mentioned that one potential function of mirror neurons might be action selection, or in the words of authors the "*…capacity of inferior premotor neurons to select actions according to gesture meanings*". Arguably, talking about "action meaning" entails the connotation of consciousness and assumptions about the other´s mental state. While viable social interactions may in any case require useful guesses on the other´s mental state, mirror neurons might very well be limited to associating the observed action with a suitable reaction, considering the given context and past experiences. Interpreting the observed action as a sensory stimulus prompting a behavioral response would be much in line with thoughts about other parts of the premotor cortex as suggested by Wise and others. For instance, in the experiments of Weinrich and Wise (1982), monkeys had to learn to carry out a particular movement in response to a specific elementary visual cue, a learned association that they found to be reflected in the discharge of neurons in F2 that started upon stimulus onset and subsided once the go signal to execute the action had been provided. Most of these neurons showed specificity for the direction of the upcoming movement direction. This is the essence of work that has promoted the idea that the premotor cortex might in general accommodate the association of sensory information and appropriate behavioral reactions as well as the ability to plan its execution considering the context. Could it be that likewise F5 mirror neurons are the basis of a context-dependent association, the only difference being that they specialize on a particular class of sensory cues, namely observed actions? Several studies seem to support this response selection hypothesis.

If mirror neurons encode the goals of observed actions, their responses should be invariant to the spatial position in which the observed action is performed. A study by Caggiano et al. (2009) clearly demonstrated that the responses of quite a few F5 mirror neurons do not show the expected invariance. In the first experiment, the visual responses of mirror neurons were determined with the demonstrator performing hand actions in the monkey’s peripersonal and extrapersonal space. Whereas hand actions in peripersonal space could in principle be influenced by the monkey´s own hand actions, this was not possible for actions in extrapersonal space. The authors found a substantial fraction of mirror neurons whose observation-related visual discharge was confined to actions in one of the two spatial compartments. Whereas 26.7% (28/105) of the mirror neurons tested preferred peripersonal space, 25.7% (27/105) favored the extrapersonal space compartment with both groups collectively categorized as “space-selective” mirror neurons. Importantly, the modulation of the visual responses of these “space-selective” mirror neurons did not depend on the metric distance between the two agents but on the ability of the observer to intervene in the demonstrated action or not. This could be established in a subsequent experiment in which the visual responses of “space-selective” mirror neurons were measured with a fully transparent plexiglas panel placed between the two agents to prevent any possible interference in the other´s action, although it was performed in the observer´s peripersonal space. As a consequence of this manipulation that deprived peripersonal space of its characteristic quality of behavioral accessibility, a significant fraction of “space-selective” mirror neurons changed their tuning. For instance, a neuron that might have responded to the vision of an action in peripersonal space stopped firing in the presence of the barrier. Hence, this study suggested that mirror neurons care about the observer´s behavioral options in view of the behavior of others rather than simply representing the other´s actions or action goals. A few years later, the notion that mirror neurons care about the operational distance between the two agents that determines whether a demonstrated action could in principle be influenced by the observer or not, found support from two other studies. In one of the two studies, 48.2% (66/137) and 25.6% (35/137) of F5 mirror neurons showed selective visual responses to action stimuli in peripersonal and extrapersonal space respectively (Bonini et al., 2014a). A second study demonstrated that also the latency of observation-related responses may depend on the spatial compartment in which an observed action takes place. This study found that visual responses of F5 mirror neurons started significantly earlier when the observed action was performed in extrapersonal as compared to peripersonal space (Maranesi et al., 2014).

Assuming that mirror neurons encode the other´s action goals, the perspective on the other´s action should not matter. However, as shown by Caggiano et al. (2011b), the observation-related discharge of most mirror neurons depends on the point of view. In this work, the authors first tested if observation-related discharge of F5 mirror neurons could be evoked by action videos. This was indeed the case. In fact, more than 40% (53/123) of all mirror neurons in F5 displayed similar responses to the observation of comparable videotaped and naturalistic grasping actions seen from the same point of view. Next, the visual responses of mirror neurons were tested when the monkey observed films that showed the same action from 3 points of view (0° = subjective view, 90° = side view, and 180° = frontal view), and the results demonstrated 74.1% (149/201) of mirror neurons exhibited a preference for one or two points of view, which meant that these mirror neurons were view-dependent. It must be emphasized that the lack of a point-of-view invariance in the vast majority of mirror neurons does not necessarily exclude the role of mirror neurons in representing the other´s action goals. The reason is that point-of-view dependent and independent mirror neurons might represent different positions in a processing hierarchy striving for invariance: responses to observed actions that are view-independent could be easily established by integrating input from a set of neurons characterized by different point-of-view preferences.

Action observation-related responses of mirror neurons are not only influenced by the spatial arrangement of actions but also by the specifics of the cognitive framework in which actions are embedded, likewise compromising the invariance of observation-related responses. A major cognitive factor that influences the responses of mirror neurons to observed actions is the value of the object grasped by the other for the observer. This was established by Caggiano et al. (2012) based on a series of 3 experiments. In experiment 1, the experimental monkey observed a demonstrator grasping a non-biological object ("neutral object") lacking any meaning for the observer without any reward given to the monkey at the end of a trial. Alternatively, the demonstrator grasped a food object which was handed over to the monkey in a subset of trials, thus achieving that the object had value for the observer. The results demonstrated that 68.5% (102/149) of F5 mirror neurons were modulated by the type of object (neutral or food) during the action observation. In a second experiment, F5 mirror neurons were tested with the monkey observing grasping actions on 2 different non-biological objects, one associated with a reward and the other one associated with no reward at the end of the trials. The results demonstrated that the observation-related discharge of 58.6% (51/87) of mirror neurons differed, depending on whether the object was associated with a reward or not. A final experiment showed that the modulation of observation-related discharge by the value of the object for the experimental monkey is gradual if the value of the object is changed in increments. Further support for the notion that the observation-related discharge of mirror neurons is modulated by the subjective value of the observed grasping act for the observer comes from a follow-up report by Pomper et al. (2020). In their study, 4 different visual cues presented early in a trial – before the presentation of an action video showing one and the same grasping act – predicted 4 different trial outcomes differing in subjective value for the observer: the need to maintain fixation of an eccentric target for a short time or a long time at the end of the trial or, alternatively, to receive a small or a large drop of fluid reward. The idea here was to scale the subjective value of a trial with the embedded action video not only quantitatively but qualitatively by introducing the possibility of outcomes experienced as punishing, namely the need to maintain eccentric fixation. The action observation responses of more than half of the F5 mirror neurons tested exhibited a clear and graded influence of the value, a conclusion that was also supported by a population analysis. Of note is the fact that the introduction of negative value levels allowed the differentiation of value and attention or arousal. Both the expectation of positive and of negative rewards (i.e. punishments) were associated with attention associated with the object of desire or rejection. Unlike reward which varied from negative to positive, attention and arousal stayed always positive with long-duration fixation and large drops associated with larger arousal or attention reactions than short-duration fixation and small drops. The statistical analysis of the discharge patterns clearly showed that the modulation of observation was better explained by the assumption of a modulatory influence of value rather than an influence of attention or arousal. Within the framework of action understanding, as discussed by Thompson et al. (2019), there is no need for the value that an observer attributes to the object manipulated by the other. However, if the information on the object targeted by the other´s action helped to define the observer´s behavioral goals, the value that the observer attributes to this object should of course matter. This perspective is clearly in line with the hypothesis that F5 helps to provide information on the other´s actions for the shaping of suitable actions of the observer – in other words, to guide reactions to the other. In order to link information on the other´s actions with the behavioral responses of the observer, taking information on the context and the internal state of the observer into account, mirroring of discharge patterns related to observation and self-action would not be needed. This is in line with the result of a very recent report that found very little evidence for mirroring in the sense of an identity of preferences for the type of action eliciting responses of F5 mirror neurons to action observation and self-action (Pomper et al., 2023). In this study, the authors compared the neural activity of F5 mirror neurons associated with three different grasping actions (lift, twist and shift) directed towards one and the same object. Only a few mirror neurons exhibited congruent preferences for the type of action during execution and observation and, moreover, usually only for very restricted periods of time. The absence of consistent congruency is hardly compatible with the idea that F5 neurons might accommodate action understanding by a neural representation of a motor vocabulary resonating with the observed action. However, choosing an appropriate action considering the other´s action does not require congruency which is why also this study is in principle reconcilable with the response-selection hypothesis.

In sum, the above-mentioned studies challenge the action understanding hypothesis while lending support to the response selection hypothesis. Yet, while in keeping with this hypothesis these studies do not clearly provide sufficient evidence to establish it beyond doubt.

1.4 Conclusion and outlook on the following sections

Since F5 mirror neurons were discovered in 1992, they have been believed to play a key role in processing information on the other´s behavior. An influential interpretation of their function that has drawn wide circles well beyond the confines of neuroscience has been the concept that these neurons might serve action understanding by mapping the observed action onto the motor repertoire of the subject, allowing the observer´s motor planning system to resonate. While the early work seemed to be in line with this idea, the results of later, much more sophisticated experiments have been taken to suggest a representation of action goals or intentions. The response selection hypothesis is a promising alternative framework for the interpretation of the properties of F5 mirror neurons. It has the advantage of being less demanding and being much more in line with the role of the premotor cortex at large. However, as yet it is supported only by several pieces of indirect evidence whose persuasive power is still insufficient. The case for a role in response selection would become much stronger if it could be demonstrated that the discharge of F5 mirror neurons reflected the choice of a self-action prompted by the interpretation of the action of another agent. And final irrefutable evidence would be obtained if one could establish that a loss of F5 mirror neurons destroyed the observer´s ability to interpret the other´s action for the guidance of their own behavior. In order to take the first step towards these goals we trained two monkeys on paradigms in which the monkeys had to choose their own action based on the action of a demonstrator monkey in varying ways as indicated by contextual information and studied the associated discharge patterns of a large population of F5 mirror neurons identified based on established criteria.

2. Materials and methods

2.1 Subjects and ethical framework

The experiments were carried out on two male rhesus monkeys (Monkey P and Monkey F, Macaca mulatta) of 10 and 11 kg respectively in accordance with German and European law and the National Institute of Health’s Guidelines for the Care and Use of Laboratory Animals. Procedures were approved by the Animal Welfare (§15)

Commission Regierungspraesidium Tübingen (reference numbers: N04/14G and N07/18G) and supervised by the Animal Welfare Officers of Tübingen University and the State Veterinary Administration (Landratsamt Tübingen). The Veterinary Service of University of Tübingen provided care in the event of illness.

