The role of F5 mirror neurons in representing different types of hand movements

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Abstract

About 30 years ago, neurons were found in the premotor area F5 of macaques that responded to both their own and observed grasping movements of the hand. These neurons were termed mirror neurons. Since then the key question has been what their function might be. Arguably the most influential attempt to answer this question has been the conjecture that mirror neurons help macaques to understand observed actions by simulating it internally by kickstarting the same mirror neurons that would be activated by a similar self-action. This idea could be understood as an adaption of the concept of motor imagery as a mental state that allows actors to prepare and initiate movements and to predict their effects. And indeed there are convincing arguments supporting the idea that motor imagery might be the basis of action understanding as posited by the action simulation theory of mirror neurons. Yet, a critical re-examination of the arguments put forward in support of the notion that mirror neurons are the basis of simulation sparks doubts and the need to consider alternative interpretations of their function.

The first chapter of this thesis provides a brief review of the discovery of the motor system and early descriptions of their properties against the backdrop of an introduction into the architecture of motor and premotor cortex, the latter involving area F5, in which mirror neurons were discovered. This introductory section is followed by a more detailed review of the studies that are relevant for the formulation of action simulation theory and its critical appraisal, a balancing presentation that also requires a consideration of work on mirror neurons, later found in other parts of the brain and several species other than macaque monkeys. This chapter ends with the conclusion that the assumption of congruence of observation and self-action related activity of mirror neurons, a major underpinning of the simulation theory needs to be re-examined and that an alternative functional role, namely a role in response selection should be considered. The second chapter, re-examines the viability of the assumption of congruence, whereas the third chapter addresses experiments designed to address the possibility that F5 mirror neurons might play a role in response selection.

In the experiments reported in chapter 2, we used rhesus monkeys that were trained to perform one out of three different grasping movements prompted by specific color cues, directed at objects whose shape and feel stayed constant. Next a human actor demonstrated the same three object-directed grasping movements to the experimental monkey. The question was to which extent the activity patterns of F5 mirror neurons, identified according to standard criteria, would resemble one another. Resorting to a sophisticated classification approach (Fisher's linear discriminant analysis), we found very little evidence for a congruence of activity patterns. Only very few mirror neurons exhibited a preference for the same type of grasping movement and then also only for very restricted phases of the overall action sequences. This chapters concludes that the widespread absence of congruence is hardly in accordance with the tenets of the simulation theory.

In the third chapter, we used a novel action selection paradigm, in which the choice of the grasping action of the experimental monkey depended on the type of grasping act carried out by a demonstrator monkey, seen before in a video on a monitor in front of the experimental animal. In other words, the observer was asked to actively simulate an observed action. In a control experiment the experimental monkey was asked to choose the type of grasping action by using information provided by a color cue. Not unexpectedly, in view of the results reported in chapter 2, the observation and self-action related discharge profiles of mirror neurons tested in this overt action simulation task lacked similarity in the vast majority of cases. For instance, when observing demonstrated actions mirror neurons could exhibit a preference for action type A but for action type B in the subsequent self-action epoch, although the experimental monkey reproduced action type A. Notwithstanding the absence of congruence, a link between action observation and execution is documented by the fact that many mirror neurons exhibited 'memory activity', i.e. discharge in the period in which the monkey had to memorize the seen action in order to reproduce it following the delivery of a go-signal. The diversity of memory activity profiles may indicate different functions such as a memory trace of the observed action or the emergence of a motor plan. Interestingly, and at odds with the classical description of mirror neurons, many of the recorded mirror neurons responded to the simple color cues calling for a particular action with quite a few even preferring dot cues.

Taken together, the results reported in chapters 2 and 3 are hardly compatible with the action understanding theory and more in line with a neuronal system that allows agents to develop action plans considering visual and possibly in general sensory input. Sensitivity to the observation of simple color cues asking for particular self-actions does not necessarily rule out a natural preference for sensory information on others´ actions, considering that the sensitivity to color cues may be a reflection of learned associations. In any case, the absence of any simple relationship between action type preferences during observation and self-action clearly indicates that the assumed role of the mirror neuron system in using information on the other´s action in order to decide on one´s own action must be a network achievement.

Chapter 1 Introduction: a brief review of mirror neurons

1.1 Mirror neurons in the F5 region of rhesus monkeys' premotor cortex

1.1.1 The anatomical location of the ventral premotor cortex F5 region

Already as early as the 17th century, British medical professor Thomas Willis at Oxford noticed a relationship between the cerebral cortex and voluntary movements by observing that cortex lesions were correlated with disturbances of the patient's body control (Willis, 1965). However, in the absence of more systematic investigations, experimental evidence which would have thrown light on the specifics of the assumed role of the cerebral cortex in motor control remained unavailable until the second half of the 19th century, when finally physiological studies of the motor function of the cerebral cortex were commenced (Bennett and Hacker, 2002).

In 1870 Gustav Fritsch and Edvard Hitzig, two young German physicians who were both associated with the Berlin Physiological Institute published results obtained with electrical stimulation of the cortex of dogs. In these experiments, they stimulated different sites of the cortical surface with galvanic currents which size was estimated by the sensory experiences evoked by the same currents when applied to the investigators' tongues. When stimulation was administered to the sigmoid gyrus next to the cruciate sulcus of the dog´s cortex they observed that animals' contralateral face or neck muscle twitched and that forepaw extension or flexion could be evoked (Fritsch, 1870; Fritsch and Hitzig, 2009; for review see Gross, 2007).

These electrical stimulation experiments demonstrated that the mammalian cerebral cortex is electrically excitable and that a specific part of it is related to movement. This notion received further support from lesioning the effective site of stimulation which compromised the ability of animals to move. Further evidence for the role of the cerebral cortex in movement control was obtained by the British physician John Hughlings Jackson who carefully studied the seizures in epilepsy patients. The key observation was that epileptic seizures due to a lesion of one hemisphere identified post-mortem had been responsible for involuntary movements of the contralateral body. Moreover, he noted that the urge to move systematically spread across the contralateral body, suggesting a correlated systematic spread of epilepsy over the motor cortex. It was this observation of this march of convulsion that led Jackson to speculate about the possibility of a topographic organization of the cortex with different parts underpinning different body movements (Jackson, 1867; for review see York and Steinberg, 2011).

Being aware of the observations of both Fritsch with Hitzig and Jackson, the Scotch physician David Ferrier replicated the stimulation experiments on dogs and several other species including monkeys. Unlike Fritsch and Hitzig, who had used monophasic stimulation, Ferrier resorted to biphasic electrical stimulation with a much longer duration of stimulation trains than used by Fritsch and Hitzig. With this approach, stimulation often evoked complex movements of body parts rather than elementary muscle twitches. Moreover, Ferrier also tried to stimulate distinct sites of the animals' cortex to test the assumption of a topographic organization of the motor cortex put forward by Jackson. Indeed Ferrier´s experiments could yield some support for Jackson's ideas (Ferrier, 1873; for review see Akkermans, 2016).

Spurred by this early evidence suggesting the existence of a cortical region devoted to movement control, Wilder Penfield and his collaborators at the Montreal Neurological Institute of McGill University set out to study the consequences of electrical stimulation of the cortex in altogether 163 conscious patients in order to guide the surgical treatment of their focal epilepsy. The justification of these experiments was the hope that electrical stimulation might allow the neurosurgeon to more reliably demarcate movement-related cortex to be avoided when removing epileptogenic tissue to prevent permanent motor deficits. By carefully mapping motor-related areas of the cortex of these patients Penfield could indeed characterize motor cortex, giving rise to stimulation-induced movements. They could also delineate neighboring cortex whose stimulation induced sensory experiences. Moreover, in full accordance with previous speculations they could establish a clear mapping of body parts onto the motor and sensory cortices with the face and the hands having a much larger representation than the trunk and other parts of the body – a topographical organization that became known as the distorted cortical homunculus (Penfield and Boldrey, 1937; for review see Schott, 1993).

Already the stimulation work of Penfield and colleagues had suggested the existence of more than one motor cortex as stimulation of a region on the medial aspect, separated from the largely lateral 'homunculus' representation, was found to give rise to much more complex movements. This second representation of movement became known as the 'supplementary motor cortex' (Penfield， Wilder and Welch， Keasley, 1951). Further evidence for a parcellation of motor cortex was provided by progress in cytoarchitectonic studies of cerebral cortex. The British anatomist Campbell used the Nissl-staining technique to reveal the detailed cellular architecture of the cerebral cortex of healthy humans, neurological patients, and various mammalian species including macaque monkeys. According to Campbell the strip of the precentral cortex whose electrical stimulation gives rise to movements of body parts has a distinct layered architecture that distinguishes it from the neighboring cortex, among others characterized by giant pyramidal-shaped neurons in layer V (Campbell, 1904).

Similarly, Brodmann, working about the same time, used Nissl stained brain sections to distinguish distinct parts of the cerebral cortex. According to him, the precentral cortex linked to motor control as revealed by electrical stimulation, comprises two parts, termed Area 6, located more rostrally and Area 4, next to the primary sulcus, adjoining somatosensory representations on the other side of the sulcus (Brodmann, 1909). It was the British physiologist Fulton who was the first to attribute different functions to these two parts of motor cortex. Using experimental lesions in nonhuman primates he established that lesions of the more rostral part of the motor cortex, corresponding to Brodmann´s Area 6 caused only minimal motor deficits in the sense of palsy, yet disorganization of acquired behavioral patterns. On the other hand lesions of the more caudal part, corresponding to Area 4 led to palsy (Fulton, 1935). Considering these differences in the consequences of lesions and cellular architecture, it became custom to distinguish a primary motor cortex corresponding to Area 4, and a premotor cortex corresponding to Area 6. The continuation of Area 6 on the mesial aspect of the hemisphere, a representation of complex movements first revealed by Penfield and colleagues, became known as the supplementary motor cortex (Geyer et al., 2012; Rizzolatti and Luppino, 2001).

However, the differentiation of these three cortical regions was by no means an endpoint as later anatomical and physiological studies quickly showed that this parcellation was still way too crude. Resorting to staining for cytochrome oxidase activity, an enzyme that represents the level of metabolic activity of cells, the macaque motor cortex was divided into seven, rather than only 3 areas (named F1-F7) (Matelli et al., 1985). Matelli’s F1 corresponds to Brodmann’s area 4 or primary motor cortex (M1). F2 is the caudal part of dorsal area 6, i.e. caudal dorsal premotor cortex (PMDc). F3 is the caudal part of mesial area 6, more often referred to as the supplementary motor area (SMA). F4 is the caudal part of ventral area 6, i.e. ventral premotor cortex (PMVc). The area that I studied in my work, area F5, corresponds to the posteroventral part of Brodmann´s area 6, i.e. rostral ventral premotor cortex (PMVr). F6 is the rostral part of mesial area 6, later also referred to as pre-supplementary motor cortex (pre-SMA) and, finally, F7 is the rostral part of dorsal area 6, the rostral dorsal premotor cortex (PMDr). It is important to note that this parcellation is by no means the reflection of an anatomical glass bead game but a concept with clear physiological implications as indicated by the example of the highly distinct properties of F5, the area I studied in my thesis which I will consider in more detail in the following.

1.1.2 The role of the ventral premotor cortex (F5) in processing information associated with hand and arm movements

Using single-unit recordings from F5, Rizzolatti and colleagues at the University of Parma found that single units in F5 exhibited responses to specific object-directed grasping acts involving hand and arm movements (Rizzolatti et al., 1988). Moreover, they observed that neurons in this part of the premotor cortex were not only sensitive to the execution of the movement but also sensitive to visual and somatosensory signals associated with the movements (Rizzolatti et al., 1981a). Quite unexpectedly it turned out that many of these neurons also responded to the passive experience of visual and somatosensory signals, with features similar to those accompanying active movements (Rizzolatti et al., 1981b). Hence, a first, arguably still pretty loose interpretation of the function of the ventral premotor cortex (F5) was a role in integrating multiple streams of sensory and motor information useful for the guidance of goal-directed arm and hand movements.

The major recipient of information processed in F5 is F1, i.e. primary motor cortex (Rizzolatti and Luppino, 2001; Schmidlin et al., 2008; Shimazu et al., 2004), which is in turn a major source of a descending pathway to the spinal cord, the pyramidal tract. F5 is one of several areas surrounding F1 that not only projects to F1 but in addition also makes direct contributions to the pyramidal tract (Matsumura and Kubota, 1979; Muakkassa and Strick, 1979). The possibility that these connections may allow F5 to contribute to the control of hand and arm movements is supported by the observation of distinct motor deficits following experimental lesions: muscimol (a GABA receptor agonist silencing neurons) was injected into F5 of macaque monkeys causing transient behavioral deficits during a 10 min duration period following the injection. The monkeys, who had been trained to perform color cue instructed flexion or extension movements around the wrist exhibited decreased movement amplitudes and velocities (Kurata and Hoffman, 1994).

The same group also trained monkeys to perform a visually guided reach task in which the monkeys had to point to a target on a screen in front of them, i.e., not requiring a grasping act. Once the monkeys had learned the task, they fitted the monkeys' eyes with wedge prisms displacing the visual scene 10° to the right and tested the ability to adapt to the shift. In the absence of muscimol injections that would reversibly deactivate the targeted site in F5, the monkeys could adapt to the prism-induced displacement of the scene in just 10-20 trials, no matter if it was to the left or the right. After the muscimol injection, however, the monkeys lost the ability to adapt to the prisms (Kurata and Hoshi, 1999). Hence, F5 is also involved in the guidance of reaching out to an object, rather than being confined to grasping it.

In any case, its contribution to the control of hand and arm movements is lateralized as demonstrated by Schieber and colleagues, who reported that unilateral inactivation of F5 with muscimol biased the monkeys to avoid using the contralateral hand (Schieber, 2000). Support for lateralization was also obtained by observing that inactivation of area F5 impaired the monkeys´ ability to establish the hand shape according to the size of and shape of the object to be grasped (Fogassi et al., 2001).

1.1.3 From F5 motor neurons to ‘mirror neurons’

In 1992 a serendipitous observation opened an unexpected perspective on the role of F5, until then thought to be confined to the control of reaching and grasping acts of the hand (di Pellegrino et al., 1992). In their experiments, the authors deployed tasks that required that the experimental monkey would retrieve food objects from a testing box. As reported earlier, neurons in F5 discharged to the grasping act. However, surprisingly, many of them also fired when the monkey observed a similar food or object-related hand movement executed by the experimenter. Later this group added that neurons in the caudal part of F5 not only discharged in response to the execution and observation of relatively elementary grasping acts, satisfying obvious behavioral demands of a monkey such as picking up a food morsel but also to more elaborated acts, not directly related to food intake like rotating an object, passing it over to others and with individual neurons exhibiting a certain degree of specificity for particular motor acts (Gallese et al., 1996; Rizzolatti et al., 1996). Struck by the fact that responses to the observation of motor acts seemed to mirror the response to their execution, Rizzolatti and coworkers dubbed these neurons ‘mirror neurons’, complementing other types of neurons in F5 confined to execution (motor neurons) or the observation of an action (action observation neurons) or activated by a goal-directed action as well as by the vision of the object goal (canonical neurons)(Rizzolatti and Fadiga, 1998).

Mirror neurons do not necessarily require visual information to respond to a particular action of others. In fact, F5 mirror neurons also respond to an action hidden to the eyes (Umiltà et al., 2001). In this work, in a fraction of trials, an occluder prevented vision of the final part of the hand trajectory to the object and the action performed on it. Hence, the monkey could only see the initial part of the motor act. About half of the tested mirror neurons kept firing at the action no matter if the vision was precluded or not. However, in the absence of an action goal pursued by the other, mirror neurons will stop discharging. This is the case if an actor mimes an object-directed action in the absence of a real object (Gallese et al., 1996). When considering the early work on F5 neurons that shaped the concept of a mirroring neuron exhibiting very similar responses to action, no matter if observed or executed, one has to touch on a potentially relevant shortcoming, namely the fact that observation related responses of mirror neurons were evoked by the vision of a human agent simulating the action of a monkey. Hence, considering the clear differences in the kinematic structure of monkey and human hand actions, similar responses to observation and execution would probably only be expected if they reflected shared overarching action concepts, e.g. in the sense of goals such as to use the hand to pick up a peanut to eat it.

Also filmed actions can activate mirror neurons, although individual neurons may have differential sensitivities for natural vs. filmed actions (Caggiano et al., 2011). Filmed actions alleviate the interleaved presentation of actions differing concerning a variable of interest while avoiding the inevitable variability of natural actions. This approach allowed a systematic testing of the role of the observer´s perspective on the action of the other (Caggiano et al., 2009). It turned out that action observation responses of a substantial fraction of mirror neurons (25.87%) were viewpoint invariant. The others exhibited a preference for either the multiple views or one of the three tested points of view (frontal, side, or subjective view).

Notwithstanding the question of the function of action observation responses of F5 mirror neurons – addressed further below – these responses exhibit an interesting difference compared to visual responses of neurons in many other regions of the visual cortex, characterized by the adaptation of their responses to the repeated presentation of the same stimulus (Caggiano et al., 2013). Not so F5 neurons whose observation responses may even grow over repeated presentations. On the other hand, local field potentials, a compound signal to which different types of neurons and their synapses in a local region around the electrode contribute, recorded concomitantly with action potentials of single units, show adaptation. This difference suggests caution when trying to rely on repetition suppression of the BOLD signal in fMRI studies of brain function as a reliable way to single out distinct groups of neurons. If in an action observation task, the BOLD signal exhibited repetition suppression it would be a reflection of a mixed bag of signals rather than a reflection of a specific contribution of mirror neurons.

1.1.4 Mirror neurons represent action goals rather than actions

Evidence for a role of the action goal rather than the kinematic structure of the action – alluded to further up - was first provided by Ferrari et al. (2005). In this work, a small object was manipulated by a hand controlling reverse or normal pliers. Although the goal was the same, the kinematic requirements were opposite. To grasp the object with the reverse pliers the hand aperture had to be opened to close the other side of the pliers on the object. A small subset (12/74) of mirror neurons exhibited similar responses to the act of grasping the object, not caring about the kinematic difference. In another study evidence for a dominated role of the action goal rather than movement kinematics was obtained by comparing responses of F5 mirror neurons to the attempt to get hold of an object by grasping it with pliers or spearing it with a stick. Again a significant group of mirror neurons responded to the observation of the action goal rather than caring about how the actor´s hand got hold of the object (Rochat et al., 2010).

Although the early work seemed to suggest that mirror neurons are the hallmark of a specific area, F5, it soon became clear that neurons with similar properties could also be found in other parts of the cortex. In fact, there are studies that demonstrated the existence of neurons with properties very similar to those of F5 mirror neurons in the inferior parietal lobule (ILP, PF/PFG), a part of parietal cortex later shown to maintain strong reciprocal anatomical interactions with area F5 (Fogassi et al., 2005; Rizzolatti and Sinigaglia, 2010). In the experiments of Fogassi and colleagues, monkeys had been trained to perform either grasping to eat or grasping to place sequences. In the grasping-to-eat task, the monkey´s hand started from a fixed initial position to reach and grasp a piece of food located in front of the monkey to finally bring the food to the mouth. In the grasping to place task, the monkey carried out a similar reach to the object and grasped it, yet then was asked to place it into a container to receive a reward. The container was positioned either close to the monkey's mouth or in front of the monkey. The mirror neurons recorded from the rostral part of the inferior parietal lobule (IPL) responded differently to self-executed grasping for placing and grasping for eating even though the two grasping acts did not differ. A comparable differentiation was found to hold for the observation of these two actions. Using the same two tasks, they also recorded neurons from the F5 region and found that F5 mirror neurons had comparable properties characterized by different responses to similar grasping actions serving different purposes (Bonini et al., 2010). Hence, this work suggests that mirror neurons care about the action purpose or goal rather than about the kinematic structure of actions or motor acts. When comparing the properties of motor neurons in F5 and the PFG region of the parietal cortex, the only difference found was that the sample of PFG motor neurons studied was characterized by a grasping to eat preference not exhibited by F5 motor neurons. This difference led the authors to suggest a particular role of PFG in planning natural actions. A similar preference for the more natural sequence (i.e. grasping for eating) holds for PFG mirror neurons in contrast to those recorded from F5. The tight anatomical connections between the PFG region and F5 alluded to before and the similarity of response patterns in the two cortical areas may suggest joined contributions to the processing of action-related information. According to Fogassi and colleagues, these action-related responses should be interpreted as signatures of specific intentions or action goals serving both action execution and observation.

The act of grasping an object action can be parsed into several semantically distinct components: initiating the sequence, e.g. by leaving a home location and adopting a particular pose of the hand, reaching out for the target object, forming a grip, touching it, and acting on it to serve different goals. The work by Fogassi and colleagues alluded to before, showed that observation-related responses of mirror neurons distinguish between motor acts embedded in different action contexts (e.g. grasping to eat vs. grasping to place), although the actual motor act of grasping may be more or less the same. In other words, the semantic context matters. This view finds further support from more recent work that showed that the entire sequence of behavioral epochs that comprise a particular motor act, matters, both for action observation and execution (Mazurek et al., 2018). This conclusion was reached by fitting hidden Markov models to the firing of mirror neurons recorded from the premotor and primary motor cortex. Markov modeling adopted for the firing of neurons is a statistical approach that uses an observable output to predict a hidden input, here, to predict the underlying hidden behavioral state from the observed discharge pattern. The authors of this work could show that hidden Markov models trained on the behavioral sequences executed by the monkey were able to predict semantically comparable sequences carried out by others in the population response involving neurons from both the premotor and motor cortex. This result is in principle in line with the idea that mirror neurons contribute to the understanding of semantically defined action goals.

Another line of work relevant to the question if mirror neurons contribute to action understanding have been experiments on intransitive actions. In linguistics, the attributes transitive and intransitive are used to distinguish verbs that act on objects or not. Similarly, actions can be object-directed or not. A particularly interesting case of the latter are pantomimed actions as they lack the object, although simulating the kinematic structure of the corresponding object-directed action and at least in human observers evoking the image of an object-directed action. Assuming those mirror neurons represented a particular kinematic structure or the meaning of an action one might expect similar responses to the observation of intransitive and transitive actions. Whether this is the case has been contentious. While the Rizzolatti group argued that mirror neurons do not respond to the observation of intransitive actions (Gallese et al., 1996), more recent work concluded that they could be driven by intransitive actions, mimicking natural grasping (Papadourakis and Raos, 2017).

One problem that may explain the differences between findings on the representation of intransitive actions is that we do not know how a monkey observer might experience the observation of a pantomimed action. It is conceivable that rhesus monkeys interpret pantomimed actions similar to human observers as the reproduction of natural object-directed action. The absence of responses to intransitive actions might be due to an inability to evoke the image of natural goal-directed action. Hence, this interpretation would be in line with the view that movement kinematics per se, as said not profoundly different between intransitive and transitive actions, do not matter.

1.1.5 Challenges for the notion that mirror neurons serve action understanding

The similarity of responses to action observation and execution led Rizzolatti and coworkers to suggest that the cortex endowed with mirror neurons might represent an action type or action goal vocabulary. Awareness of a particular action type would be elicited indiscriminately by execution as well as by observation due to the activation of sets of specific elements of this vocabulary. Assuming a prime role of F5 in the planning and execution of actions, one might argue that the vocabulary´s ability to accommodate action observation might depend on its role in motor planning. This perspective nourishes the intriguing speculation that understanding the other´s action might be due to activating the observer's motor vocabulary in F5. Or in other words, action understanding might be based on allowing the observer´s machinery for motor planning to resonate with the action of the other and to activate a motor plan underlying a simulation of the other´s action (Gallese and Goldman, 1998).

Although intriguing, this resonance or simulation hypothesis faces a number of challenges. First of all, it is not at all apparent how 'action understanding' in the sense of awareness of distinct action types could be demonstrated in experiments on monkeys. As shown by Fogassi et al. (2001) lesions of F5 lead to disturbances of object-directed hand movements. However, if this deficit was associated with an inability to understand the other´s actions remained an open question as no attempts were made to probe understanding. And as addressed in much more detail in chapter 2 of this thesis one may rightly wonder if the assumed matching of observation and execution-related mirror neuron activity, arguably a major requirement of the simulation concept is given. In these experiments, two monkeys manipulated three identical objects in three different ways （lift, twist or shift). On the population level F5 mirror neurons could almost perfectly distinguish the three action types both during observation and execution. However, the population code for execution matched the code for observation only poorly. And the vast majority of individual neurons lacked matching. Only very few neurons exhibited a matching of their codes for short segments of the overall action period. This result is obviously at odds with the resonance or simulation hypothesis which posits matched code for action observation and execution as the basis of action understanding.

But there are more challenges such as the finding that observation-related responses depend on the spatial position in which the other´s action unfolds, hardly compatible with the resonance hypothesis. Position dependence of action observation responses of F5 mirror neurons was demonstrated by Caggiano et al. (2009), who studied monkeys watching human actors grasping objects that were positioned either in the observers' peri-personal space or outside, i.e. in extra-personal space. They found that about one-quarter of F5 mirror neurons preferred observed grasping actions in peri-personal space, another quarter preferred extra-personal while the remainder lacked a clear preference. Peri-personal space is characterized by the agent´s ability to reach objects and manipulate them. In fact, it turned out that the preference of F5 mirror neurons for observed actions unfolding in peri-personal and extra-personal space respectively is determined by the observer´s principal ability to reach the object, the other is acting on. This was demonstrated by introducing a transparent barrier that did not affect the vision of the actor´s action in the observer´s peri-personal space, yet eliminated his ability to interfere. In reaction to this manipulation, a significant fraction of mirror neurons with spatial tuning lost their ability to distinguish between peri-and extra-personal space. Hence, it is the operational and not the metric distance that matters. Understanding the purpose of the other´s action should not depend on the observer´s distance from it. However, deciding on a particular reaction to it should be. Hence, this work suggests that F5 mirror neurons may play a major role in response selection, a possibility that I explored in the experiments that are at the center of chapter 3 of this thesis.

Further support for the notion that mirror neurons care about the position of objects serving as targets of hand actions was provided by work carried out a few years later by Bonini et al. (2014a). Using a linear multielectrode probe for neuronal recordings they found that 48.2% (66/137) of F5 mirror neurons were selective for observed actions in peri-personal space and 25.6% for actions in extra-personal space. They also obtained evidence in accordance with earlier demonstrations of a view-dependency of action observation responses by Caggiano et al. (2009). According to Bonini and coworkers, a larger percentage of mirror neurons (54/178, 30.3%) than non-mirror neurons (21/185,11.4%) preferred the subjective (or first person) view. This preference may explain that execution-related responses of mirror neurons are stronger if carried out with light on, i.e. with visual feedback on the hand movement.

Like any purposeful action also the observer´s response to an observed action has a value that will influence the selection of an appropriate response. And assessments of the potential utility of a response will of course be influenced by information on the value of the other´s action for the observer. Hence seeing that the other is dealing with an object of relevance also to the observer will be more likely to elicit a behavioral reaction than an action directed at an object completely irrelevant for the observer.

Indeed the observation-related responses of F5 mirror neurons are modulated by the subjective value of the observed action (Caggiano et al., 2012). In this study, three experiments were carried out that tested mirror neurons' responses to the observation of objects assumed to have a different value. In the first experiment observation related responses of a majority (61%) of the studied mirror neurons were stronger for food as compared to non-food objects. Only a small subgroup (7%) of neurons showed a preference for grasping the non-food object while the remaining 32% did not exhibit a preference. In the second experiment, the monkeys grasped two cylinders (i.e. non-food objects) with only one of the two associated with a reward. In this case, about half (46%) of the tested mirror neurons exhibited a preference for the observation of rewarded grasping while 13% preferred the non-rewarded grasping act. Finally, in a third experiment, an object could be associated with two different levels of subjective reward (dry food or fruit – the latter assumed to be preferred by the observer) or no reward at all. When comparing responses to observed actions many mirror neurons showed selective tuning for the level of reward. These findings suggest that during action observation F5 mirror neurons have access to key information needed to shape the behavioral responses of the observer.

1.1.6 Mirror neurons may project to the spinal cord and, moreover, exhibit discharge suppression upon action observation

Mirror neurons in F5 may project to the spinal cord as demonstrated by the fact that they could be antidromically activated by stimulation of the brainstem pyramids. While the execution-related responses of these spinal cord projecting mirror neurons do not differ from previous descriptions, typically characterized by an action-related activation, observation-related responses of about one-quarter of these spinal cord projecting mirror neurons exhibited an action-related discharge suppression (Kraskov et al., 2009). Neurons with very similar properties were not confined to F5 but also found in M1, i.e. the primary motor cortex (Vigneswaran et al., 2013). One may wonder why neurons – by many implicated in action understanding – should project to the spinal cord, there arguably controlling motor networks. And in the same vein, one may wonder why a system contributing to action understanding based on simulation or resonance should exhibit qualitative different responses to action execution and observation. On the other hand, a neuronal system controlling execution-related circuitry in the spinal cord considering information provided by the other´s action would be pretty much in line with the response selection concept.