2.2 Surgical procedures

All single units presented in this study were recorded from area F5 of the left hemispheres of the two monkeys. The position of the circular recording chamber was selected such as to allow access to F5 adjoining the arcuate sulcus, premotor area F5, and larger parts of the frontal eye field (FEF) lying on the other side of the sulcus.

To localize F5, the monkeys underwent a brain MRI scan before surgery, and the MRI images were further processed with the Horos™ software (sponsored by Nimble Co LLC, Maryland, United States) running on Mac OS. Based on the scans and expedient information retrieved from the DTI Atlas of (Calabrese et al., 2015) and the online Scalable Brain Atlas (Bakker et al., 2015), the coordinates of F5 were determined. The MRI images were further used to reconstruct a 3D model of the skull and the brain resorting to the SolidWorks CAD program (Dassault Systèmes SolidWorks Corporation, Massachusetts, United States). Based on the coordinates of F5 and the 3D models, the headpost and the recording chamber, customized to the individual anatomy, were manufactured by the workshop using titanium (grade 5). The stereotactical implantation was performed in two steps. In the first step, the basis of the chamber and the headpost basis were attached to the skull with titanium screws and then fully covered with skin in order to ensure the undisturbed integration of the implants and in particular of the screws into the bone. In the second step, following the firm integration of the implants into the bone after 3 – 6 months, the skull was trepanned and the head post basis was supplemented by the actual holder part, penetrating the skin.

Surgical anesthesia was initiated with atropine (0.025mg/kg), ketamine (10mg/kg) and xylazine (0.8mg/kg), administered i.m. that allowed intubation and the subsequent mechanical ventilation with isoflurane (0.8% - 1%) whose anaesthetic-analgetic effects were complemented by the continuous delivery of remifentanil (1 - 2µg/kg·min) to ensure balanced anaesthesia. Vital signs such as blood oxygen saturation, the concentration of CO_2 in the exhaled air, heart rate, blood pressure, and body core temperature were continuously monitored. During surgeries, the monkey's head was firmly fixed in a standard stereotactic frame. In the first surgery, the skin was incised over the future position of the basis of the chamber and headpost, and the flaps were gently moved to the sides to allow the stereotactical insertion of the implants and their fixation to the skull with titanium bone screws. Finally, the skin was closed layer by layer. In the second surgery, the skin located over the chamber basis was reopened, the bone inside the chamber basis was trepanned and the basis was complemented by its upper part, later carrying a micromanipulator for the guidance of sets of several electrodes. Finally, following a small cut of the skin over the headpost foot, the actual post was firmly screwed to the foot. After each surgery, the monkeys received carprofen (4mg/kg, once/day) and buprenorphine (0.01mg/kg, 2 - 3 times/day) for pain relief until all signs of pain had disappeared. Full recovery was required before the monkeys were used again within the framework of the experiments.

As said, the identification of the region of interest was guided by the information provided by the anatomical MRI scans. Valuable information for the verification of the location and extent of F5 was then provided by the delineation of the location and course of the arcuate sulcus resorting to information accrued by the later electrode penetrations and the different characteristics of neurons in F5 and FEF when asking the monkey to perform saccades or grasping actions. The posterior position of the arcuate sulcus was also identified by microstimulation and observing either arm, hand, face, or mouth movements or FEF-typical eye movements. Based on these different sources of information, the exact location of F5 and its boundaries could be determined.

2.3 Experimental setup

The monkeys had first been trained to voluntarily enter the primate chair. The next step was to train them on a grasping task that required the manipulation of three different objects according to visual information displayed on a monitor screen in front of the experimental monkey (Figure 2A). The monkey's right hand could move forward freely, whereas a baffle plate in front of the left hand prevented this hand from moving outside the confines of the chair. Within reach of the monkey’s right hand, there was a ‘grasping table’, a plastic plate tilted towards the monkey by about 55° relative to the horizontal that accommodated three objects - a home button on the midline close to the monkey´s body and two cylindrical objects of identical shape right and left of it, farther away from the monkey, yet still conveniently accessible by the hand. All three objects were fitted with mechanoelectrical sensors that detected the beginning and end of object manipulations. The cylindrical object on the right contained a tiny LED in its top that shone green when turned on. It accommodated a lift of up to 2.6cm against gravity. The

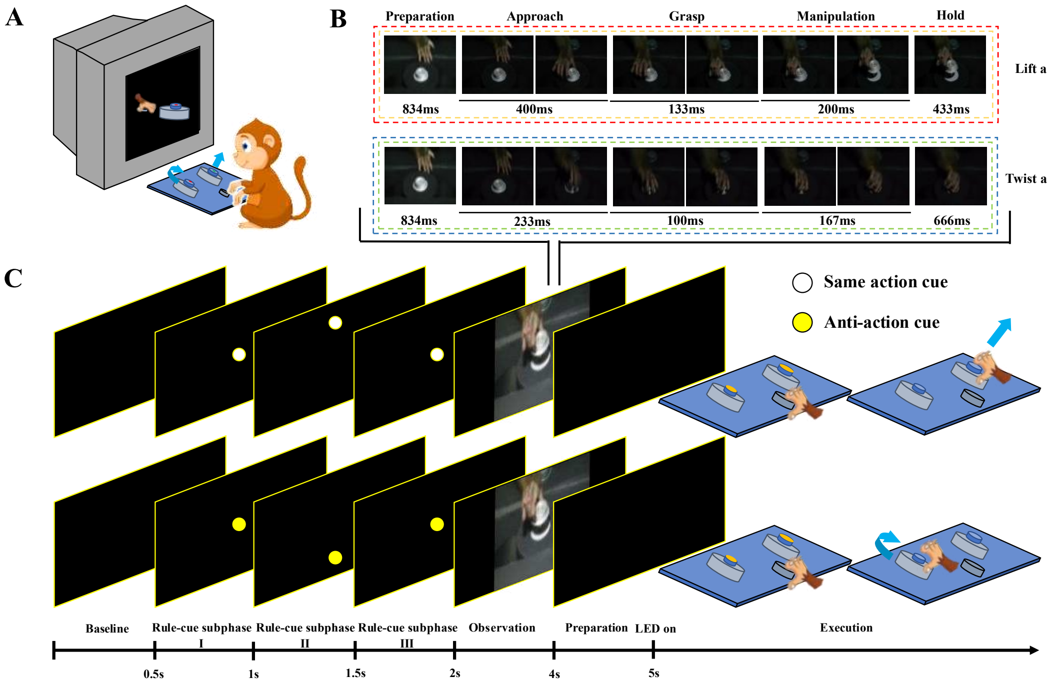

Figure 2 Paradigm of the experiment. (A) Schematic diagram of the monkeys’ tasks. (B) Two types of video clips were displayed in the experiment. The duration of the video clips was 2 seconds and could be subdivided into 5 epochs: preparation, approach, grasp, manipulation, and hold. The upper row represents the lift action video and the lower row represents the twist action video. (C) The procedure of the example trials, in which the lift action video was displayed. The white dot stands for the “same action” rule – the monkey needed to simulate the action displayed in the video; the yellow dot indicates the “anti-action” rule – here, the monkey needed to perform the action option not displayed in the video. (1) Baseline phase (0-0.5s), the monkey pressed the home button, and the screen was black. (2) Rule-cue subphase I (0.5s – 1s), a white/yellow dot appeared in the center of the black screen. (3) Rule-cue subphase II (1s – 1.5s), the white/yellow dot jumped up / down 10° visual angle. (4) Rule-cue subphase III (1.5s – 2s), the white/yellow dot appeared in the center of the black screen again. (5) Observation phase (2s – 4s), a lift action video was displayed on the screen. (6) Preparation phase (4s – 5s), the lift action video was over and the screen became black again. (7) The LED light in the center of the two objects was switched on as the ‘go’ signal. (8) The monkey detached his hand from the home button and lifted (white dot) / twisted (yellow dot) the object. If the monkey successfully finished the action, he would get a drop of water as the reward.

object on the left had the same visual and haptic appearance as the one on the right, yet accommodated a different manipulation, namely a 70° clockwise rotation against a spring force. This object also contained an LED in its top, which shone red when turned on. The reason to have LEDs shining different colors in the two objects was that the monkeys had also been used in an additional experiment (outside my own project), and observation-related responses of F5 mirror neurons had been compared under two conditions in which either the observed grip or the LED color mattered for the observer. In my own experiments, turning the two LEDs on simultaneously served as a "go signal" to start the required object-related execution phase (see below). The diameter of the two objects was 4.5cm and their height was 1.8cm. The distance between the centers of the two cylindrical objects was 17.5cm. The distance between the monkey's chest and the midpoint of the two cylindrical objects was about 30cm. There was a 15-inch monitor (Dell, Texas, United States, E156FPf) facing the monkey's head with the distance between the monkey's eyes and the monitor amounting to 44cm. The perpendicular to the monkey's forehead, the position of the home button, and the midpoint between the two cylindrical objects were congruent with the perpendicular to the monitor.

The monkeys' behavior was monitored online utilizing two infrared cameras: One camera was aimed at the monkey's face and body to monitor the monkey's body movement, and the other – one which was the basis of an in-house eye tracking system – was aimed at the monkey's right eye. The sampling rate of the eye tracking system was 50Hz and the latency of the measurement system for online control of eye position was 150ms.

The control of the experiment and the recording of behavioral data, including the recording of eye position data as well as the acquisition of electrophysiological data (see below) were based on the in-house open-source software Nrec (https://nrec.neurologie.uni-tuebingen.de, created by F. Bunjes, J. Gukelberger et. al) running under Debian Linux on a PC.