Differences between pyramidal tract mirror neurons in F5 and M1 were worked out in more detail in a follow-up study. In this work, two monkeys were trained to execute reach-to-grasp actions directed at two potential target objects, a 3D trapezoid and a sphere with the former demanding a precision grip and the latter a whole hand prehension. Alternatively, the monkeys observed a human actor carrying out similar reach-to-grasp actions. On the population level, they found a relatively close correspondence in amplitude and discharge profile when comparing activity evoked by observation and execution in F5. Not so in M1, where the correlation between execution and observation-related responses was much weaker and particularly weak in the early stage of the movement. The observation-related discharge exhibited by M1 pyramidal tract mirror neurons resembled activity profiles observed in a variant of the task in which neither the monkey nor the human agent moved their hands (no-go task). The authors argue that this similarity may suggest that the major purpose of observation-related activity may be to help to withhold self-movement if required by the context. In any case, the clear differences between M1 and F5 suggest different functional roles (Jerjian et al., 2020).

Neurons in M1 sensitive to action observation have also been described by others. For instance, Dushanova and Donoghue (2010) reported that M1 neurons may respond to the observation of simple reaching movements directed at spatial targets. According to these authors, this holds for roughly 75% of neurons in the hand and arm representation of M1. About one-third of them exhibited the same direction preferences for execution and observation, whereas the others were characterized by differences. The population of M1 neurons responsive to execution and observation can predict the direction and trajectory of reaching movements, no matter if the actor is the experimental animal or the observed agent. If the observation-related population signal execution is a reflection of the 'understanding' of others' reaching movements or a way to facilitate the observer´s action repertoire based on learning and mental rehearsal remains a matter of speculation. The aforementioned work of Kraskow and colleagues speaks against such a role.

1.1.7 Mirror neurons may predict others´ future actions

Mirror neurons are not confined to processing a current action, no matter if the processing may lead to 'understanding' or the selection of an appropriate response. Rather they may also reflect the absence of a particular action (an 'in-action') as the context may require withholding it. Moreover, mirror neurons may provide information on current actions but also predictive information on upcoming actions (or in-actions). These are the key conclusions suggested by work, carried out by Bonini et al. (2014b) who trained monkeys to execute a particular action whenever one variant of an auditory cue was provided and to withhold it ('in-action') in case of the second variant. Using this paradigm they could separate two groups of mirror neurons, one, activated whenever the action took place and a second activated whenever the action had to be withheld. In observation trials, responses in both groups started well before the start of the action/ in-action, shortly after the delivery of the instructive cue, i.e. the discharge predicted the subsequent action or inaction(Bonini et al., 2014b). Moreover, the predictive discharge started earlier in case the observed action unfolded in the monkeys' extra-personal space rather than in his peri-personal space.

1.2 'Mirroring' is a widespread property of neurons in the cerebral cortex

As discussed in the preceding sections, mirror neurons were originally found in F5, i.e. the ventral part of premotor cortex, and somewhat later also in parts of the parietal cortex and the hand representation of the primary motor cortex adjoining F5. In fact, in more recent years it became clear that the list of cortical areas accommodating neurons characterized by the mirroring property is not confined to just three. In the following paragraphs, I will provide a brief account of the various cortical areas beyond F5, here also providing additional information on M1 and the mirror neuron region of the parietal cortex, with a focus on similarities and differences that may help us to better understand the purpose(s) of mirroring.

Other areas in which such neurons were found are area AIP, located in the anterior part of the intraparietal sulcus (Ferroni et al., 2021), area F2 – also referred to as dorsal premotor cortex (Papadourakis and Raos, 2019) – as well as frontal cortex close to the midline, varyingly referred to as pre-supplementary cortex, area F6 (Livi et al., 2019) or medial premotor cortex (Breveglieri et al., 2019).

The pre-supplementary cortex (area F6) has been known as an element in a cortical network for the control of grasping characterized by the vision and grasping of objects (Lanzilotto et al., 2016). However, later work could demonstrate that F6 is not confined to the execution of object-directed motor acts. Similar to F5, also neurons in F6 may exhibit responses to action observation. According to Lanzilotto et al. (2016), one of the key features of F6 is the existence of two populations of neurons with visual sensitivity. One group emphasizes the vision of the action and the other group the vision of an object. Action-related neurons may encode the agent's own action, the other´s action or both. Object-type neurons may encode the object relevant to the agent, the other, or both. Hence, there seems to be both action and object mirroring.

In a subsequent study Ferroni et al. compared the preferences of mirror neurons in F6 with those in F5 and AIP, the latter – as already said also known to accommodate neurons that are characterized by the mirroring property (Ferroni et al., 2021). This comparison was based on adopting a go/no-go grasping task in which either the experimental monkey played the active part or a human actor that was observed by the monkey. At the population level, clear differences between areas were found: in execution trials, mutual information allowing the observer to distinguish between go and no-go trials became significant first in F6 then in F5, and last in AIP. On the other hand, information on the type of object and the grip afforded by the object was earlier in AIP and F5 than in F6. In the observation task, the major difference between areas was the dominance of facilitatory responses in F5 relative to the other. In general, the authors emphasize the greater similarity of responses between areas AIP and F5, both located on the lateral aspect of the hemisphere, linked by their interest in object and grip preferences compared to the medial area F6, probably more involved in the decision as to whether an action is released or suppressed.

Neurons with mirroring properties have also been found in the medial frontal cortex (mPFC), a region that adjoins F6 rostrally (Ninomiya et al., 2020). According to the work carried out by the Isoda laboratory, mPFC is characterized by neurons that are qualitatively reminiscent of those in F5 with many neurons activated by the agent´s self-action, others preferring observed actions and finally, mirror neurons responsive to both. In quantitative terms, there are differences between mPFC and F5 insofar as the latter two types of neurons are more frequent in mPFC. F5 projects to mPFC and observation-related activity in the mPFC depends on input from F5. This was established in recent work which showed that selective blockade of information flow from F5 to mPFC, resorting to an innovative double viral vector infection approach, impaired the processing of observed, yet not executed actions. The same group has implicated mPFC in autism (Yoshida et al., 2016), based on the serendipitous observation of a macaque monkey presenting an autistic phenotype. When exploring mPFC with micro-electrodes it turned out that neurons driven by others' actions were almost absent. Genetic analysis revealed the presence of a coding variant of the serotonin receptor 2C and other genes linked to human neuropsychiatric deficiencies.

Information on the other´s behavior and behavioral goals also seems to be available in the lateral prefrontal cortex. This is the conclusion of a study carried out by Falcone et al. (2016) who trained monkeys on a task that required monitoring the other´s choices and the consequences of these choices. Considering the acquired information allowed the observer to adjust his/ her own behavioral choices for her/ his own benefit. While the monkey performed this task the authors recorded the activity of neurons in the lateral prefrontal cortex. They found that the neurons' firing rate reflected not only the monkey actors' choices but also those of the observed human actor´s recent and future choices (Falcone et al., 2016).

Not only the ventral part of the lateral premotor cortex, i.e. F5, but also the dorsal lateral premotor cortex (PMd) contains neurons with mirror properties. This was shown by Raos et al. who recorded from this region in monkeys performing or observing grasping tasks (Papadourakis and Raos, 2019). They identified neurons with properties very similar to those in F5. Moreover, also the frequency of mirror neurons was comparable to the one of mirror neurons in neighboring F5, explored by the authors for comparison, deploying the same tasks. However, mirror neurons in the F5 region exhibited a stronger action type selectivity than those in the dorsal premotor cortex, whereas mirror neurons in PMd were found to show earlier response, both during action execution and observation than F5.

Unlike the previous authors, Cisek and Kalaska (2004) did not study neurons during actions directed at natural objects but reaching movements directed at elementary spatial cues, following a delay in response to a ‘go’ signal. They found that the majority of PMd neurons recorded showed similar response modulation, no matter if the experimental monkey executed the task or observed a human agent carrying out a similar task. Rather than suggesting a link between these responses to action or action goal understanding, the authors argued that the observed responses might be reflections of mental rehearsal of a seen action (Cisek and Kalaska, 2004).

In a relatively recent publication, Cirillo et al. (2018) provided support for Cisek´s finding that neurons in the dorsal lateral premotor cortex may be activated by actions directed at spatial targets, no matter if the experimental monkey executes or observes the action. Yet, in contrast to the former, Rossella et al. emphasized a substantial degree of self-other differentiation of neuronal responses. Neurons encoded either the future choice of the animal, of the human actor, or both. This result suggests the notion that these neurons may underpin independent representations of the future actions or intentions of the two agents involved (Cirillo et al., 2018).

1.3 Mirror neurons in species other than macaque monkeys

1.3.1 Marmosets

There is very preliminary evidence for the existence of mirror neurons in the marmoset, a new world monkey species, among others characterized by a flat (i.e. lissencephalic) cerebral cortex. Because of the lack of sulcal landmarks and in particular the absence of an arcuate sulcus, it is not possible to delineate the ventral premotor cortex as a potential homologous of the macaque monkey area F5. This is why Suzuki et al. (2015) first identified neurons in the superior temporal sulcus (STS), also identifiable in the marmoset, activated by the observation of grasping actions. Then by injecting a retrograde fluorescent tracer into this region, they could identify a group of labeled cells in the ventral lateral frontal cortex, an anatomical connection interpreted as analogous to F4/F5 in macaque monkeys. Neurons in the labeled region of the ventral lateral frontal cortex were described as being activated by self-actions and in some cases also by observed actions, reminiscent of the key properties of F5 mirror neurons in the macaque (Suzuki et al., 2015). As rigid controls and quantitative criteria were lacking this finding will require verification by future work.

1.3.2 Rodents

Tombaz et al. (2020) used two-photon microscopy to measure Ca2+ signals as a proxy of electrophysiological signals in neurons in the posterior parietal cortex and secondary motor cortex (M2) of awake mice engaged in pellet reaching or running on a wheel. Not surprisingly they reported activity evoked by these movements. However, contrary to the expectation, neurons in both regions were not significantly activated by observing other animals' movements. Hence, this study suggests that action recognition must take place elsewhere in the rodent´s brain (Tombaz et al., 2020).

In contrast to Tombaz and colleagues, Viaro et al. (2021) reported evidence of activity in a frontoparietal network that was evoked by action observation. In this work they studied the rat´s forelimb representation in motor cortex, resorting to micro electrocorticography and single-unit recordings during a self-grasping vs. other-grasping task, reminiscent of the classical tasks used in early work on monkey area F5. In fact, they could identify neurons activated by self-actions as well as action-observation in the region explored, reminiscent of monkey mirror neurons (Viaro et al., 2021).

Numerous neuroimaging studies – mostly resorting to functional magnetic resonance imaging (fMRI) –have tried to reveal a mirroring network in the human brain. Any useful attempt to summarize and evaluate the evidence obtained would require careful consideration of the peculiarities and limitations of this work that are introduced by the reliance on the fMRI method which is outside the scope of a thesis that focuses on the analysis of information of indubitable neuronal origin. A useful account of human studies can be found in a recent review (Iacoboni, 2009). I confine myself to briefly touch on studies that have tried to lead the emergence of empathy – the ability to feel the other´s pain – back to the activation of a mirroring mechanism, among others involving the human cingulate cortex. This seems worth mentioning because of its obvious relationship to the concept of action understanding. Support for this notion comes from recent work on the rat´s anterior cingulate cortex (ACC)(Carrillo et al., 2019). In these experiments, the animals experienced foot shocks that evoked pain.

Alternatively, the experimental animals could observe that other rats received foot shocks, an experience that elicits a freezing reaction in the observer. A subset of ACC neurons recorded from the ACC, termed 'emotional mirror neurons' by the authors, discharged not only when the rat experienced self-pain but also observed another rat in pain. Deactivating the ACC region by muscimol injections substantially reduced the freezing, suggesting that the rat ACC accommodates a causal mechanism based on these emotional mirror neurons. One might argue that selecting a specific behavior –here freezing – in response to observing a particular interaction of the other with their environment, responsible for the experience of pain –seems to be conceptually closer to response selection than to action understanding.

1.3.3 Songbirds

I may finally mention that even species that are phylogenetically much more distant from primates than rodents, namely birds, make use of neurons that are characterized by mirroring. Here this mechanism and the underlying neurons seem to play an important role in the song system. This was established by Prather et al. (2008) who found a group of neurons in the telencephalic HVC (caudal nucleus of the ventral hyperstriatum) of the swamp sparrow, a species of songbird in which HVC neurons may exhibit very similar discharge patterns associated with the production of a song and to listening others singing the same song, an example of auditory-vocal mirroring. It has been suggested that these neurons play a role in vocal learning (Mooney, 2014; Prather et al., 2008).

1.4 Conclusion and outlook on the following chapters

In summary, previous work on mirror neurons – and in particular the early studies – have prompted fascinating concepts on their functional role. Yet, one might criticize that the concepts were not necessarily based on sufficiently solid and convincing data. The major goal of my studies, reported in the following two chapters, was to add experimental findings, based on novel paradigms, that might allow me to explore the possibility that F5 mirror neurons contribute to response selection. This question is at the center of chapter 3, whereas chapter 2 explores the justification of the assumption of similarity of action observation and execution, a prerequisite for a role in action understanding as compared to response selection and more general functions in sensorimotor transformations.

Chapter 2 Poor matching of action codes challenges ‘mirroring’ in macaque F5 mirror neurons

2.1 Introduction

Mirror neurons have been defined as neurons that modulate their discharge rate not only in response to the execution of an action directed toward an object but also in response to the observation of a similar action carried out by others (Gallese et al., 1996; Rizzolatti et al., 1996). The discovery of neurons characterized by this intriguing combination of response preferences in premotor cortical area F5 of macaque monkeys (di Pellegrino et al., 1992) attracted great interest, which resulted not only in a large number of subsequent studies in which the properties of these neurons were explored in more detail (Rizzolatti et al., 2014) but also in the discovery of mirror neurons in other cortical areas of monkeys (Fogassi et al., 2005; Papadourakis and Raos, 2019) and species, including humans (Mukamel et al., 2010).

Down to the present day, researchers studying mirror neurons have been mesmerized by the possibility that these peculiar neurons might play a role in processing information on the other´s actions, rather than being confined to action planning and/or control, the latter perhaps suggested by their occurrence in area F5, formerly thought to be confined to motor planning (Rizzolatti et al., 1988). Nevertheless, why should the information on the other´s action be processed by neurons in a motor area? A possible answer is that the observation of others´ actions might resonate in neuronal machinery, at other times controlling similar actions of the observer. In other words, action understanding is thought to arise from allowing the observer´s action planning and execution machinery to simulate the other´s action (Rizzolatti et al., 1996).

Simulation of others’ action based on F5 mirror neurons would require that the activity of these neurons is the same (or similar enough), no matter if ignited by the desire to engage in a particular action or in its observation. This is the tenet of the mirror mechanism (Rizzolatti and Sinigaglia, 2016). Although some studies suggested that indeed a substantial fraction of mirror neurons might satisfy this hypothesis (di

Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996), others have remained skeptical, considering inconsistencies and limitations of the experimental evidence available that may not suffice to support the strong requirements of the mirror mechanism (Cook and Bird, 2013; Csibra, 2007; Hickok, 2009; Thompson et al., 2019). For instance, whereas Gallese and colleagues found that about a third of mirror neurons exhibited the same grip type preferences during execution and observation (Gallese et al., 1996), a recent study reported similar grip type tuning during execution and observation tasks only at the population level. However, in the latter study, the relationship of grip type preferences for execution and observation at the single-cell level was reported to be random (Papadourakis and Raos, 2019). The lack of consistent evidence for mirroring grip type preferences might be because mirror neurons serving the assumed mirror mechanism may not care about specific grip types but about action goals, independent of a consideration of low-level features that matter in the description of specific grip types (Rizzolatti and Sinigaglia, 2010). In accordance with this possibility, previous studies could indeed show that the response of some mirror neurons to executed or observed actions can be largely independent of the effector used — hand, mouth, or tools including reverse pliers — as long as the action goal was maintained (Ferrari et al., 2005; Rizzolatti and Sinigaglia, 2016; Rochat et al., 2010).

Encouraged by these results, it was concluded that mirror neurons may indeed encode an action goal as basis of action understanding (Rizzolatti and Sinigaglia, 2016). However, while certainly intriguing, this conclusion suffers from a shortage of expedient experimental data needed to critically assess it. For instance, responses to execution and to observation should not only match for the preferred goal identified by the strongest modulation but also for others, albeit giving rise to less discharge modulation. In order to test this prediction we decided to carry out a rigorous comparison of responses to three different action goals by asking the experimental animal to manipulate a visually identical object in three different ways, namely to lift it, to twist it, or to shift it. This enabled us to characterize an action tuning, while avoiding a confounding influence of different objects. The actions differed in the ultimate action goal given by different end positions and the type of manipulation requiring different grip types. We reasoned that if mirror neurons were the substrate of a mirror mechanism supporting a representation of action goals, then this experimental paradigm should reveal it. And since the mirror mechanism demands a matched code between action execution and observation, it should be possible to decode the specific action from the activity of mirror neurons during observation if we knew how the same action was encoded during execution.

We found that population-based decoders, trained on action observation trials predicted observed actions with a reliability of up to 100% in dissociating the three different action types. However, decoders trained on execution trials predicted observed actions only poorly (up to 55%; chance level 33.3%). Even in a small subpopulation, in which execution-trained decoders predicted observed actions equally well as observation-trained decoders, this prediction was confined to a segment of the overall action epoch. Hence, these results prompt a reconsideration of the role of mirror neurons beyond the tight bounds of a mirror mechanism.

2.2 Material and methods

2.2.1 Experimental design

The experiments were performed on two adult male rhesus monkeys (Macaca mulatta). All experiments were approved by the local animal care committee (Regierungspräsidium Tübingen and Landratsamt Tübingen) and conducted in accordance with German and European law and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, and carefully monitored by the veterinary service of the University of Tübingen.

2.2.2 Behavioral tasks

Three objects to be manipulated by either the monkey or a human actor were fixed on a table and aligned parallel to the ground. The table rotated twice between trials (in the same or in opposite directions and for variable durations) to place randomly one of the three objects in front of the respective actor. The objects were metallic discs identical in shape and size (diameter 3.5 cm, height 0.5 cm). In the execution task, the center of the object at stake had a distance of 23 cm from the monkey's eyes. In the observation task, in which a human standing opposite to the monkey acted on the relevant object, the distance to the monkey was 49 cm. Each object accommodated only one specific type of action: it could be either lifted (2.7 cm up), twisted (50° clockwise), or shifted (1.9 cm to the right). Note that the identical appearance of the three objects denied any clue on the type of action they accommodated.

In the execution task, the rotation of the table and the execution of the action on the target object by the monkey took place in darkness. In the observation task, a light was switched on when the object was rotated in place so that the monkey could observe the experimenter standing opposite to him (light was turned off with object release or if a trial was aborted).

A trial started when the object was rotated in place and the actor had pressed the start button. A variable time (1-1.5 s) after trial start, an LED next to the positioned object that had halted in front of the respective actor, was turned on for 0.1 to 0.3 s. The LED color indicated the manipulation to be performed: green or yellow for a lift, white or blue for a twist, red for a shift (in 5% of trials a yellow LED, noninformative for the type of object and located in a slightly different position was turned on; these trials were not analyzed here). The actor was allowed to release immediately the start-button to approach, manipulate, and finally hold the object against a resistance (against gravity for lift, against a spring for twist and shift). After a variable time after object hold start (0.3-0.8 s), the monkey — no matter if he had performed the action or had observed the human action — received water (or occasionally also units of a banana flavor high caloric drink) as reward, followed by the actor releasing the object. The amount of fluid per trial varied between 0.15 and 0.5 ml across sessions but was the same across the six actions (3 for each task) within a session.

The task events 'start-button press´, 'start-button release´, 'LED onset´, ‘touch of object´, 'start moving the object´, 'reaching the hold position´, 'reward´, and 'withdraw from the object´ by releasing it, were registered by mechanoelectrical sensors. A trial was aborted (and a beep was given as feedback to the monkey) if the start-button was released within 100 ms after LED onset (to motivate the monkey to use the LED), if the object was released before reward delivery or if a timeout was reached 10 s after trial start. To motivate the monkey even more to use the LED in the execution task, another timeout was active in 30% of trials (session-dependent rarely up to 100%) for the time period between 'touch of object´ to 'start moving the object´ : 0.15 s (rarely 0.1 s) for a twist and a shift, 0.35 s (rarely 0.3 s) for a lift). In the observation task, a trial was also aborted (and a beep was given as feedback to the monkey) if the monkey did not attend to the action as indicated by gaze not staying inside a given fixation window (see below). For the control of the experiment and the recording behavioral data, we deployed in-house open-source software (nrec: https://nrec.neurologie.uni-tuebingen.de, developed and maintained by F. Bunjes, J. Gukelberger, V. Gueler) running under Debian Linux on a standard PC.

2.2.3 Measurement of eye movements

Eye position was recorded using an in-house video eye-tracker based on pupil detection in infrared light, operating at a sampling rate of 50 Hz, in one monkey and by permanently implanted search coils in the other monkey. Eye position recordings were calibrated at the beginning of each experimental session by asking the monkey to fixate a target dot displayed on a monitor, seen by the monkey in the table plane after redirection by a mirror in front of the monkey. In the table plane, the target dot (red color, 0.1° radius) appeared within the range the human actor performs his action, allowing the reliable association of object position and eye movement records. As the monkey´s head was painlessly fixed (see below) during the experiment, eye position corresponded to gaze position. In the action observation task, attention to the action was ensured by making the delivery of reward contingent on gaze staying within a fixation window of ±7° vertically and ±5° horizontally relative to the center of the relevant object.

2.2.4 Surgical procedures

The animals were implanted with a titanium post accommodating the painless fixation of the head and a titanium recording chamber overlying area F5 of the left hemisphere. The correct position of the chamber was determined using information from a pre-surgical anatomical MRI scan. All surgical procedures were conducted under strict aseptic conditions deploying combination anesthesia with isoflurane (0.8%) and remifentanil (1–2 microgram/kg·min) with full control of relevant physiological parameters such as body temperature, heart rate, blood pressure, PO_2, and PCO_2 were monitored. Postoperatively, buprenorphine was given until signs of pain were gone. Animals were allowed to recover fully before starting the experiments.

2.2.5 Electrophysiological recordings

Extracellular action potentials were recorded using glass-coated tungsten electrodes (0.5- to 2-MΩ impedance; Alpha Omega) using a multielectrode system equipped with up to eight probes (Alpha Omega Engineering). Action potentials of individual neurons were discriminated online resorting to template matching provided by Alpha Omega´s Multi Spike Detector.

The mirror neurons reported in this study were recorded from area F5 of the left hemispheres of the two experimental animals. Area F5 was targeted, guided by presurgical MRI, information on the location of the arcuate sulcus provided by electrode penetrations and a consideration of the response properties of neurons in F5 and in FEF as well as characteristic behavioral reactions to microstimulation with saccades evoked from the FEF and arm, hand, face, or mouth movements elicited when stimulating F5.

2.2.6 Mirror neuron criteria

Except for the classification analysis to be described later, we used software based on MATLAB (2019a). For the analysis of discharge patterns, only well-isolated single units from area F5 were considered for which at least eight valid trials per condition were available. A trial was ´valid´ if the aforementioned sequence of task events (´start-button press´ to 'withdraw from the object´) was registered by the sensors without technical malfunction. A neuron was classified as an F5 mirror neuron resorting to established criteria (e.g., Pomper et al., 2020). To this end, a task was subdivided into 5 epochs: baseline (-750 ms to -250 ms from LED onset), approach phase (from start-button release until touching the object), manipulation phase I (from touching the object until moving the object), manipulation phase II (from moving the object until holding the object in its target position), hold phase (from holding the object in its target position until 150 ms later). A Friedman test with the factor 'epoch´ was performed for each action (lift, twist or shift) separately. A neuron had a motor or a visual response if the Friedman test was significant for at least one action (Bonferroni correction, alpha = .05/3) in execution or observation, respectively. If a neuron had a motor and a visual response, it was classified as a mirror neuron. Note that in accordance with standard procedures this classification did not require that the modulation affected the same epoch or same direction (i.e. discharge rate increase vs. decrease).

2.2.7 Action preference analyses

Action preference was determined for each neuron and time bin separately for execution and observation based on three pairwise comparisons between two conditions (execution and observation) deploying Wilcoxon rank-sum tests: lift (L) vs. twist (T), lift (L) vs. shift (S), T vs. S, Bonferroni-corrected for the 3 comparisons and 24time bins. An action was considered the preferred one if its discharge rate was significantly above the other two. If two actions were not statistically different but significantly above the third one, both were considered the preferred ones. In total, this resulted in three classes of narrow action preference (L, T, S) and another three classes of combined action preferences (LT, LS, TS). We finally asked if a mirror neuron had the same action preference for observation and execution.

2.2.8 Classification analyses

We used a linear discriminant classifier (Fisher 1936) by running the ‘classify´ function available in MATLAB 2021a, in order to explore if the information offered by either single mirror neurons or the population discharge, predicted specific action types.

For a given time bin, the classification was performed on the average discharge rate of a neuron over the course of the time bin, using the same number of trials for each task condition. The number of trials for each task condition considered for single neuron classification was neuron-dependent and corresponded to the minimum number of trials per condition across both tasks and all conditions. For population classification, the number of trials for each task condition was eight, since only units with at least eight trials per condition were considered. The linear discriminant classifier was trained on 75% of trials selected randomly for one task (observation or execution) and the model obtained was then tested on the remaining trials of the same task, or in case of across-task classification, it was tested on all trials of the other task. For example, the model trained on discharge rate during execution was used in an across-task comparison to determine, how well it predicted the action from the discharge rate acquired during observation. The whole sequence of model calculation, within and across-task prediction, was repeated 200 times by randomly selecting new sets of training trials. The bin-wise means of the 200 iterations were taken as the performance of the classifier for individual neurons.

For the population classification, each neuron´s activity was used as a feature of the classifier. Six trials (75%) of a given task were used for training and the remaining two trials were used for testing. In the across-task classification, all eight trials of the other task were used for testing. The remaining procedure was like the single neuron classification.

2.3 Results

2.3.1 Paradigm

Two rhesus macaques were trained to perform the behavioral paradigm depicted in figure 1. The paradigm consisted of two tasks that were performed in two separate blocks (Fig. 1A): an execution task, in which the experimental monkey had to perform one out of three possible actions on objects with identical visual appearance (Fig. 1B, top row), and an observation task, in which he was asked to pay attention to the same actions carried out by a human actor in front of him (Fig. 1B, bottom row). Which action the object at stake afforded was indicated by a color cue provided by an LED next to the respective target object (Fig. 1A). The target object was always the object coming to hold right in front of the respective actor (the monkey or the human) after an initial random rotation (in darkness) of the table carrying the three identically looking objects, carried out in order to ensure that memories of previous action type choices were useless. Distinct events allowed the separation of six epochs (Fig. 1C).

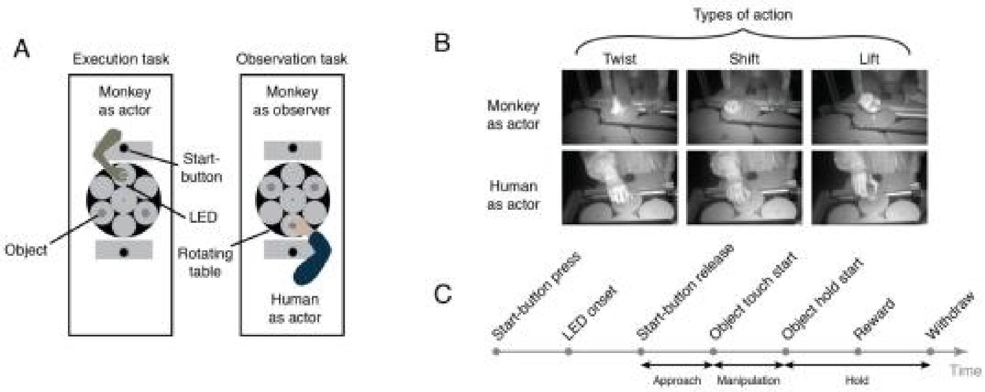

Figure 1. The behavioral paradigm. (A) Experimental setup with three identical objects positioned on a rotating table in front of the actor. Each object could be acted on in only one way. The type of action required was cued by the color of an LED next to the object. After LED onset, the actor was allowed to release the start button. (B) Photographs of the three actions in execution and observation tasks at the time when the object was held in its target position. (C) The sequence of events in a trial.

Out of 240 neurons recorded from two monkeys, 177 (74%) met the mirror neuron criteria outlined in the Methods and were used for the following analyses. As summarized in Table 1, the time between two adjacent events varied depending on the trial, type of action, and task. In order to make the timing of events comparable, we transformed the absolute times between events to a relative time by dividing the time interval between every two adjacent events into quartiles. Hence, each trial was divided into 24-time bins between the time of pressing the start button and the time of withdrawing from the object.