2.4 Electrophysiological recordings

Extracellular action potentials were recorded by means of commercial glass-coated tungsten electrodes whose impedance was 0.5mΩ or 1mΩ (Alpha Omega Engineering, Nof HaGalil, Israel, 0.5 mΩ: catalog No. 366-130605-00, 1mΩ: catalog No. 366-130610-00). Four independent electrodes were arranged in a rectangular/squared order with a minimum distance of 1 mm between adjacent electrodes in the grid of the multi-channel micromanipulator which was then mounted on the recording chamber. The electrodes could be moved independently with micrometer precision under the control of the electrode positioning system (Alpha Omega Engineering, Nof HaGalil, Israel, Serial No. EPS000421). The electrodes were inserted through the intact dura mater into the cortex. The signals provided by the electrodes were amplified and fed into an online spike detection system (see below) which isolated and sorted the action potentials by comparing incoming action potentials with an action potential template set up by the user based on the first action potentials of a spike train. The aforementioned electrode positioning system, the data acquisition as well as the real-time detection and sorting of spikes were accommodated by the AlphaLab Server (Alpha Omega Engineering, Nof HaGalil, Israel).

2.5 Visual stimuli

The visual stimuli in this research were two video clips that showed the actions of a monkey´s hand and arm carried out to lift and twist respectively the aforementioned cylindrical object on the grasping table. The actions replayed in the videos had been performed by one of the two monkeys employed in the experiment. The monkey's actions were filmed from a frontal point of view (Caggiano et al., 2011a) using a Samsung NX300M camera with a resolution of 1920x 1080 pixels in a noncompressed format. The light sources and positions of the lights used for filming were the same. The original videos were edited by Matlab 2018 (MathWorks, Massachusetts, United States) to generate the clips used in the experiments. Processing involved downsampling the resolution, cropping the segment of interest as specified further below, and rendering the background homogenous. The video clips are available for download at https://zenodo.org/records/14788883 (DOI: 10.5281/zenodo.14788882). The edited video clips' resolution was 700 × 400 pixels and their duration was 2 seconds (60 frames). When the video clips were displayed on the monitor they had an extent of 32° × 18° (width v.s. height) as seen by the experimental monkey.

The videos started showing the demonstrator monkey´s right hand placed on the home button with the cylindrical object within reach and the LED on the top of the cylindrical object turned off. Both the lift action video and the twist action video could be subdivided into 5 epochs (Figure 2B): (1) the preparation epoch (lift video and twist video: duration was 834ms or 25 frames), starting with the monkey's hand placed on the home button and pressing it down against a spring to close contact. For this phase, the videos were identical no matter what kind of action was shown later; (2) the approach epoch (lift video: 400ms or 12 frames, twist video: 233ms or 7 frames), starting with the hand leaving the home button until the hand touching the object; (3) the grasp epoch (lift video: 133ms or 4 frames, twist video: 100ms or 3 frames), encompassing the time from the hand touching the object to the hand starting to move the object; (4) the manipulation epoch (lift video: 200ms or 6 frames, twist video: 167ms or 5 frames), from the hand starting to move the object until completing the object lift or twist respectively (i.e. the object reaching the end position of the respective manipulation); (5) the hold epoch (lift video: 433ms or 13 frames; twist video: 666ms or 20 frames), during which the object had to be held in the end position.

2.6 Behavioural task

2.6.1 Eye movement calibration

At the beginning of the experiment, on each day an eye movement calibration session was performed. In this session, the monkey had to fixate a red dot, that appeared in varying positions on a 26°x18° grid with a width of 13° horizontal/ 9° vertical, centered on the monitor midpoint. As the radius of the dot was 0.2° visual angle and its contrast relative to the uniformly black screen was low, the fovea had to be deployed for fixation. Proper fixation of the dot was assumed by the experimenter whenever the monkey made a short-latency saccade to the dot appearing in a new position and in this case rewarded by a drop of water (about 0.2ml), manually released by the experimenter. The dot appeared in each position 3 times yielding a data set of 3 times 9 spatial positions that were then subjected to a two-dimensional regression analysis that provided a precise and reliable identification of eye position. The nonlinear transformation between measured values between the horizontal xm(p) and vertical ym(p) components of the eye position records furnished by the video eye tracker and the horizontal and vertical components of the gaze angle xg(p) and yg(p) respectively was given by the following equations:

$$xg = a1 + a2 * xm + a3 * ym + a4 * xm^2 + a5 * ym^2 + a6 * xm * ym;$$

$$yg = b1 + b2 * xm + b3 * ym + b4 * xm^2 + b5 * ym^2 + b6 * xm * ym.$$

As the monkey´s head was painlessly fixed via the implanted headpost during experiments, eye position corresponded to gaze position.

2.6.2 Main behavioral tasks

In this research, my main interest was the question of how information drawn from an observed action of another agent might guide an observer´s (re-) action. More concretely, depending on the prevailing rule the observer was asked to reproduce the action of the demonstrator acting in the video ("same action" task; i.e. reproducing either a twist or a lift) or, alternatively, to switch to the one out of two possible actions not performed by the demonstrator ("anti-action" task; e.g. in case the demonstrator performed a lift action, the observer was required to carry out a twist action). The anti-action variant was realized in two ways, dubbed the "video-blocked paradigm" and the "rule-blocked paradigm" respectively. In the "video-blocked paradigm", the observer saw a demonstrator performing one and the same action over a block of 50 trials. Whether to reproduce the observed action or to switch to the other action was decided by a rule cue, delivered before the video clip. As the action visible later in the video was the same from trial to trial, the observer could commence planning his own action as soon as the rule cue had become available, in fact without having to pay attention to the video. However, in the "rule-blocked paradigm", in which a particular rule (i.e. either the "same action" or the "anti-action" rule) was in effect for all trials of a block, planning of the self-action could start only once the type of action carried out by the demonstrator had become clear. The experimental monkey quickly learned whether he performed in the one or the other type of blocked paradigm after a few trials by realizing that either the cue or the video clip did not change from trial to trial. Note that both variants reduce the amount of ad hoc information that needs to be collected by the observer in a given trial as either the cue or the kind of observed action can be predicted. Rather than running trials of blocks allowing the prediction of the one or the other, choosing both the rule cue and the type of observed action at random in a given trial would have eliminated any prior information on the required transformation of observed actions into self-action. Unfortunately, although trained over many weeks on this randomization variant, the performance of the two monkeys never consistently exceeded chance level, which is why we had to resort to the blocked paradigms set out above.

Each trial in the two paradigms consisted of the same sequence of phases: baseline phase + rule-cue phase + observation phase + preparation phase + execution phase (Figure 2C). These phases were delimited with the help of signals delivered by mechanoelectrical sensors that detected particular changes in the state of the home button and the objects (e.g. the object movement having reached the mechanical stop, corresponding to the movement endpoint). To ensure that the monkey watched the visual stimuli on the screen of the monitor, the eyes had to stay inside a position window centered on the target position. Note that the position window metric differed for different fixation periods during the trial (see details below) and, moreover, that the window was not activated right at the onset of a particular fixation period but only a short time later, a "grace period" allowing the monkey to home in a given window. If the monkey did not cope with the phase-specific behavioral and eye position requirements, the trial was aborted, counted as an invalid trial and the fluid reward denied. However, if the monkey met the requirements, he was rewarded with the delivery of a drop of water (0.3ml) at the end of a trial and the trial was considered for later analysis. The behavioral data were time-stamped and simultaneously acquired along with neuronal electrophysiological recordings for offline data analysis. The 5 phases of the task are described in more detail as follows.

Baseline phase (0-0.5s): This phase which marked the beginning of a trial was initiated once the monkey had pressed the home button. During this phase, the screen of the monitor was uniformly black. The phase ended when 500 ms after its start and a white or yellow dot of the radius of 1° visual angle appeared in the center of the screen. The dot color informed the monkey about the specific task. Moreover, the presence of the dot entailed fixation requirements described in more detail below.

Rule-cue phase (0.5s - 2s): The color of the dot ("cue") informed the monkey about the type of action to be executed (Figure 2C). The white color cue indicated that he was supposed to reproduce the observed action (“same action” condition) whereas the yellow color cue told him to switch to the other action (“anti-action” condition) in the later execution phases of the trial. The rule-cue phase involved 3 different subphases (I-III):

Rule-cue subphase I (0.5s – 1s): A white (“same action” cue) or yellow dot (“anti-action” cue) appeared in the center of the black screen. During subphase I, the dot lay in the center of a fixation square window of ±5° visual angle and the grace period was 350ms.

Rule-cue subphase II (1s – 1.5s): This subphase started with a jump of the cue to a more peripheral position. Whereas the white dot jumped up +10° visual angle, the yellow dot jumped down -10° visual angle. During this subphase, the eye fixation square window was ±9° visual angle and remained centered on the dot (i.e. it jumped with the dot). The grace period was 300ms in this subphase. Hence both color information and jump direction alike informed the monkey about how the observed action was to be transformed into a self-action. The reasoning underlying this redundancy was that it might help the monkey to comply with the task demands. Note, however, that we never tested if this doubling of information was really necessary.

Rule-cue subphase III (1.5s – 2s): The white or yellow dot appeared again in the center of the black screen. During this subphase, the eye fixation square window was ±7.5° visual angle and the grace period was 300ms.

Observation phase (2s – 4s): A lift or a twist action video was displayed on the screen. During this phase, the eye fixation square window was ±10° visual angle in most sessions and ±15° visual angle in the others. The grace period was 100ms in this phase. Note that this phase lacked the presence of a central fixation dot. Hence the monkey had to home in the display center, a task that was not too demanding given the fact that even the ± 10° fixation covered most of the video (32° × 18°, width vs. height) whose scrutiny was essential for correct task performance. Note that the LED in the object manipulated by the demonstrator monkey was turned off in the main tasks in which the observed action mattered.

Preparation phase (4s – 5s): The video was over and the screen became black again. During this phase, the monkey needed to keep pressing the home button from the beginning of the trial to the end of this phase.

Execution phase (5s -): The LED lights on top of both the ‘lifting’ and the ‘twisting’ object were simultaneously switched on, which was the ‘go’ signal requesting the self-action in accordance with the prevailing rule, and the LED lights were on for 0.5s. As the ambient illumination was turned off, the self-action was carried out in the darkness. Hence, the visibility of the objects and the hand were reduced, albeit not fully eliminated, and therefore, arguably reducing the impact of visual feedback on the self-action. The self-action started with the monkey detaching his right hand from the home button, choosing the object accommodating the type of action required, i.e. lifting or twisting the respective object and finally holding it in the end position for at least 1s but not longer than 1.5s before being allowed to release the object and return the hand to the home button. The self-action had to be completed within 3.5 s after the ‘go’ signal. If not, the trial was aborted. In case the monkey had successfully complied with all trial demands he received a fluid reward at the end of the trial.

In the analysis of neural responses to both action observation and self-action, we distinguished 4 epochs: (1) the approach epoch, starting with the monkey’s hand leaving the home button and ending with the hand touching the object; (2) the grasp epoch, from the hand touching the object until the hand starting to move the object; (3) the manipulation epoch, from the hand starting to move the object until finishing the lifting or twisting movement (the object reaching the end position); (4) the hold epoch, the hand holding the object at the end position.

Video-blocked paradigm: In this paradigm, one session comprised 2 blocks, and each block contained 50 trials. In each block, the “same action” cue or the “anti-action” cue was randomly displayed in the rule-cue phase. In one block, only the lift action video was displayed in the observation phase, whereas in the other block, only the twist action video was presented in the observation phase. Table 2 provides a graphical summary of the structure of this paradigm. Appendices 1 and 2 provide exemplary trial lists for two blocks in which the lift and the twist video respectively were displayed.

Rule-blocked paradigm: In this paradigm, one session involved 2 blocks again, and each block contained 50 trials. However, now in one block, only the “same action” cue was presented in the rule-cue phase, whereas in the other block, only the “anti-action” cue was displayed in the rule-cue phase. In each block, the lift action video and the twist action video were randomly displayed in the observation phase. Table 2 also accommodates this paradigm and Appendices 3 and 4 present exemplary trial lists.

Table 2 Schematic flow chart of the video-blocked and rule-blocked paradigms

Video-blocked paradigm	Block A (50 trials)	Baseline (0.5s)	Same action cue or Anti–action cue (1.5s)	Lift video (2s)	Preparation (1s)	Lift action or Twist action
	Block B (50 trials)	Baseline (0.5s)	Same action cue or Anti–action cue (1.5s)	Twist video (2s)	Preparation (1s)	Twist action or Lift action

Rule-blocked paradigm	Block C (50 trials)	Baseline (0.5s)	Same action cue (1.5s)	Lift video or Twist video (2s)	Preparation (1s)	Lift action or Twist action
	Block D (50 trials)	Baseline (0.5s)	Anti–action cue (1.5s)	Lift video or Twist video (2s)	Preparation (1s)	Twist action or Lift action

2.7 Data analysis

2.7.1 Selecting mirror neurons

First, we determined whether neurons responded to either video observation or during motor execution, following the previously introduced procedure (Pomper et al., 2023). To this end, we split both the observation phase and execution phase into four epochs (approach, grasp, manipulation and hold), respectively. Then we determined whether the neuron responded during one of the four epochs comprising the observation phase by performing a Friedman test against the baseline (first 500ms of each trial). We performed this test separately for each of the four conditions in the two paradigms. If the Friedman test was significant at the level 0.05/4 for any of the four conditions, we considered the neuron to respond during video observation. We performed the same procedure for the execution phase. If the neuron responded both during video observation and self-action execution, we considered it to be a mirror neuron. Importantly, the criterion did not take into account whether the tuning preference was the same for observation and execution.

2.7.2 Data preprocessing for principal component analysis (PCA)

Our study encompassed the video-blocked paradigm and the rule-blocked paradigm. Each paradigm involved 2 blocks (Blocks A and B in the video-blocked paradigm, Blocks C and D in the rule-blocked paradigm) and each block encompassed 2 conditions (see Table 2). For each neuron, we smoothed the spike trains with a Gaussian kernel with a standard deviation of 50 ms and averaged them over trials for a particular condition. Furthermore, we followed the preprocessing procedure of (Churchland et al., 2012) for each experimental block: We soft-normalized each neuron's response by dividing its response by a factor given by the maximal firing rate of the neuron in the block + 5. Dividing by the maximal firing rate ensured that the population response was not dominated by neurons with particularly high average firing rates. To retain some information about the average firing rate, the small integer was added to the denominator. Then for each neuron and time point, we subtracted the mean over the two conditions in the block. This allowed us to focus on differences in the neural response for the two conditions without considering response patterns that were apparent in both conditions. The reasoning here was that processes related to deciding between the two actions should be reflected by neural signatures that differed between the two conditions.

2.7.3 Joint PCA

The goal of the joint PCA was to visualize the neural activity related to action selection and to infer whether the activity was related to the rule cue or self-action in the video-blocked paradigm and the observed video or the self-action in the rule-blocked paradigm. The PCA was conducted separately for the two paradigms, and for the four different temporal segments of the trial: the rule-cue phase, observation phase, preparation phase and execution phase. To simplify notation, we only present the procedure for the preparation phase in the rule-blocked paradigm. We collected the data for Blocks C (it refers to Block 1 in the following context) and D (it refers to Block 2 in the following context) in the data matrices X_1, $X_2 \in \mathbb{R}^{2T\times N}$, respectively. In the case of the preparation phase, T = 1000. Say that we are interested in the principal component $\omega \in \mathbb{R}^{N}$ that explains the highest amount of variance in Block 1, i.e.

$$\arg\max_{\|w\|=1} \|X_1 w\|^2 = \arg\max_{\|w\|=1} w^\intercal X_1^\intercal X_1 w. \quad (1)$$

The vector w is given by the eigenvector of $X_1^T X_1$ corresponding to the largest eigenvalue. To find a component that maximizes the variance in both blocks, we could modify equation (1) to

$$\underset{\|w\|=1}{\arg\max} \|X_1 w\|^2 + \|X_2 w\|^2 = \underset{\|w\|=1}{\arg\max}\, w^\intercal C w, \quad (2)$$

with $C := X_1^T X_1 + X_2^T X_2$. Since C was symmetric, eigendecomposition on C yields the desired component just as in the one-block case. We modified equation (2) to obtain components that explained variance in both blocks in the same way.

The experiment had two conditions per block. Block 1 contained the conditions “Lift Video + Lift Action” and “Twist Video + Twist Action”. Block 2 contained “Twist Video + Lift Action” and “Lift Video + Twist Action”. More compactly, write $X_1 = (LL, TT)$ and $X_2 = (TL, LT)$, where the brackets denote the concatenation of the submatrices. The projections of X_1 and X_2 onto w are both vectors of dimension $2T$. We can measure the similarity of the projections by their squared L_2-distance $\|X_1\omega - X_2\omega\|^2$. We then expand this distance to

$$\|X_1 w - X_2 w\|^2 = \sum_{i=1}^{2T} ((X_1 w)_i - (X_2 w)_i)^2$$

$$= \sum_{i=1}^{T} ((LLw)_i - (TLw)_i)^2 + \sum_{j=1}^{T} ((TTw)_j - (LTw)_j)^2.$$

$$= \|LLw - TLw\|^2 + \|TTw - LTw\|^2.$$

If the L_2-distance $\|X_1 \omega - X_2\omega\|^2$ is small, the projections of LL and TL are similar, and the projections of TT and LT are similar. Thus, the component ω projects conditions with in which the same self-action was performed closely together. Then we could conclude that the component’s variance must stem from tuning to the self-action, not from tuning to the video identity.

In contrast, consider the matrix $X_2^* := (LT, TL)$ in which the ordering of the conditions is flipped. Then if a component ω the L_2-distance $\|X_1\omega - X_2^*\omega\|$ is small, the component is not strongly tuned for the identity of the self-action. The component’s variance must be due to the video’s identity.

Building on equation (2), we obtained formulas for components that explain variance in both blocks, while being tuned to only one modality. The first self-action-tuned component is given by

$$\underset{\|w\|=1}{\arg\max}\ \|X_1w\|^2 + \|X_2w\|^2 - \|(X_1 - X_2)w\|^2,$$

and the first video-tuned component is given by

$$\underset{\|w\|=1}{\arg\max}\ \|X_1w\|^2 + \|X_2w\|^2 - \|(X_1 - X_2^*)w\|^2.$$

We arrived at the final formulation of our objective function. This formulation does not require prespecifying the modality to which the component should be tuned. We aim to find the component ω that satisfies

$$\begin{aligned}
&\underset{\|w\|=1}{\arg\max}\ \|X_1w\|^2 + \|X_2w\|^2 - \min\{\|(X_1 - X_2)w\|^2, \|(X_1 - X_2^*)w\|^2\} \\
&\qquad = \underset{\|w\|=1}{\arg\max}\max\{\|X_1w\|^2 + \|X_2w\|^2 - \|(X_1 - X_2)w\|^2, \\
&\qquad\qquad \|X_1w\|^2 + \|X_2w\|^2\| - \|(X_1 - X_2^*)w\|^2\} \\
&= \underset{\|w\|=1}{\arg\max}\max\{w^\intercal X_1^\intercal X_1 w + w^\intercal X_2^\intercal X_2 w - w^\intercal (X_1 - X_2)^\intercal (X_1 - X_2)w, \\
&\qquad w^\intercal X_1^\intercal X_1 w + w^\intercal X_2^\intercal X_2 w - w^\intercal (X_1 - X_2^*)^\intercal (X_1 - X_2^*)w\} \\
&= \underset{\|w\|=1}{\arg\max}\max\{w^\intercal X_1^\intercal X_2 w + w^\intercal X_2^\intercal X_1 w, w^\intercal X_1^\intercal X_2^* w + w^\intercal X_2^{*\intercal} X_1 w\} \\
&= \underset{\|w\|=1}{\arg\max}\max\{w^\intercal (X_1^\intercal X_2 + X_2^\intercal X_1)w, w^\intercal (X_1^\intercal X_2^* + X_2^{*\intercal} X_1)w\}. \qquad (3)
\end{aligned}$$

Utilizing $X_2^* = -X_2$, (3) became

$$\begin{aligned}
&\underset{\|w\|=1}{\arg\max}\max\{w^\intercal|(X_1^\intercal X_2 + X_2^\intercal X_1)w, w^\intercal (X_1^\intercal (-X_2) + (-X_2)^\intercal X_1)w\} \\
&\qquad = \underset{\|w\|=1}{\arg\max}\ |w^\intercal C w|, \qquad (4)
\end{aligned}$$

where $C\colon = X_1^\intercal X_2 + X_2^\intercal X_1$. Of course, C is symmetric. The eigenvector of C corresponding to the largest eigenvalue maximizes the expression arg $\max_{\|\omega\|=1} \omega^\intercal C\, \omega$. The eigenvector of C corresponding to the smallest eigenvalue maximizes the expression arg $\max_{\|\omega\|=1} \omega^\intercal (-C)\, \omega$. Then the eigenvector corresponding to the eigenvalue with the largest absolute value maximizes equation (4). Conveniently, the sign of the eigenvalue indicates whether the eigenvector maximized or minimized arg $\max_{\|\omega\|=1} \omega^\intercal C\omega$. In the terminology used above, the sign of the eigenvalue determines whether the corresponding eigenvector is self-action-tuned or video-tuned.