	Monkey			Human		
	twist	shift	lift	twist	shift	lift
Button press to LED onset	3295 [1206, 4728]	3611 [1249, 5224]	3607 [1360, 4974]	1274 [1029, 1508]	1288 [1030, 1515]	1276 [1029, 1509]
LED onset to	297	303	292	461	452	473

Button release	[226, 394]	[231, 407]	[220, 385]	[336, 669]	[325, 676]	[337, 690]
Button release to touch	162 [111, 219]	182 [111, 248]	178 [110, 244]	276 [205, 409]	300 [214, 424]	315 [231, 457]
Touch to hold	235 [140, 624]	117 [59, 667]	325 [203, 649]	132 [74, 292]	121 [61, 294]	291 [182, 508]
Hold to reward	566 [329, 804]	570 [328, 805]	569 [328, 804]	570 [329, 806]	569 [327, 804]	569 [329, 805]
Reward to withdraw	666 [296, 1301]	740 [317, 1365]	472 [297, 703]	386 [230, 795]	436 [280, 797]	440 [265, 877]

Table 1. The average duration (in ms) across trials of each task period per action for monkeys and humans. The lower and upper bounds of the 95% confidence intervals are shown in brackets.

2.3.2 Only a minority of mirror neurons exhibit congruent action tuning in at least one time bin

Similar to previous approaches to mirror neurons, we identified the preferred action of a neuron as the single action or a pair of actions (in case the two actions were not statistically different) that yielded a discharge rate that was significantly larger than those associated with another action type (s) (see Methods). We assessed the preferences independently for each of the 24 bins. In the execution task, most neurons had a preferred action in at least one time bin (n = 137, 77%). In the observation task, this proportion was slightly less (n = 104, 59%). In total, it was common that the preferred action differed across the time bins (see the exemplary neurons in fig. 2).

The comparison of action preferences between execution and observation showed that a quarter of all mirror neurons (n = 41, 23%) had the same action preference in at least one time bin. Some neurons maintained the same preference across multiple time bins (such as the neurons depicted in Fig. 2A), whereas others had the same preference in only one time bin (such as the one in Fig. 2B). The remaining neurons that had a preference in at least one time bin during observation (n = 63, 36%) had either no preference or a different preference in that time bin during execution (such as Fig. 2C). In short, only a minority of mirror neurons exhibited the same action preferences for execution and observation. In these neurons, however, congruent preferences were usually confined to restricted periods of time.

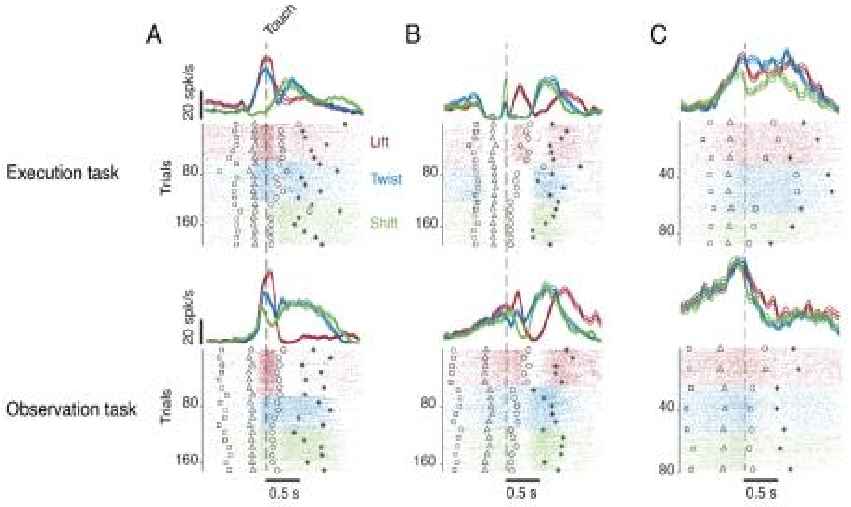

Figure 2. Exemplary F5 mirror neurons. Spike density functions and raster plots are aligned to the time of touch start (vertical dashed line). (A) A neuron prefers a lift at the time of touch, a twist, and a shift in both tasks. (B) A neuron prefers a shift during execution and a lift during observation at the time of touch, but a lift after touching in both tasks. (C) A neuron that has no or different preference(s) during execution and observation. In A-C, markers indicate the time of four events around the time of touch: LED onset (square), start-button release (triangle), object hold start (circle), and reward (star). For better visualization, the events are shown for only about 10% of trials.

2.3.3 The action encoding in the execution and observation tasks transfers only incompletely to the respective other task

The question is if an ideal observer, having to consider all mirror neurons rather than being able to select the minority exhibiting congruent action preferences (as indicated by the preliminary analysis), would still be able to predict the type of action carried out? In order to address this question, we resorted to a linear discriminant classification of actions, allowing us to determine if the neural code of actions in the execution task would be able to predict the actions during observation. In the first step, we asked how well the three actions could be decoded during the execution task. We trained a classifier for each neuron and time bin based on 75% of the execution trials (as described in more detail in the Methods) and examined how well it predicted the action in the remaining trials (exe ® exe, blue curve in Fig. 3A). We found that the mean performance of the classifiers calculated across all mirror neurons increased sharply in the third time bin after LED onset (Fig. 3A). This increase continued with a lower slope until it reached its maximum of about 42% successful discrimination (chance level 33.3%) in the first time bin after the object had been moved to its final position.

In a second step, we asked how well the three actions could be decoded during the observation task. We trained a classifier for each neuron and time bin based on 75% of the observation trials and examined how well it predicted the action from the remaining trials (obs ® obs, green curve in Fig. 3A). The rise of discrimination performance started three time bins later, in the second time bin after start-button release. However, the level of maximal discrimination achieved (~42%) was comparable to the execution decoder's performance and the peak was reached at the same time. In sum, the actions could be read out from the discharge rate of mirror neurons during both execution and observation tasks equally well at least in one time bin after the object had been moved to its final position.

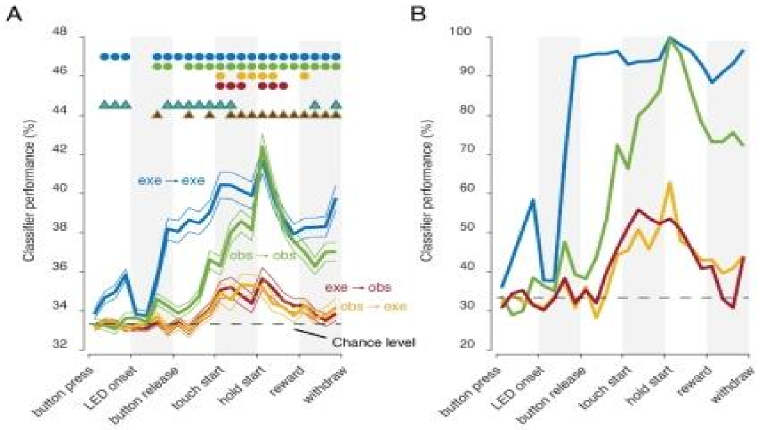

Figure 3. Classification of the three actions. (A) Single neuron classification results (mean ± s.e.m, n = 177 neurons) per time bin, execution-trained classifiers tested with execution trials (exe ® exe, blue), tested with observation trials (exe ® obs, red). Observation-trained classifiers tested with observation trials (obs ® obs, green), and execution trials (obs ® exe, orange). Colored dots indicate time bins with significant performance above chance (Benjamini-Hochberg-corrected one-sided signed-rank tests). Two colored triangles indicate time bins with significant differences in the performance between the corresponding colors (Benjamini-Hochberg-corrected two-sided signed-rank tests). Shaded gray areas are for better visual dissociation of the task periods, each consisting of four time bins. (B) Population classification results per time bin. Same as A, but here, all neurons (n = 177) constructed the 177 features of a classifier.

We finally asked if the encoding of the actions during the execution and observation tasks matched. Therefore, we applied the classifiers trained in one task to the other task respectively (exe ® obs, the red curve in Fig. 3A; obs ® exe, the orange curve in Fig. 3A). Regardless of the direction of the test (exe ® obs or obs ® exe), we observed an increase in the discrimination performance that started in the third time bin after the start-button release and reached the highest level of performance while the manipulation was carried out. However, the maximum level of performance attained was only ~36% for both transfer directions, which was significantly below the exe ® exe and the obs ® obs discrimination performances. Hence, there is a match between the codes for the three actions in the execution and observation tasks, yet the match is poor.

A biologically plausible way to read out actions offered by a population of mirror neurons would be to rely on the collective activity of all, rather than on individual neurons. Therefore we trained a linear discrimination classifier on the discharge rates of all 177 mirror neurons, separately for execution and observation, in a bin-wise manner, fully analogous to the procedure deployed for the single neuron classification. As shown in figure 3B, we found a very high performance for both the exe ® exe and the obs ® obs classifications with a time course comparable to the single neuron-based results. Shortly after reaching the final position of the object, the performance was optimal (100%) for both the exe ® exe and the obs ® obs classifications. The classifiers trained on data from one task and tested on data from the other task gave a much weaker performance of about 55% to 60%. In sum, as expected, the population classifiers performed much better than the single-neuron classifiers. However, similar to the single neuron classifications, the across-task performances were consistently below the within-task performances.

2.3.4 There is a cluster of time bins with a matched code

It is conceivable that the across-task performances of all single neurons were consistently (across neurons and time bins) weaker than the within-task performances. Alternatively, the sample of mirror neurons studied might comprise a subset of mirror neurons characterized by perfect classification transfer across tasks, albeit restricted to particular time bins. In order to decide between these two scenarios, we compared the across-task discrimination performance of individual mirror neurons to the within-task performance. Rather than using the absolute discrimination level, we chose the difference relative to the chance level for a particular time bin shown in Fig. 3A. As the concept of a *mirror mechanism* posits that the observation performance can be led back to an activation of a motor representation, we restricted this analytical step to a comparison of the exe ® obs and the obs ® obs discrimination performance.

Figure 4A depicts the resulting scatter plot of the exe ® obs discrimination performance as a function of the obs ® obs discrimination performance with individual data points representing individual neurons and time bins out of the 12-time bins covering the complete action sequence from start-button release until reward delivery. Data points indicating the same discrimination performance for within-task and across-task classifications would fall on the unity line (45 degrees line). On the other hand, a dissimilar discrimination performance would result in data points that are distributed around the exe ® obs chance level (0 degrees line). If across-task performances were consistently weaker than within-task performances, we would expect the data points to be distributed around a regression line with a slope lower than 1. In fact, the regression line based on all data points had a slope of only 0.25 (Fig. 3A), indicating a relatively weak transfer of decoding.

In order to assess how data points were distributed with respect to these reference lines, we transformed each data point to polar coordinates and created a distribution of angles. An obvious caveat is that meaningless data points were contained, characterized by the lack of discrimination performance in the reference case, i.e. the obs ® obs classification. As there is no a priori criterion that would allow one to distinguish good discrimination performance from insufficient performance, we compared the influence of increasingly restrictive thresholds on the obs ® obs performance (starting from 5% above chance level to 20% in steps of 5%).

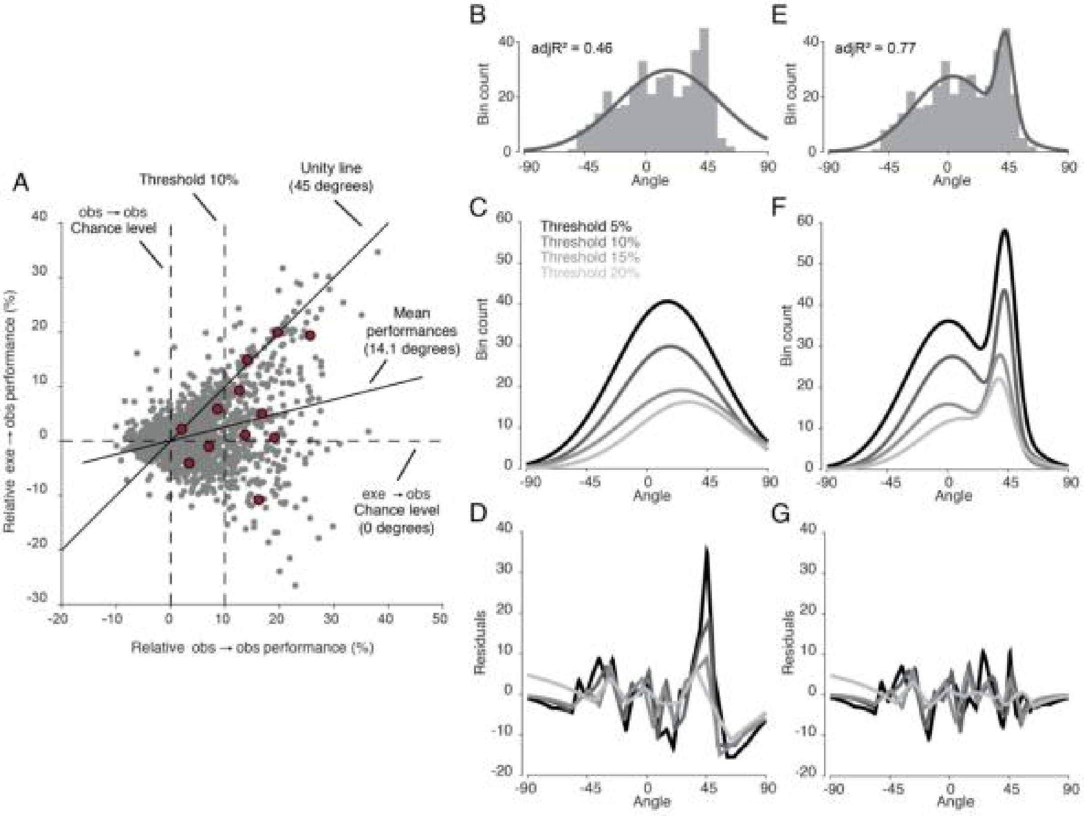

Figure 4. Identification of matching bins. (A) Each dot in the scatter plot represents the exe ® obs performance (related to the red curve in fig. 3(A) and obs ® obs performance (related to the green curve in fig. 3(A) of a neuron in a time bin with respect to chance level. Each neuron contributed to this plot with 12 time bins between start-button release and reward. The red dots indicate the data points of an example neuron. Threshold 10% is one instance of the four filtering thresholds. The mean performances line (14.1 degrees) is a regression line of all the data points. (B) The gray histogram shows the distribution of the angles of each dot above threshold 10% in A. The envelope depicts the single-Gaussian fit. (C) Single-Gaussian fits for four different thresholds. (D) The residuals of the single-Gaussian fits for the four thresholds. (E) The two-Gaussian fits for the four thresholds. (F) Two-Gaussian fits for the four thresholds. (G) The residuals of the two-Gaussian fits for the four thresholds.