The eigenvector corresponding to the eigenvalue with the second-largest absolute value yields the direction that maximizes (4) while being orthogonal to ω. Then we obtained the principal components by performing eigendecomposition on C and ordering the eigenvectors by their corresponding absolute eigenvalues. For each component, the sign of the eigenvalue determines its tuning.

In practice, we additionally sparsified the weights of each component to ensure that the neural behavior was actually shared between blocks at the single-neuron level. Without sparsification, it was possible that neurons which were activated in only one block still contribute to the joint components. This effect could bias the estimation of the variance explained by the different modalities. To address this issue, we explicitly penalized non-zero weights for neurons that did not preserve their behavior across blocks. After computing a component ω by performing the eigendecomposition explained above, we used ω as the initial value for the optimization problem

$$\underset{\|w\|=1}{\arg\max}\, w^{\intercal} s C w - \beta \frac{\frac{1}{T}\mathbf{1}(X_1 - sX_2)\|w\|_1}{\|w\|_1}. \qquad (5)$$

Here, $1 := (1, \ldots, 1)$ and $s = 1$ if ω is tuned to the self-action and -1 otherwise. Thus, s simply switches the signs of C and X_2 to conveniently impose the constraints discussed above. Comparing (5) to (4), the term that maximizes the variance remains unchanged. We only introduced L_1-sparsification on ω, such that dimensions in which X_1 and X_2 differed more are penalized more strongly. The loss is not convex, but when starting gradient descent in the analytic solution of the non-sparsified objective function, we always found good components. For each component, we chose the smallest beta such that the regularization term was smaller than the average across neurons during the baseline period. This procedure effectively removed the contribution of neurons that responded differently in the two blocks, except for differences due to noise.

2.7.4 Generalized linear model (GLM)

The objective of the GLM was to test the influence of rule type (same vs. anti-action) and self-action on single-neuron responses during the cue and the observation phase respectively, and we fitted the GLM parameters for each neuron separately. For original spike counts, we used a Poisson response distribution. The baseline-corrected spike trials were not restricted to integers anymore, which is why modeled them using a Gaussian distribution. First, we estimated the null model parameters using only the blocking variable ”video” as a regressor. Then we fitted the full model that included the self-action type and the video-action interaction. We compared the two models using a Likelihood-Ratio test to determine whether the neuron was modulated by either the self-action or the video-action interaction. In other words, we tested if the neuron’s response could be explained solely by the observed video against the alternative hypothesis that the necessary self-action or a video-action interaction also influenced the response significantly. If the test was significant, we flagged the neuron as responsive and analyzed the GLM parameters. The combination of the parameters β_{A} for the self-action and β_{AV} for the video-action interaction summarized the neuron’s behavior: If $\beta_{\mathrm{AV}} = 0$, we concluded that the neuron’s response was determined by the self-action. If $\beta_{\mathrm{AV}} = -\beta_{\mathrm{A}}$, the neuron only responded to the self-action in one of the two blocks. In this case, the neuron preferred a specific combination of rule cue and self-action. Finally, if $\beta_{\mathrm{AV}} = -2\beta_{\mathrm{A}}$, the neuron’s response could be explained by the rule cue type alone. Pragmatically, we distributed the neurons by putting each into the closest bin. In other words, we determined

$$\arg\min(\beta_{AV} - 0, \beta_{AV} - \beta_A, \beta_{AV} - 2\beta_A)$$

and then classified the neuron accordingly.

3. **Results**

We recorded 859 neurons in the video-blocked variant of the experiment and 288 neurons in the rule-blocked variant in separate sessions. Respectively, 500 (58.2%) and 228 (79.2%) of them were classified as mirror neurons and used in the analysis.

3.1 Planning the upcoming action dominates the population response already during action observation

First, we inquired whether the observation-related responses of mirror neurons were mainly determined by the visual stimulation or also depended on the context of the action video, namely the rule to imitate the action demonstrated in the video or to perform the anti-action, i.e., the action complementary to the one shown in the video. Moreover, we asked if a possible influence of the rule was dependent on the need to attend to the video, required only in the rule-blocked variant of the paradigm. These questions could be answered by inspecting the time courses of the principal components obtained for the two variants of the experiment shown in Figure 3 (video-blocked variant) and Figure 4 (rule-blocked variant). Both figures convey that the first principle component dominating the observation phase can in fact be attributed to the choice of the type of action (twist vs. lift) in both monkeys, independent of the observed action type and independent of the initial rule to perform the same or the anti-action (see Tables 3, 4 for the variance explained by this and the following 3 principle components). This analysis clearly establishes that already during action observation, the activity of mirror neurons is largely dominated by planning the upcoming action. Also during the subsequent preparation phase, between the end of the video and the go-signal for the self-action and the subsequent execution phase, it is the type of action that dominates the population activity. In the video-blocked variant, in which the rule cue needed to be monitored in order to decide on the same or the anti-action on a trial-by-trial basis, principal components 1 and 2 in monkey P and component 2 in monkey F differentiate between these two choice options in the rule-cue phase, a differentiation that is conserved in the subsequent observation phase but lost thereafter. PCA of data from the rule-blocked variant lacked components related to information on the need to perform the same or the anti-action. This is to be expected as the monkey´s action in a given block unfolded in either a stable same-action or anti-action mode without the need for further deliberation. On the other hand, in the rule-blocked case, PCA revealed weak information of the type of video in the observation phase, a signature that was clear in monkey P (Table 3).

3.2 A subpopulation of mirror neurons responds differently to the same video depending on the rule

As said before, principal component 2 of the PCA applied to the observation phase of the data from the video-blocked variant clearly reflected the rule-based decision

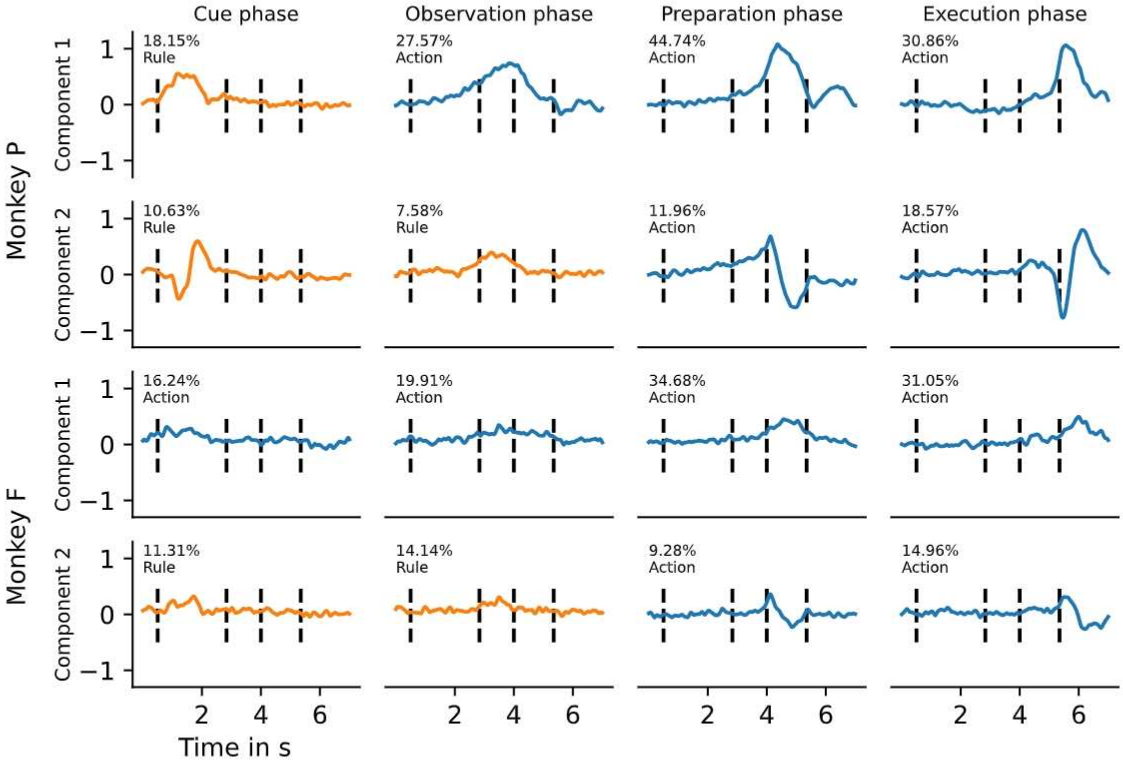

Figure 3 Joint principal components for the video-blocked paradigm. Components are tuned either to the self-action type or the rule type. The top two rows correspond to monkey P and the bottom two rows correspond to monkey F. In each column, components were calculated using spiking data for the specific phase only. Still, the plot shows the projection of the entire data onto the component. Dashed lines indicate the start of the cue, observation, preparation and execution phases, respectively. The numbers in the plot convey the proportion of explained variance of the component and the key words underneath the numbers show the modality that the component is tuned to. Orange line, rule type; Blue line, self-action type.

Table 3 Proportion of variance explained by the first four components in the video-blocked paradigm

	Monkey P		Monkey F	
Phase	Action	Rule	Action	Rule
Cue	8.3%	35.9%	22.7%	17.4%
Observation	27.6%	16.9%	28.6%	23.0%
Preparation	56.7%	6.7%	49.8%	8.5%
Execution	59.5%	1.6%	52.7%	7.5%

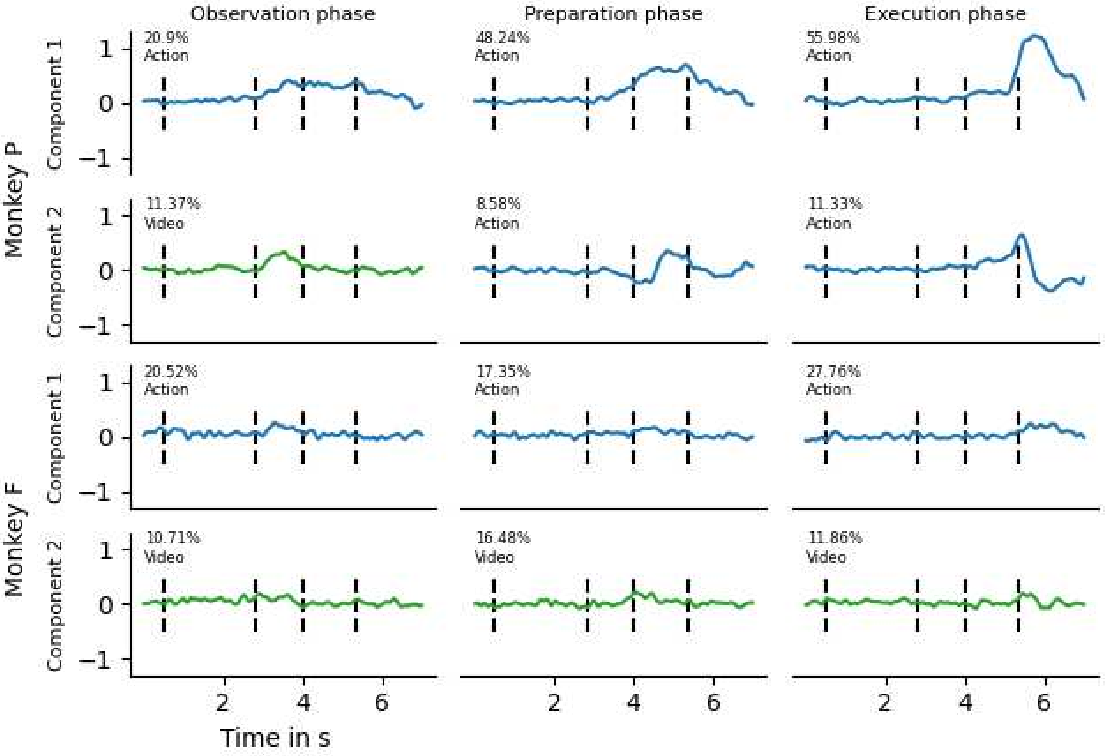

Figure 4 Joint principal components for the rule-blocked paradigm. Components are tuned either to the self-action type or the video type. The top two rows correspond to monkey P and the bottom two rows correspond to monkey F. In each column, components were calculated using spiking data for the specific phase only. Still, the plot shows the projection of the entire data onto the component. Dashed lines indicate the start of the cue, observation, preparation and execution phases, respectively. The numbers in the plot convey the proportion of explained variance of the component, and the key words underneath the numbers show the modality that the component is tuned to. Blue line, self-action type; Green line, video type.

Table 4 Proportion of variance explained by the first four components in the rule-blocked paradigm

	Monkey P		Monkey F	
Phase	Action	Video	Action	Video
Observation	28.5%	18.5%	28.5%	20.5%
Preparation	60.5%	5.8%	26.2%	29.8%
Execution	75.7%	0.0%	36.4%	20.3%

whether to simulate the action or not, although the contribution to the total data variance was relatively small. Relatively weak decision-related information might be expected if the information on the decision to simulate or not were dependent on a subset of neurons whose decision-related information was diluted at the population level. In order to determine if only a set of F5 mirror neurons fired more based on whether the observed action needed to be imitated or not to carry out the lift or twist action, we fitted a GLM with the two variables, i.e. rule type (same action vs. anti-action) and self-action (lift vs. twist) to the spike counts of individual neurons during the observation phase. In total, 21.4% (81) of the population from monkey P and 10.7% (13) of the population from monkey F responded differently to the same video, depending on the rule cue and/or upcoming self-action, i.e., they exhibited significant non-visual tuning. We then grouped the neurons with significant non-visual tuning into three categories based on the respective GLM parameters. The first group, comprising neurons tuned to the rule type, responded differently to the same video based on whether the observed action had to be imitated. The second group consisted of neurons whose discharge profiles were best explained by the upcoming self-action. These neurons responded differently to the same video based on the later self-action. Finally, a third group of neurons was tuned to a specific rule-action combination. The results from the grouping procedure are shown in Figure 5. We plotted the differences in spike counts between the same-action condition

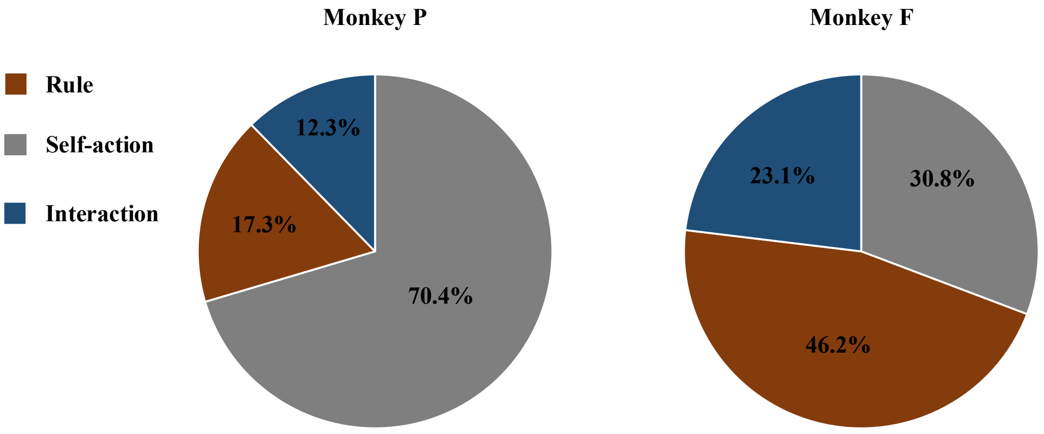

Figure 5 Three types of mirror neurons that responded differently to the same video in the video-blocked variant. These mirror neurons could be divided into three types: rule-tuned, self-action tuned and interaction-tuned.

and anti-action condition for all neurons tuned to the rule type or an interaction of the rule type and self-action (Figure 6, orange bars). A shift to the right of the origin would indicate a preference for the same action condition, and one to the left for the anti-action. In fact, the distribution turned out to be shifted to the left (two-sided Wilcoxon test p=0.037) in accordance with stronger activity associated with the anti-action condition. To summarize, we identified a subgroup of F5 mirror neurons that changed their response to the visual stimuli based on whether the observed action had to be imitated or not, exhibiting a weak bias towards responding more strongly if the observed action was not to be imitated.

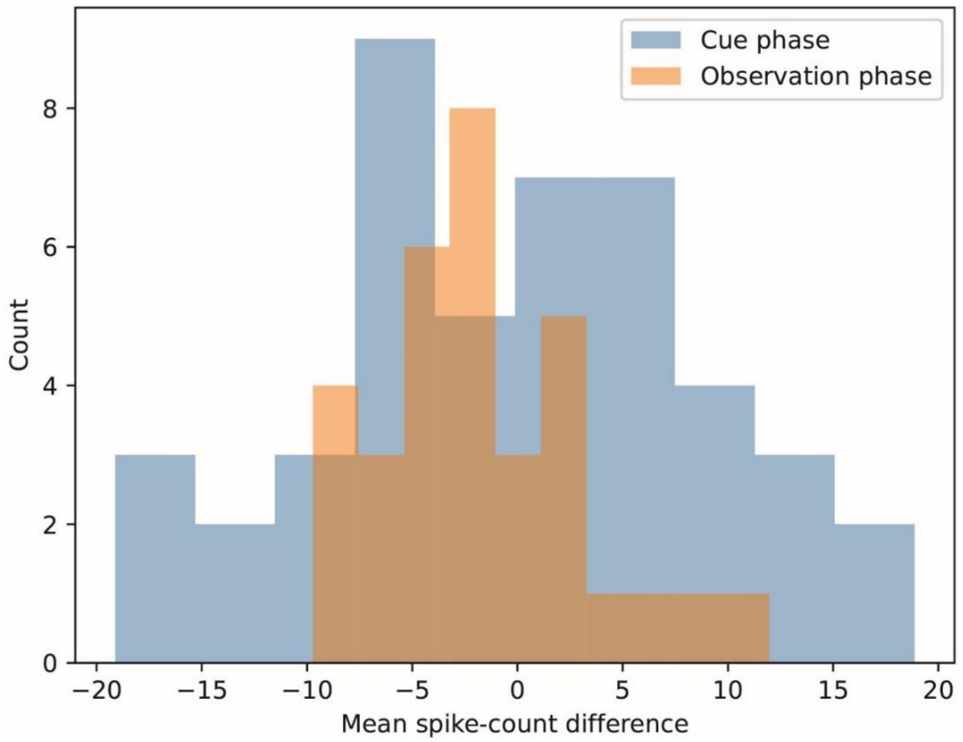

Figure 6 Histogram of the difference in mean spike count between same-action and anti-action conditions for all rule-tuned neurons in the video-blocked paradigm. Positive values indicate a preference for the same-action rule, and negative values preference for the anti-action rule. The blue bar stands for the rule-cue phase and the orange bar stands for the observation phase.

3.3 The observation responses of mirror neurons depend more strongly on the rule during active observation

The preceding section of data from the video-blocked variant presented evidence for a subpopulation of F5 mirror neurons whose activity during the (passive) observation of the action videos was modulated by the rule-based decision to perform the same action or the anti-action. We wondered whether the discharge profiles of a separate set of mirror neurons might show a comparable influence on the decision in the observation phase of the rule-blocked variant when separating all trials into two pools based on the action video, in every case requiring active observation. Similar to the aforementioned analysis of neurons tested in the video-blocked variant, we fitted a GLM with the parameters rule type and self-action type to the instantaneous discharge rate of each neuron. As trials characterized by a particular type of action video could come from either the same or the anti-action block, we performed a baseline correction of spike trains in order to account for possible changes in activity levels between blocks. Next, we fitted a Gaussian-error model to the data since the assumption of Poisson distribution of spiking was no longer satisfied after baseline correction. Finally, to accommodate a fair comparison between the two variants requiring passive and active observation respectively, we refitted the GLMs for the video-blocked variant, using the same method. We found that during active observation, the differences between neural responses to the same video in different conditions (same action condition vs. anti-action condition) were larger than those during passive observation. This effect was consistent in monkey P (23.8% vs. 20.6%) and monkey F (28.6% vs. 9.1%). This indicates that attention plays a role in the neuronal responses of mirror neurons to action-observation.