As shown in Fig. 4B, the distribution of angles for a performance threshold of 10% clearly had a bimodal shape with a broad mode around zero and a narrower mode around 45°, the latter indicating a group of time bins with a complete match of classification performances. In accordance with the visual impression, trying to fit the distribution with a single Gaussian for all the thresholds (Fig. 4C) resulted in substantial residuals concentrated around 45 degrees, as shown in Fig. 4D, no matter which threshold was chosen (mean adjusted R^2 across thresholds = 0.51). A two-Gaussian fit of the same distribution (10% threshold) resulted in a better fit that centered with a broad mode at 3.4 degrees and a second, narrower mode at 42.8 degrees (Fig. 4E). Figure 4F shows a consistent pattern of two-Gaussian fits (mean adjR^2 = 0.76) across the four thresholds with robust means close to 0 and 45 degrees (inset), devoid of any concentration of residuals around particular angles (Fig. 4G). Hence, there is a distinct class of matching bins characterized by matched codes across the two tasks.

2.3.5 A small subpopulation exists with a matched population code confined to the manipulation epoch

The question remains how the matching bins are distributed across neurons, and whether a subpopulation of neurons exists whose classification performances match throughout the action sequence. To this end, we separated matching bins from nonmatching bins, i.e. bins in which observed actions were discriminated by a matched code as opposed to a nonmatched code. To this end, we relied on the location of the trough between the two peaks of the two-Gaussian fit as the boundary between matching and nonmatching bins.

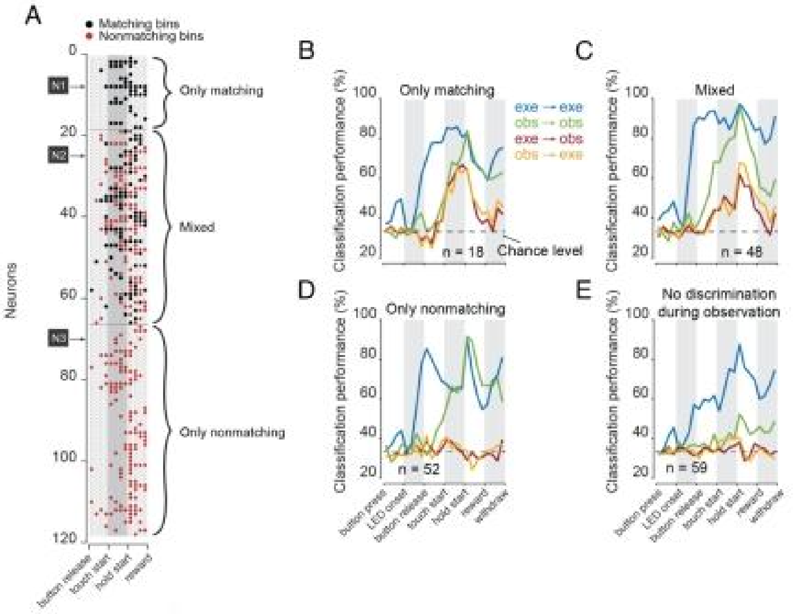

Figure 5. Subpopulations of mirror neurons. (A) Mirror neurons that discriminated the three actions 10% better than chance (n = 118) are divided into three categories: neurons with only matching bins, mixed neurons with matching bins and nonmatching bins, and neurons with only nonmatching bins. The arrows indicate the three exemplary neurons shown in figure 2. (B) The population classification performance of the three subpopulations in A: (B) neurons with only matching bins, (C) neurons with matching and nonmatching bins, (D) neurons with only nonmatching bin plus (E) neurons that did not have any time bins with significant action discrimination during observation.

Considering the occurrence of matching and nonmatching bins in individual mirror neurons revealed four categories of neurons. Figure 5A distinguishes three of the four categories obtained for a performance threshold of 10%: a minority of 'matching neurons’ with only matching bins (10.5% of all mirror neurons), many more neurons with matching and non-matching bins ('mixed neurons´, 27.1%) and neurons with only nonmatching bins (29.4%). The fourth category (not shown in Fig. 5A) was neurons without any bin in which the observed actions could be dissociated (33.3%). Assuming that a biologically plausible readout would have to rely on the collective activity of distinct subsets of neurons, population classifications were carried out separately for each of these categories of neurons. For the subpopulation of neurons with only matching bins, we found a matched action code mainly during the manipulation epoch (Fig. 5B). The performance of exe ® obs and obs ® obs classifications started to be similar shortly before the actor touched the object and remained similar until the object was in its end position. After this point, however, the obs ® obs performance remained high while the exe ® obs performance gradually dropped to the chance level. In the subpopulation of mixed neurons (Fig. 5C) the exe ® obs performance also increased during action observation, but it remained well below the obs ® obs performance, indicating an only partially matching code. Not surprisingly, neurons with only nonmatching bins had an exe ® obs population performance at chance level (Fig. 5D) and neurons that had neither a matching bin nor a nonmatching bin (i.e., did not discriminate the three actions during the observation task) showed a poor obs ® obs performance (Fig. 5E). Hence, the population classification revealed that only a small subpopulation of mirror neurons exhibits a matched action code, which is mainly confined to the manipulation epoch.

2.4 Discussion

We report that three actions performed on visually identical objects could be decoded from the discharge rates of a population of F5 mirror neurons during both execution and observation. In both tasks, the decoding performance was optimal shortly after the end of the manipulation. A substantial degree of independence of the action codes for observation and execution is indicated by the fact that the ability of the action code for execution fell short of the quality of the one for observation when trying to predict the observed action. In other words, the level of action discrimination during observation achieved by the population of F5 mirror neurons, in its entirety, cannot be led back to a plain activation of a motor representation as assumed by the mirror mechanism. While not necessarily excluding a mirror mechanism, the ability to attain the necessary level of action discrimination during observation would in any case require supplementary analysis of visual inputs.

We considered the possibility that only a subpopulation of mirror neurons might fulfill the tenets of the mirroring concept. We could indeed unravel a small subset of mirror neurons that exhibited matched action codes for execution and observation for restricted epochs of the actions. We found neurons with matched action codes in one or more time bins in about one-third of the mirror neurons, with the number of bins per neuron varying. However, most of these neurons (73%; 'mixed neurons´) also contained nonmatching bins, i.e., bins in which the observed actions were discriminated according to a code that differed from the one for execution. Correspondingly, when treated as a population acting collectively, these 'mixed´ mirror neurons were characterized by a code for observed actions that only partially matched the one for executed actions, again in contrast to the requirements of the mirror mechanism. Only a minority of 10.5% of mirror neurons contained matching bins for particular segments (i.e. well-defined relative time bins), but no discrimination of observed actions at other times. Population classification of this small subpopulation showed a matched action code mainly confined to the manipulation epoch. If this time restriction is disregarded, this subset of mirror neurons satisfies the requirements of the mirror mechanism, which leads the sensitivity to observed actions back to an activation of a motor representation of the same actions. Hence, by enabling a veridical activation of motor programs underpinning an agent´s own goal-directed actions, this subpopulation of mirror neurons might serve the understanding of others´ actions as suggested by Rizzolatti and Sinigaglia (Rizzolatti and Sinigaglia, 2016).

Yet, there are two caveats that complicate the acceptance of the conclusion. The first one is that any system for readout would need to know how to listen selectively to matching mirror neurons while ignoring all the others, unable to support veridical motor resonance. Certainly distinct anatomical or physiological features, if available, might allow the identification of matching mirror neurons. Our inability to identify such characteristic features may simply be a consequence of the well-known difficulty of extracellular recordings of neural activity from the brain of behaving animals to provide reliable information on the layer or type of a cortical neuron. The second caveat is that the readout mechanism would not only have to selectively listen to the subpopulation of matching neurons, but in addition, be able to tune in to the right time epoch while ignoring others. However, how the epoch could be labeled in order to ensure the necessary temporal selection remains completely unclear. Hence trying to argue for a specific role of matching mirror neurons rather than taking them as outliers of a broad population of neurons sharing a common function does not seem to be particularly parsimonious.

We may note that the small proportion of 7.5% of all F5 neurons qualified as matching mirroring subpopulation in our study is in the same range as the proportion of 'strictly congruent´ mirror neurons (5.5%, 29/531) reported by Gallese and colleagues (Gallese, 1996) and the proportion of neurons with matched grip type preference reported recently by (Papadourakis, 2019). Although these numbers may not be directly comparable, considering differences between paradigms and analytical methods, the previous work concurs with our conclusion that only a small fraction of neurons may comply with the demands of a mirror mechanism. Moreover, the problem of how to filter out pertinent information from subpopulations lacking distinctive characteristics also pervades previous work trying to identify neurons compatible with the mirroring concept.

Trying to defend the existence of a small fraction of mirror neurons complying with the tenets of the mirroring concept inevitably leads to the question of what the function of all the other mirror neurons might be. The standard answer is that they are intermediate or hidden layer units in a network that tries to combine visual and motor information in order to accommodate the mirror mechanism (Elshaw et al., 2004; LeCun et al., 2015). This will certainly remain a valid possibility as long as we lack pertinent information allowing us to decide if matching mirror neurons have indeed distinct positions in the network or not. An alternative possibility is that the emergence of matching mirror neurons is an incidental and ultimately irrelevant consequence of a network that tries to associate visual information on observed actions with action commands. In order to allow visual information on observed actions to select and/or to shape action plans, matching would not be needed. An observed action is relevant for the observer and needs to be taken into account when weighing behavioral options. The notion that F5 mirror neurons might be instrumental in selecting and/or shaping actions and re-actions of course builds on the well-established role of the ventral premotor cortex in action control (Fogassi, 2001; Kraskov et al., 2009; Umilta et al., 2007; Wise et al., 1986).

In fact, a number of findings on action observation responses of F5 mirror neurons could be easily accommodated by the action selection framework. For instance, to select a response to an observed action, the angle from which the other is seen matters. Hence, it is not surprising that observation responses of mirror neurons have been shown to depend on the viewpoint (Caggiano, 2011). Action selection would benefit from anticipating actions chosen by the other. And indeed, the discharge of mirror neurons has been shown to reflect the expectation of an upcoming action (Maranesi et al., 2014), the expected final action goal the other is pursuing (Bonini et al., 2010), or the mere assumption of an ongoing action (Umiltà et al., 2001). The agent´s action choice will of course not only depend on information on the other´s action but to be useful it needs to depend on information on the relevance or value of the other´s action, including information on past experiences or information on the viability of potential action options. An example of the latter is the distance between the observer and the object, which has been demonstrated to modulate action observation responses of mirror neurons (Caggiano, 2009). As the distance of an object manipulated by the other is decisive for the question if it could be reached by the observer one may assume that a distance depending change of observation-related activity will also have an impact on the motor outflow from F5. During an action selection process that requires a decision between options, the respective value of competing options matters (Cisek, 2007). Assuming that the other´s actions influence the agent´s action choice, it is not surprising that action observation responses of mirror neurons are influenced by information on value (Caggiano et al., 2012; Pomper et al., 2020). One may correctly argue that all these observations are circumstantial, lacking the quality needed in order to firmly establish the action selection hypothesis based on irrefutabile causal evidence. However, if we believe that Ockham´s razor, i.e. to the principle of parsimony, has virtue in trying to solve a scientific problem, the action selection hypothesis may be taken as a useful pointer to a new, promising path towards a better understanding of a class of neurons that has remained enigmatic to the present day.

Chapter 3 The response of monkey F5 mirror and non-mirror neurons in action observation and color cue-based action selection tasks

3.1 Introduction

As discussed in detail in the **Chapter 1** general introduction, mirror neurons (MNs) are a distinct group of neurons, originally found in the monkey ventral pre-motor cortical region F5. These neurons fire not only when an agent performs a grasp but as well when the same agent observes another individual carrying out a similar grasping act (Di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996).

Chapter 2 addressed the question if the assumption of the similarity of action execution and observation responses is justified. As discussed there, this was important as similarity of responses is required by the action understanding hypothesis, i.e., the possibility that an observer will be able to understand the goal of the other´s action by simulating it, based on activation of the motor vocabulary offered by F5 mirror neurons. The work addressed in this chapter aimed at providing information on the alternative interpretation of mirror neurons, discussed in the general introduction, namely a possible role in action selection, guided by information on the other’s action.

MNs differentiating between peri-personal and extra-personal space have been found (Caggiano et al., 2009) and we know that most MNs exhibit view dependency (Caggiano et al., 2011). And further studies show that the observation-related discharge of MN is also influenced by the value the observer attributes to the observed action (Caggiano et al., 2012) and, finally that such observation-related activity may guide decisions (Lemus et al., 2009; Romo et al., 2004). All these properties are compatible with a role in response selection, yet not specific enough to allow definite conclusions. In an attempt to obtain more substantial arguments for a possible role of MN in response selection, we devised a paradigm in which action observation was essential for the choice of a subsequent action of the observer. More specifically, we designed a novel paradigm that required the scrutiny of an observed action in order to simulate it successfully as compared to producing the action learned to be associated with a particular color cue.

The majority of MNs tested with this paradigm exhibited not only the standard responses to action observation and execution but also a clear discharge modulation - either a discharge suppression or excitation - at some point during the pre-go period, i.e. in the period, in which the monkey had obtained the information on the appropriate reaction, yet had to wait for the go-cue. The emergence of such pre-go activity is in principle in accordance with the notion that F5 MNs are involved in response selection. Yet, rather than indicating a role in preparing the observer´s own action based on the observed action, pre- go activity might reflect the reactivation of an action type memory, fed by the observed action.

3.2 Material and methods

3.2.1 Animal and surgery

All the experiments were carried out on two adult male rhesus monkeys (both with weights around 11.5 kg during experiments). The head holders for the painless immobilization of the head during experiments were implanted under combined anesthesia with isoflurane and remifentanil. In the same surgery we also implanted titanium chambers over the left premotor cortex, later accommodating a microelectrode positioning system. The chambers were positioned and shaped considering the prospective location of F5 in 3D reconstructions of a monkey´s brain based on high resolution MRI scans. Monkeys were supplied with opiate analgesics to alleviate postsurgical pain and used again only after full recovery. All experimental protocols and procedures were approved by the regional animal care committee (Regierungspräsidium Tübingen, Abteilung Tierschutz) and fully complied with German and European law.