3.4 The population response is dominated by self-action during the preparation phase

In the rule-blocked variant, PCA had documented weak, albeit clear video-type related information during observation. We next asked if this information was maintained in the subsequent preparation period. As dominant information on the motor plan underlying the later self-action already emerged in the observation phase and was maintained during action preparation (principal component 1), one could doubt the necessity of preserving information on the video in the preparation phase preceding the go-signal. Figure 4 shows that the first component found for the preparation phase is indeed attributed to self-action rather than the observed video in both monkeys. When considering not only the first two but the first four components, the dominance of self-action is retained in Monkey P, whereas in Monkey F slightly more variance is attributed to the observed video than to the self-action (Table 4). As alluded to earlier, self-action also dominates the preparation phase in the video-blocked variant in both monkeys, no matter if one considers only the first or the first four components (Figure 3, Table 3). These results clearly indicate that the neural activity in the preparation phase is mainly related to planning and preparing the upcoming self-action, rather than to the processing of visual information on the observed action.

3.5 Both the rule and the self-action may impact the discharge of mirror neurons in the rule-cue phase

Finally, we asked if F5 mirror neurons encode information on the rule to perform the same or the anti-action based on the information provided by the rule cue at the beginning of the trial. Since the rule cue became uninformative after a few trials in the rule-blocked variant, we addressed this question, considering only the video-blocked data. We analyzed neural activity in the rule-cue phase resorting to the PCA as described earlier in order to estimate the impact of the rule cue. The result is shown in the left panels of Figure 3. It demonstrates that in both monkeys, the population signal differentiates between the two possible rules and the consequences arising from them. Whereas a significant fraction of the activity of mirror neurons derived from monkey P reflected information related to the rule cue, quite surprisingly, neurons from monkey F exhibited both rule and self-action-related activity. In order to further explore this finding, we took a closer look at single neurons by fitting the rule-cue phase spike trains of individual neurons from both monkeys with a GLM that involved the two variables of interest, the rule type and the later self-action.

Fits were classified according to the significance of the two variables and the neurons accordingly divided into three pools reflecting the significant impact of the rule, the self-action or both. The results are summarized in Figure 7. They show that the rule is the most significant variable for the majority of neurons recorded from monkey P but for none of the neurons from monkey F. Conversely, self-action dominates almost twice as many neurons from monkey F as from monkey P. The single neuron analysis is therefore in line with the population analysis based on PCA. We therefore conclude that the recorded mirror neuron populations from the two monkeys differ in their tuning. This incongruence might reflect contrasting strategies: Possibly, monkey F used the implicit information from the block design in more cases, allowing him to ignore the information from the video. On the other hand, monkey P might retain information from the rule type to later integrate it with information from the video to select the appropriate response.

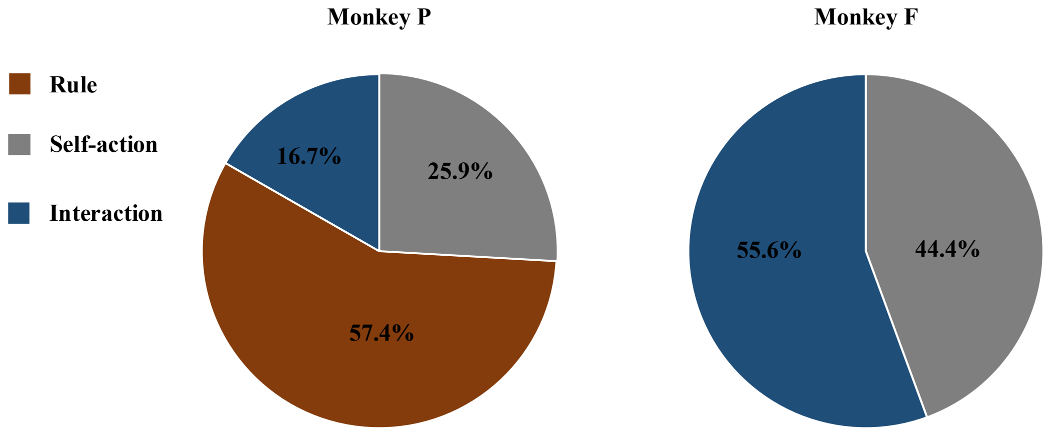

Figure 7 Three types of mirror neurons that distinguish different rules in the video-blocked variant. These mirror neurons could be divided into three types: rule-tuned, self-action tuned and interaction-tuned.

We have established that the rule influences neural responses during both the rule-cue phase, and the observation phase. Building on this finding, we were interested in whether there existed a population preference for the same-action condition over the anti-action condition. To investigate this question, we computed the mean firing rate difference between the same-action condition and the anti-action condition for all neurons tuned to the rule type and the rule-action interaction respectively. Figure 6 (blue bars) displays a histogram of this difference. A shift of the difference to the right of the origin would indicate a preference for the same action condition at the population level, and vice versa. The distribution being roughly symmetric around the origin suggests that there is no systematic bias toward a preference for the same-action condition or the anti-action condition. Further, the one-sample Wilcoxon test did not find a significant shift (p=0.99). Hence, the assumption of an action bias as an explanation of the availability of information on the later self-action has to be discarded.

To sum this part up, we found that a subpopulation of mirror neurons responded to the presentation of the rule cue. However, we obtained no evidence for a preference for the same action cue or the anti-action cue.

4. Discussion

This work was guided by the idea that mirror neurons in F5 might help the observer use information about the other´s action to select and shape the observer´s self-action in a context-dependent manner. In order to critically examine this idea, we studied rhesus monkeys in two paradigms in which the subject´s self-action depended on the behavior of a demonstrator monkey shown in a video clip. The assumed importance of the situational context for the choice of action was captured by contrasting rules that flexibilized the connection between action observation and self-action. More specifically, we asked the experimental subjects to distinguish two types of manipulative hand actions of a demonstrator monkey and to reproduce the seen action or to carry out the alternative action depending on the rule. In the first variant of the task, the "video-blocked" paradigm, the action seen in a block of consecutive trials was kept constant. As the presented action could be predicted after the first few trials in this variant, the observer could plan his own action early on based on the rule to reproduce the shown action or to choose the alternative action. In other words, no attention was required to the video. This was different in the second variant, the "rule-blocked paradigm", in which the rule to carry out either the same or the alternative action was kept constant. Hence, the planning of the own action required full attention to the demonstrated action in order to choose the type of self-action called for by the rule cue.

In an attempt to identify the main drivers of neuronal activity as well as their temporal evolution, we resorted to a novel analytical approach to the population of F5 mirror neurons recorded. It enabled us to retrieve a limited number of readily interpretable informational dimensions governing the population activity, irrespective of the presence of highly idiosyncratic discharge patterns at the single-neuron level. The procedure chosen is similar to the demixed principal component analysis (dPCA) (Kobak et al., 2016, Michaels and Scherberger, 2018), which performs an ANOVA-style decomposition of neural data. However, we had to take a slightly different route, considering that we had to choose a subset of experimental variables for each paradigm, therefore making a conventional ANOVA decomposition unsuitable. This approach allowed the conclusion that the responses of mirror neurons to the other´s action are dominated early on by planning and preparing the later self-action, no matter if there was a need to allocate attention to the other´s action. Even in the rule-blocked paradigm, in which the planning and preparation of the self-action depended on the observed action, hence requiring attention to the other´s action, the impact of visual information on the population activity in the observation phase remained surprisingly minor compared to the influence of planning and preparing the self-action. A persisting influence of the rule in the observation phase and an early self-action-related signal seen already in the rule-cue phase in case of the video-blocked paradigm may not be unexpected. After all the information provided by the action video did not really matter for the preparation of the later self-action which was fully determined by the combination of the rule-cue based instruction and a reliable prediction of the upcoming action. A necessary restriction applies to a first or very few first trials of a block in which attention to the video was needed in order to find out which of the two action video variants should be expected in the remainder of a given block. Hence, while an attention effect may have mattered in the beginning of a block, it is unlikely to have influenced the block responses, arguably dominated by a multiple of trials in which the action video type was known beforehand, hence, no longer requiring attention.

To the best of our knowledge, this is the first study to investigate the effect of attention on mirror neurons in macaque area F5 at the single-neuron level. In previous studies of non-human primate mirror neurons, the observation of the other´s action and the execution of a self-action were relatively independent, and the monkey's selection of an action did not depend on the demonstrated action, which meant that the monkey did not need to pay attention to it and passive observation of the other´s action was fully sufficient. In the present study, however, attention to the videos was required in the rule-blocked paradigm, in which a clear impact of the action video on the observation-related discharge was found. The notion that attention modulates the discharge evoked by the observation of actions is in line with the literature on the human mirror neuron system as studied by non-invasive approaches, in particular based on the BOLD response studied in functional magnetic resonance imaging. As reviewed by Kemmerer (2021), attention is one of the multiple factors modulating action observation-related activity. As discussed earlier (see Introduction) previous work on mirror neurons in monkey area F5 has established that spatial position, viewpoint and subjective value are factors influencing the neural activity of mirror neurons during action observation (Caggiano et al., 2009, Caggiano et al., 2011b, Caggiano et al., 2012) and as shown by the present study, attention and the prediction or expection of a particular visual stimulus extend this list and further underpin the justification for doubting the viability of the ‘resonance’ hypothesis of the role of mirror neurons while on the other hand, fully compatible with a role in shaping one´s self-action, taking the action of others into account. Importantly, rather than being a slave of the other´s action, mirror neurons afford a highly flexible linkage that reflects the observer´s needs and expectations when planning actions that promise appropriateness and usefulness. This line of thinking might in principle be in accordance with the view laid out by Schaffelhofer and Scherberger (2016). These authors addressed the question of how grasping acts are shaped by visual information on the object to be grasped. Based on the recordings of neurons in AIP, F5 and M1, they argued that F5 represents an intermediary stage between area AIP, seen as a quasi-visual area devoted to the extraction of visual object features and primary motor cortex M1, dominated by signals reflecting the movement of a grasping hand. The intermediate character of F5 –in principle in line with its anatomical position between AIP and M1– was given by the transient sharing of visual information on an object to be grasped with AIP and a later switch to planning and execution related activity. Note here, though, that in this work visual responses were evoked by particular objects to be grasped by the observer and not by a transitive (object-directed) action of another agent. Hence, one may wonder to what extent mirror neurons may have contributed to an F5 population signal that was based on a mixed bag of F5 neurons without further differentiation of distinct categories of mirror neurons. However, as discussed in the introduction, F5 accommodates functionally differing types of neurons such as mirror neurons or motor neurons. And of particular interest with regard to the Schaffelhofer and Scherberger study is a third group of F5 neurons, the so-called “canonical” neurons (Rajmohan and Mohandas, 2007). They are of interest as they are known to be tuned to the vision of graspable objects rather than to the observation of grasping acts and it seems plausible that they may have determined the reported sequence of early visual and later motor information in F5. Canonical neurons are believed to determine the hand shape allowing a reliable grasp of an object using the information on the visual features of the object, hence they accommodate the visual guidance of movements. However, to the best of my knowledge, it is not known if their basic visuo-motor properties are influenced by the many other factors known to modulate mirror neuron activity, possibly allowing them to contribute to deciding on the object to be grasped. If this were the case, they might be seen as neurons tapping a specific source of visual information needed to plan successful object-directed actions. In fact, mirror neurons might be seen as neurons serving the same goal, yet drawing on a complementary source of information, namely visual information on the grasping acts of the other, and two groups of neurons together could provide a firm basis for the subject´s behavioral decisions to the promising reward. We may finally add that in this scenario, it may in the end be irrelevant if canonical and mirror neurons can indeed be seen as qualitatively distinct groups of neurons rather than being members of a continuum of visuo-motor neurons in F5, weighting the various streams of visual information in an individually differing, graded manner.