3.2.2 Setup

The experimental monkeys learned to voluntarily enter the primate chair and to allow the experimenter to fix their head using the chronically implanted head holder, a measure needed in order to control the monkey´s gaze. The primate chair was placed in the light-proof experimental chamber with the monkey facing the display with his head at 43cm distance with the monitor center aligned with the midpoint between the monkey's two eyes. Below and in front of the monitor, within an area the experimental monkey could explore with his hand (peri-personal space), we placed a 'grasping' table, a platform with a central home button and two objects to be manipulated right and left of this button. Note that the two objects had identical shape and haptic appearance, yet accommodated two different grip types, a lift (right object) and a twist (left object) respectively. Both objects were fitted with LEDs, integrated into their top panel. The LED of the left object shone red when turned on, the LED of the right object green. Their color mattered in the 'color mapping task' to be described further below, whereas in the main task, their simultaneous lighting up served as go-cue. Both objects were equipped with switches that allowed the measurement of movement onset and end. The home button that the monkey had to press down in order to start a new trial and to release upon reaching out for an object was likewise armed with switches allowing the determination of the timing of home button release. The monkeys' eye movements were monitored by an infrared sensitive video camera operating at a temporal resolution of 50Hz. The calibrated eye position record provided by the camera was used to ensure that the monkey´s gaze stayed within a fixation window (details further below) around a central dot that served as fixation cue and – depending on its color – provided instruction to the observer. The provision of a unit of reward (a drop of water or diluted fruit juice, depending on a monkey´s preferences) at the end of a trial required that the monkey had followed the prevailing rule on how to choose his action and successfully implemented the sequence of requirements from trial onset to end as detailed further below. The motivation to work on the fluid rewards was stimulated by restricting free fluid access to weekends and vacation periods. Otherwise, the animals had to work for their daily needs. The strict control of a monkey´s weight and relevant physiological parameters ensured that these needs were indeed met. Otherwise, complementary fluid was provided. Data collection, control of experimental parameters including the timing of the action videos were controlled by in-house developed software running in a Linux environment (‘Nrec’, see https://nrec.neurologie.uni-tuebingen.de/nrec/nrec).

3.2.3 Paradigm

In this study, the experimental monkey had to observe a filmed demonstrator monkey grasping a cylindric object (same appearance as the ones on the grasping table) which he either lifted or twisted. In the ‘action simulation’ task (see Fig. 1) the observer was required to carry out the object-related action corresponding to the one seen in the film. To this end he had to choose one of two identically shaped objects positioned next to each other on a grasping table in front of him. He had learned that the left object accommodated a twist, the right one a lift. Hence, the experimental monkey was required to decide on the relevant object and the associated action type (i.e. lift vs. twist) it accommodated. Twist and lift trials were presented randomly interleaved. As said, the start of a new trial was determined by the experimental monkey. As the animal was eager to receive rewards, intertrial intervals were usually short on the order of 0.5-1.5s with little variability.

In the second task, the ‘color mapping task’, the relevant object and associated action type was indicated by the color of the LED integrated into the top panel of the object in the demonstrator video. This LED shone in one of two flavors (green vs. red), randomly chosen in both the action simulation and the color mapping task. Yet the color mattered only in the latter. As soon as the LEDs of the two objects on the grasping table were turned on simultaneously after the presentation of the demonstrator video, the experimental monkey had to select the object whose color matched the one seen in the demonstrator video and to carry out the action, the color-cued object accommodated, rather than to simulate the demonstrated action. As said earlier, in the main task the simultaneous lighting up of the LEDs was the signal for the monkey to start simulating the observed action. In both tasks, the twist action required a 30° clockwise rotation of the object until a mechanical end point was reached, whereas the lift had to traverse 30mm before a stop was reached.

Whether to simulate the action or to choose the action based on color was determined by an instruction cue provided by the color of the fixation dot: the paradigm started with an initial fixation period in which the experimental monkey had to look at the fixation dot presented in the monitor center. If the fixation dot was white, it meant that the monkey had to simulate the seen action. However, if it was blue, the monkey was expected to choose the action by mapping the object color in the demonstrator video on the object on the grasping table. Action simulation and color mapping trials were presented in a block design. Independent of the prevailing instruction, one second after the end of the action video, the LEDs of both objects on the grasping table were turned on. As already said, this served as the signal to initiate the action, no matter if the observed action or the color information decided on the observer´s response. The sequence of events in a trial is depicted in Fig. 1 B for the action simulation.

All trials started with the observer monkey pressing the start button (home position). After having pressed it for 0.5s, the central fixation cue, a dot of 0.5° diameter appeared. As said before, its color indicated if action simulation or action choice based on the color of the object LED were required. The observer had to keep fixation on the dot for 0.5s. Then the dot jumped 10° up, stayed in the new position for 0.5s, before returning to the center where it stayed for another 0.5s until. Its disappearance coincided with the start of the action video. Note that the back-and-forth jump of the task cue had no relevance for the two tasks discussed here but had been introduced in order to promote the monkey´s willingness to comply with a related, but different task, not addressed in this thesis. The observer´s gaze had to stay within a relatively large fixation window of 15° centered on the fixation/ task cue. The purpose of this generous window was to ensure that the monkey´s gaze would be directed at the central region of the monitor presenting the demonstrator action as soon as the action video started. During its presentation and during the subsequent parts of the paradigm, fixation requirements were further relaxed to the extent of the video clip. The action videos (see Fig. 1, bottom) presented on the monitor 43cm in front of the observer showed the grasping action (hand and distal arm) of one of the experimental monkeys (Monkey 1 ‘Pollux’) filmed from the top and front. Note that the overall duration of the two action types (lift vs. twist) and the duration of the various action phases (see Fig. 1 for further information) shown in the videos differed. Independent of condition and action type, the monkey was required to complete the action by holding the respective object in its final position for 100 msec before releasing it again. In order to receive a unit of fluid reward at the end of a trial the monkey had to choose the right object and the action it accommodated and, moreover, to comply with all other behavioral requirements from the start to the end of a trial.

Unfortunately, it turned out that ability of the two monkeys to switch between the two tasks without an accompanying increase in errors remained poor regardless of all training efforts. This is why I had to give up the color mapping task as described before and replace it by a much simpler variant. Rather than exposing the observer to the same action videos in the main and the color mapping task (note: that only the color of the instruction cue differed), I had to resort to a simpler, and less demanding variant of a color mapping task, lacking the identity of visual stimuli. As depicted in Fig. 1C, I decided to test if F5 neurons, responsive to an observed action might also be responsive to a color cue presented in an object plus hand context. The observer saw the other´s hand pressing the home button next to the standard object also used in the other tasks. After 167ms, the LED in the top panel was turned on, turned off again 667msec later and the video ended yet another 1166ms later. Depending on the LED color being red or green the experimental monkey had to decide on the action in the subsequent self-action that did not differ from the main task.

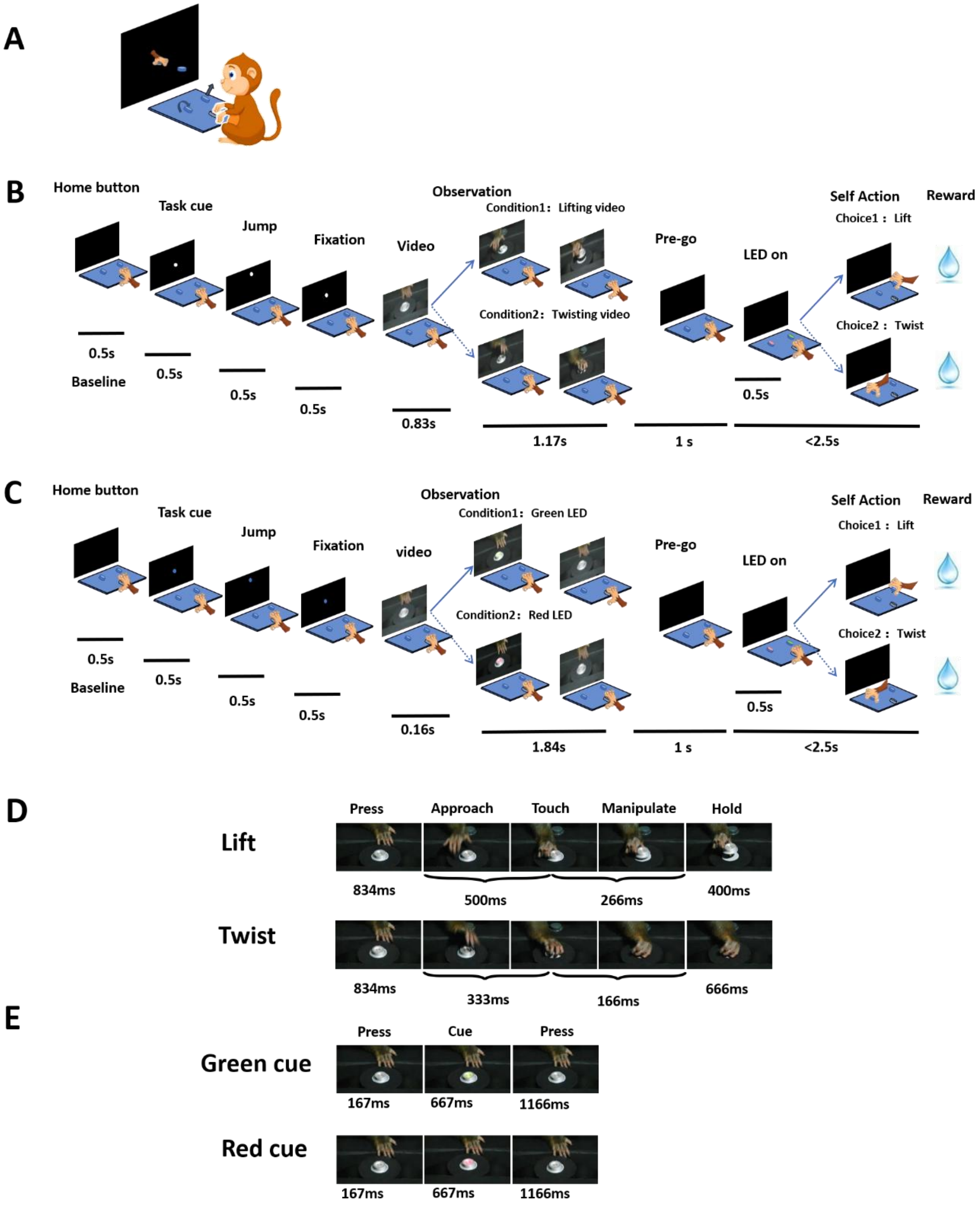

Figure 1 Action simulation paradigm. (A) Setup: the monkey sat in front of a monitor and a grasping table. Timeline of events in (B) the action simulation paradigm and (C) the color mapping paradigm (original variant). Snapshots representing the major phases characterizing the (D) lifting and twisting action seen by the observer with the numbers underneath indicating their respective duration. (E) Stimuli seen by the observer in the simplified variant of the color

mapping paradigm. The numbers beneath the frames indicate the duration of the key phases in the video (see Methods for further details).

3.2.4 Training

Both monkeys were trained step by step. First, they learned that a simple button press was needed to get a reward. Next, they learned to carry out alternative lifting or twisting actions on the same objects also later used in the actual experiments, following the occurrence of a go cue. Once monkeys could successfully perform types of actions, I started to train them on the most demanding behavioral requirement, namely to select the self-action based on the information provided by the demonstrator video. During the early part of this training phase, this required that the monkey had a chance to see blocks of trials presenting one and the same action video, i.e., always a lift and always accompanied by the information to rely on the action rather that the color cue. Once the necessary association between seen action and appropriate self-action was established, I could proceed to randomized trials requiring that the monkey had to pay attention to all information in order to arrive at the right action choice and finally receive the desired reward. In later parts of the training, the association of color and action type was established by presenting one and the same color and grip type repetitively over longer series of trials. However, as noted earlier only the simplified variant of the color mapping task could be implemented successfully.

3.2.5 Recording

Extracellular action potentials were recorded with glass-coated microelectrodes (impedance 0.5-1 MΩ) via a multi-electrode micro-array with up to 8, independently movable probes (Alpha Omega Engineering). The electrodes were guided by metal tubes touching the dura and inserted through the intact dura. The depth of each electrode was independently monitored and controlled by micromanipulators (EPS™ - Electrode Positioning System). Spikes of well identified single units were sorted online resorting to template matching accommodated by the Alpha Omega (AlphaLab SNR, Alpha Omega

Engineering). The neurons recorded were from the F5 region of ventral premotor cortex of the two monkeys' left hemispheres. This region was identified based on its location in the posterior bank of the caudal arcuate sulcus and adjoining gyral cortex, estimated by presurgical MRI and verified by the depth of neuronal activity. Further information was obtained by micro-stimulating the area explored and using evoked saccades as indication that the boundary to the frontal eye field (FEF), adjoining F5 more rostrally and dorsally had been crossed. Finally, the characteristic properties of F5 neurons provided additional physiological evidence on recording sites lying within the confines of this area.

3.2.6 Data analysis

Raw spike train data for individual neurons and conditions was prepared for visual inspection in raster plots and associated spike density functions. The latter was obtained by convoluting single trial spike trains with a 15ms Gaussian kernel calculating the across trial means and standard deviations as function of time. The mean discharge rate in the first 500 msec of a trial, in which the monkey fixated the white central dot served as baseline for a first statistical assessment of discharge rates in periods of interest, described below. The analysis task-related discharge was confined to correct trials as the number of error trials was relatively small (mean percentage of correct trials over all sessions: in monkey P 86.51% for action selection task, 80.31% for color task.; in monkey F 82.27% action selection task, 78.8% for color task), impeding a separate analysis.

As the two types of hand actions demonstrated in the video had different timing, also the duration of the three action phases distinguished (hand approach, object manipulation and object hold) differed. In case of the lift action the following time windows were chosen: approach phase (2.83s-3.23s), manipulation phase (3.23s-3.6s) and holding phase (3.6-4s). The following 1s duration pre-go period was divided into three 333ms time windows. The final self-action period was divided into the same three phases as the initial visual period, namely into an approach, manipulation and a holding phase, their respective duration being determined by the trigger signals provided by the grasping table on the start of the hand movement (release of home button), the hand contact and finally contact to the mechanical stop. In the twist action video, the 3 phases had the following durations: approach phase (2.83s-3.17s), manipulation phase (3.17s-3.34s) and holding phase (3.34-4s). The pre-go period was again divided into three 333ms time windows and the subsequent three phases of the self-action period (approach, manipulation, holding) were again delimited by the trigger signals provided by the grasping table. As said in the beginning, the baseline activity level was determined in a window that started with the monkey pressing the home button and ended 500ms later with the appearance of the fixation dot. The statistical analysis of mean discharge levels in these phases (or combinations thereof) resorted to non-parametric (Kruskall-Wallis) ANOVAs ($p<0.05$) with Benjamini Hochberg corrections. Note that the final analysis of phase specific action type preferences using non-parametric Wilcoxon comparisons with Benjamini Hochberg corrections of phase specific discharge associated with the two types of actions also considered a period during action observation ('Press', duration 500ms), during which the demonstrator monkey pressed the home button, waiting for the cue to start the action). Hence altogether 11 trial phases were distinguished and considered in this latter analysis. Data analysis was based on self-written Matlab scripts (2018a).