The analysis showed that the proportion of variance explained by the self-action-tuned modality was available already well before the observation phase in the video-blocked paradigm and first emerged as early as in the observation phase in the rule-blocked paradigm, and it gradually increased towards the execution phase in the video-blocked paradigms, regardless of whether only the first component or the first four components were taken into account. This increase in variance explanation, reflecting an increasing role of self-action related information in the neuronal pool, might be seen as a consequence of an increasing consolidation of the action plan over time. This view is in line with the interpretation of the findings of other studies, which have demonstrated an emergence and strengthening of discharge reflecting behavioral decisions in neurons in area F5 based on sensory information input (Romo et al., 2004, Pardo-Vazquez et al., 2008). Again, all in all, this gradual building up of action-related information based on earlier sensory and cognitive information is in accordance with the notion that F5 matters for the context-dependent selection of actions.

Of course, context-dependent behavioral choices under natural conditions draw on much more information and, moreover, they are probably rarely as simple as in the paradigms used in this study, requiring a binary decision between two possible actions. For instance, as recently shown by Lanzarini et al. (2025), premotor cortical neurons may exhibit very different preferences when studied in much more natural contexts, allowing the monkeys to move freely in larger compartments as compared to being studied under conventional restrained conditions. While the interpretation of these differences between freely moving context and restrained context are not clear yet, they cleary suggest – much in line with the gist of the present study – that responses of mirror neurons are able to adjust to the needs of the given context, compatible with a role in shaping and guiding behavioral decisions. What is needed in order to unmistakably accept this role is data from causal experiments. Will blocking information processing in F5 by reversibly lesioning F5 disrupt the monkey's ability to interpret the other´s action in order to arrive at viable behavioral decisions? We had an opportunity to carry out a few pilot experiments towards the end of my doctoral project in one of the two monkeys, resorting to muscimol injections. In fact, we could not see any effect on the monkey´s performance. Does this mean that the response selection concept has to be taken as falsified? This conclusion would certainly be premature as the data pool was still. Moreover, resources possibly contributed by the F5 in the other hemisphere may have been sufficient to maintain the original level of performance and after all, additional redundancy may be based on contributions from other areas. Hence, while our work has been able to provide answers to important questions supporting the (re-) action concepts, it has in fact opened up many more questions. But this is the nature of any scientific endeavor.

5. Summary

Mirror neurons have been believed to be crucial in processing information about others' behaviors , and the mainstream interpretation of their function is action understanding by mapping the observed action onto the motor repertoire of the subject, allowing the motor planning system of the observer to resonate. However, some recent studies using more sophisticated experiments have not been in line with it. The response selection hypothesis is a promising alternative theoretical framework to better interpret the properties of mirror neurons, but only several pieces of indirect evidence support it until now. In order to provide persuasive evidence, we trained two macaque monkeys using two paradigms (video-blocked paradigm and rule-blocked paradigm) in which the monkeys had to choose their action according to the demonstrator monkey's transitive hand action in varying ways indicated by contextual information, and investigated whether the activity of F5 mirror neurons reflected the selection of a self-action based on the interpretation of another agent's action.

We recorded 859 neurons in the video-blocked paradigm and 288 neurons in the rule-blocked paradigm. Respectively, 500 (58.2%) and 228 (79.2%) of them were classified as mirror neurons and used in the analysis. We employed a novel analytical approach incorporating dimensionality reduction, allowing us to assign the information encoded by the population of F5 mirror neurons to the behavioral rule, observed action or the executed action. This approach enabled us to reveal the main drivers of neural activity and to trace their temporal dynamics, regardless of the presence of highly variable tuning at the level of individual neurons. The analysis showed that the influence of visual information on the activity of the F5 mirror neuron population is unexpectedly limited during the action observation. Instead, the dominant driver of neural activity during the action observation is the planning of the observer's forthcoming action and the dominance gradually increased towards the execution phase. The gradual accumulation of action-related information according to earlier sensory and cognitive information is in line with that F5 matters for the context-dependent selection of actions.

Zusammenfassung

Man glaubt, dass Spiegelneuronen für die Verarbeitung von Informationen über das Verhalten anderer von entscheidender Bedeutung sind, und die gängige Interpretation ihrer Funktion ist das Verstehen von Handlungen durch die Abbildung der beobachteten Handlung auf das motorische Repertoire des Subjekts, wodurch das motorische Planungssystem des Beobachters in Resonanz treten kann. Einige neuere Studien mit ausgefeilteren Experimenten konnten diese Annahme jedoch nicht bestätigen. Die Reaktionsauswahlhypothese ist ein vielversprechender alternativer theoretischer Rahmen für eine bessere Interpretation der Eigenschaften von Spiegelneuronen, wird jedoch bisher nur durch einige indirekte Belege gestützt. Um überzeugende Beweise zu liefern, trainierten wir zwei Makaken mit zwei Paradigmen (Videoblock-Paradigma und Regelblock-Paradigma), in denen die Affen ihre Handlung entsprechend der transitiven Handbewegung des Demonstrator-Affen auf verschiedene, durch Kontextinformationen angezeigte Weise auswählen mussten. Außerdem untersuchten wir, ob die Aktivität der F5-Spiegelneuronen die Auswahl einer eigenen Handlung basierend auf der Interpretation der Handlung eines anderen Agenten widerspiegelte.

Wir haben 859 Neuronen im videoblockierten Paradigma und 288 Neuronen im regelblockierten Paradigma aufgezeichnet. Davon wurden 500 (58,2 %) bzw. 228 (79,2 %) als Spiegelneuronen klassifiziert und in der Analyse verwendet. Wir verwendeten einen neuartigen analytischen Ansatz mit Dimensionsreduktion, der es uns ermöglicht, die von der Population der F5-Spiegelneuronen kodierten Informationen der Verhaltensregel, der beobachteten Handlung oder der ausgeführten Handlung zuzuordnen. Mit diesem Ansatz konnten wir die wichtigsten Treiber neuronaler Aktivität offenlegen und ihre zeitliche Dynamik verfolgen, ungeachtet der stark variablen Abstimmung auf der Ebene einzelner Neuronen. Die Analyse zeigte, dass der Einfluss visueller Informationen auf die Aktivität der F5-Spiegelneuronenpopulation während der Handlungsbeobachtung unerwartet begrenzt ist. Stattdessen ist der dominierende Treiber neuronaler Aktivität während der Handlungsbeobachtung die Planung der bevorstehenden Handlung des Beobachters, und diese Dominanz nimmt zur Ausführungsphase hin allmählich zu. Die schrittweise Ansammlung handlungsbezogener Informationen auf Grundlage früherer sensorischer und kognitiver Informationen steht im Einklang mit der Annahme, dass F5 für die kontextabhängige Auswahl von Handlungen von Bedeutung ist.

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7. Statement of individual contribution

I hereby declare that I designed the study together with my thesis advisor, Prof. Thier. I have carried out the experiments, collected the data, and produced this work entitled “Predictable Context–Based Encoding of Observed Actions in Mirror Neurons of Macaque Premotor Area F5”, submitted for the award of a doctorate from the Medical Faculty on my own. I have used only the resources and aids indicated. I received support from Alexander Lappe (HIH) on statistical aspects of the project, including the methods part in the thesis. Prof. Giese gave advice on statistical methods and the interpretation of the results.

Date 28.09.2025

Signature

8. Acknowledgments

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and I am sincerely thankful for all the help and dedication he has shown throughout this process.

9. Appendices

Appendix 1 Lift action video block - exemplary list (first 50 trials)

Video type	Action type	Cue jump direction	Cue color
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Twist	-10	Yellow
Lift	Lift	10	White
Lift	Twist	-10	Yellow

Appendix 2 Twist action video block - exemplary list (first 50 trials)

Video type	Action type	Cue jump direction	Cue color
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Twist	10	White
Twist	Twist	10	White

Appendix 3 “Same action” cue block - exemplary list (first 50 trials)

Video type	Action type	Cue jump direction	Cue color
Lift	Lift	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Lift	Lift	10	White
Twist	Twist	10	White
Twist	Twist	10	White

Appendix 4 “Anti-action” cue block - exemplary list (first 50 trials)

Video type	Action type	Cue jump direction	Cue color
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow
Twist	Lift	-10	Yellow
Lift	Twist	-10	Yellow

Exemplary trial lists

(1) Video type: the type of action video presented on the monitor in the action observation phase. “Lift” or “Twist” indicates the lift action video or the twist action video respectively.

(2) Action type: the action the experimental monkey had to perform in the execution phase. “Lift” or “Twist” indicates that the monkey had to lift or twist the object respectively.

(3) Cue jump direction: the direction of cue jumping in the rule-cue subphase II. “10” indicates that the cue jumped up 10° visual degree and “-10” down 10° visual degree.