3.3 Results

In the action simulation task, both monkeys reached a performance of 80% correct trials after about 3000 training trials. Once the monkeys reached this performance level, I started to record single units in F5. In total, 613 single units were recorded from the left hemispheres of the two monkeys, 549 neurons from monkey P and 64 neurons from monkey F.

As said earlier, in order to further characterize the recorded neurons, only data from correct trials were used, i.e. trials in which the monkeys had chosen the behavior as required by the prevailing rules. For instance, if the condition rule called for action simulation and the demonstrator monkey shown in the movie carried out a lift action, the observer had to choose the object, accommodating the same action and of course to carry it out.

3.3.1 The types of F5 neurons as characterized by the action-simulation paradigm

As reviewed in chapter 1 F5 is a region located in the ventral part of premotor cortex characterized by a dominance of neurons responsive to hand and mouth movements. Unlike F5 motor neurons that present action-related discharge but lack responses to the observation of motor acts carried out by others, mirror neurons combine both. Finally, some neurons in F5 are purely visual, responding to the observation of a graspable object (canonical neurons) only. In my study, the recorded neurons could exhibit activity in any of the three major periods considered, the observation period, the self-action period as well in the interval between the two – i.e. in the pre-go period. Already a brief look at the pool of neurons suggests that any combination – i.e. activity in only one, in various combinations of two periods or in all three could be found.

Figures 2 and 3 present exemplary neurons that give an idea of the diversity of activity patterns found in F5. Whereas the ones compiled in Fig. 2 are non-mirror neurons exhibiting a preference for one of the three periods, those shown in Fig. 3 were categorized as mirror neurons due to the presentation of combinations of observation and self-action evoked discharge. The neurons presented in panels A and B of figure 2 are purely visual neurons, characterized by observation-related discharge and no or at best very little discharge modulation beyond the observation period. Note that in both cases the visual responses were phasic, peaking at the onset of the demonstrated action in neuron A and a bit earlier– at the time of video start (the other´s hand still in the starting position) – in neuron B.

Figure 2 (C), (D) depict two neurons characterized by activity in the pre-go period while lacking activity evoked by action observation or execution. In both cases the discharge rate started to build up right after the end of video about 1s before the onset of the self-action, peaking about the time of the go-cue, followed by a rapid decline of the discharge rate to baseline levels before/ around the time of the onset of the self-action. In both neurons, the pre-go discharge was action-type specific with C exhibiting a preference for the lift action and D for the twist action.

Finally, panels E and F present typical motor neurons characterized by a restriction of the discharge to the self-action period. In neuron 5 (E) the action related discharge was relatively weak and not clearly specific to the type of action, followed by a much stronger broad burst of activity after the end of the self-action, continuing beyond the delivery of the reward. This later broad burst turned out to be lift specific. Neuron 6 (F) exhibited a clearer self-action related burst of activity that was twist specific followed by a later smaller burst around the time of the reward, the latter lacking action-type specificity. The bottom line is that even neurons whose responses are confined to 1 of the 3 major periods exhibit highly idiosyncratic features.

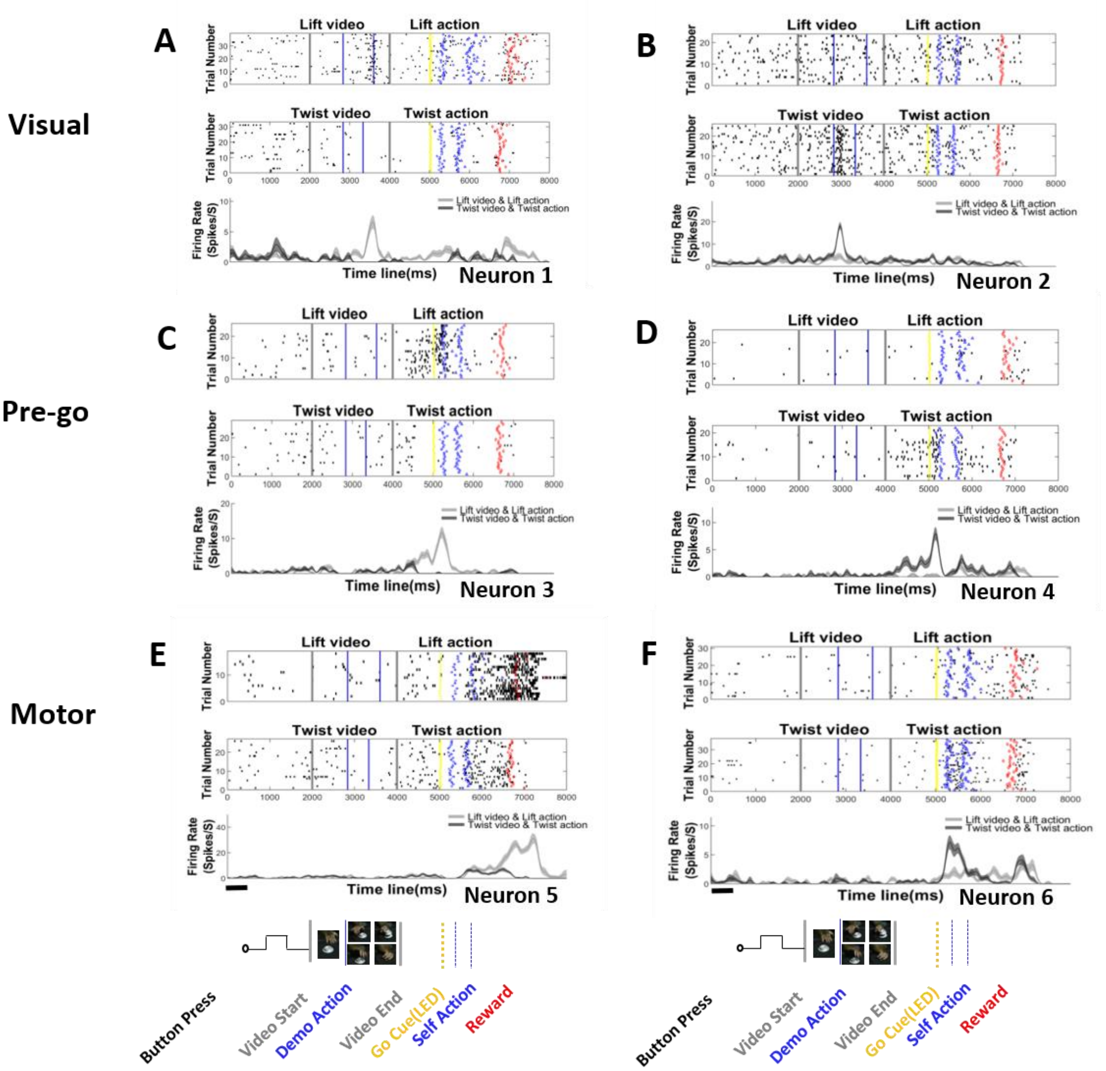

Figure 2 Examples of F5 non-mirror neurons. Each neuron is represented by a raster plot (top panels) and spike density functions (kernel 15ms; bottom panels, plots of means and standard deviations). (A), (B) Visual neurons, (C), (D) Pre-go neurons, (E), (F) Motor neurons. See the text for a detailed discussion of their features.

Figure 3 presents the discharge profile of four F5 mirror neurons, identified as such because of the yoked presence of observation and self-action related discharge. During action observation, the neuron shown in A (neuron 1) preferred the object lift and the one depicted in B (neuron 2) the object twist. Before the onset of the self-action, both neurons exhibited a gradual build-up of activity that started right after the end of the action video. Whereas neuron 1 showed an activity peak before the go signal, followed by a rapid decline of the discharge rate thereafter, the activity profile of neuron 2 peaked later, namely in the self-action phase. In both neurons the action type preference characterizing action observation was maintained. Note, however, that neuron 1 also exhibited a clear, yet smaller phasic activity increase in the second part of a trial, associated with action twists, delayed by several 100ms relative to the profile associated with lift actions. These two neurons are presented in Fig. 3 as 'simple' mirror neurons, a provisional attribute chosen to capture the fact that the observation and execution-related responses were characterized by preference for a particular action type that is maintained throughout a trial and by more or less clear bi-modal profiles with one peak in the observation part and a second peak in the following pre-go – self-action part of the trial. In contrast 'complex' mirror neurons as exemplified by neurons 3 and 4 (Fig. 3 C, D) lacked these features. While their response profiles deviate from baseline levels in both the observation part and the subsequent pre-go – self-action part, such deviations were rarely monophasic and, moreover, characterized by unstable and very often phase-dependent preferences for particular action types.

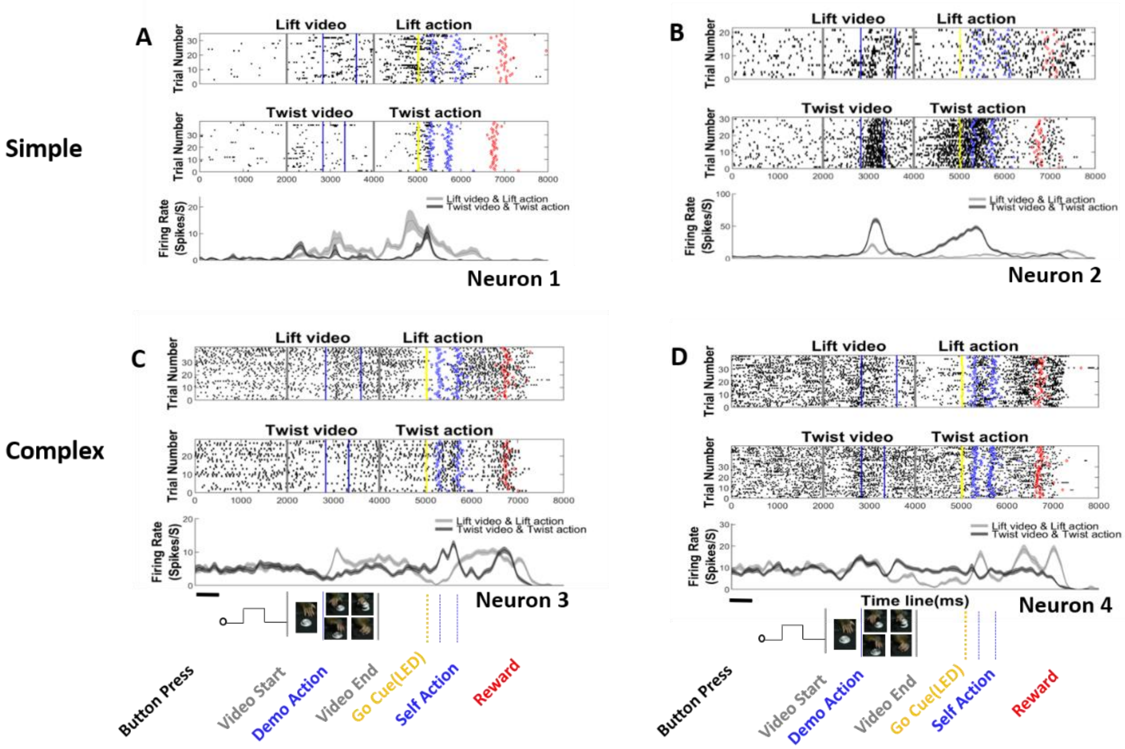

Figure 3 Examples of F5 mirror neurons. Each neuron is represented by a raster plot (top panels) and spike density functions (kernel 15 ms; bottom panels, plots of means and standard deviation) (A), (B) ‘simple’ mirror neurons, (C), (D) ‘complex’ mirror neurons. See the text for a discussion of the examples and the meaning of the two attributes (i.e. simple vs. complex).

3.3.2 Towards a quantitative analysis of the preferences of F5 mirror and non-mirror neurons

The discussion of the example neurons in the preceding chapter suggests that a qualitative assessment of discharge profiles by eye has virtue. Yet, the pronounced diversity of individual discharge profiles, even seen in seemingly simple cases as those in Fig. 3 (A)(B), all the more in 'complex’ mirror neurons as in Fig. 3 (C) (D), also clearly indicates the limitations of this approach.

Hence, as a first step towards a more objective and quantitative evaluation I decided to determine the mean firing rates in four interested phases (pre go, approach, manipulation and hold) in both the observation and execution parts of the trials. In the self-action case, the measure of pre-go/ pre-stimulus activity was based on the discharge mean in the full 1 sec period before the go-signal and in the observation case it was based on the 1 sec period before the onset of the action video (the composite of the 0.5 periods of target jump and subsequent fixation). In order to compare the phase specific rates with the mean baseline firing rate I resorted to a non-parametric (Kruskall Wallis) ANOVA ($p<0.05$) with Benjamini Hochberg corrections. The statistical tests were carried out independently for the two types of actions.

A neuron was classified as a mirror neuron if it exhibited significant activity in at least one of the three action phases (approach, manipulation, hold) both during action observation and execution. In accordance with previous work, I did not require congruency of the significant action phases during observation and self-action. Moreover, action type specificity or – in the case of specificity – congruency of action type preferences for observation and self-action were not required. Based on these requirements, out of the 613 neurons recorded, 223 (36.37% of the whole population) were classified as mirror neurons with a complement of 390 non-mirror neurons (63.63%). Monkey P contributed 197 mirror neurons (out of 549 F5 neurons; 35.88%) and monkey F 26 mirror neurons (out of 64 F5 neurons; 40.63%).

In an attempt to reveal patterns of preferences for particular phases of the action simulation task in the two groups of neurons, I considered all 11 phases specified in the methods section (3.3.6 data analysis) and asked if they differed for the two action types, i.e., lift and twist, resorting to non-parametric paired (Wilcoxon) t-tests, $p<0.05$, again followed by the Benjamini-Hochberg procedure in order to reduce the false discovery rate. The result is plotted as a heatmap in Fig. 4 A in which yellow indicates a significant preference for the lift, blue for the twist and white the absence of a significant preference. Note that the latter might indicate the absence of a discharge modulation relative to the level of baseline activity or a deviation from baseline that did not differ for the two action types. Unfortunately, further elucidation of these two possibilities was not possible as Fig.4 A indicates that even activity in the baseline phase could exhibit action type dependency, therefore devaluating the significance of differences of later discharge phases relative to baseline. As the two action types took turns randomly interleaved, a differential impact of unspecific time-dependent factors on baseline firing can be excluded and a reverberation of the preceding action type on the discharge in the baseline period must be suspected. Fig. 4 B presents a chart in which individual columns convey the percentages of neurons that exhibited a significant preference for the twist (left panel) and the lift action (right panel) respectively as function of trial phase for both mirror and non-mirror neurons. As already said, even in the baseline period a few neurons exhibited a preference for the one or the other action type. Fig. 4B clearly conveys that independent of trial phase, the percentage-based share of mirror neurons exhibiting action type preference exceeded the one of non-mirror neurons.

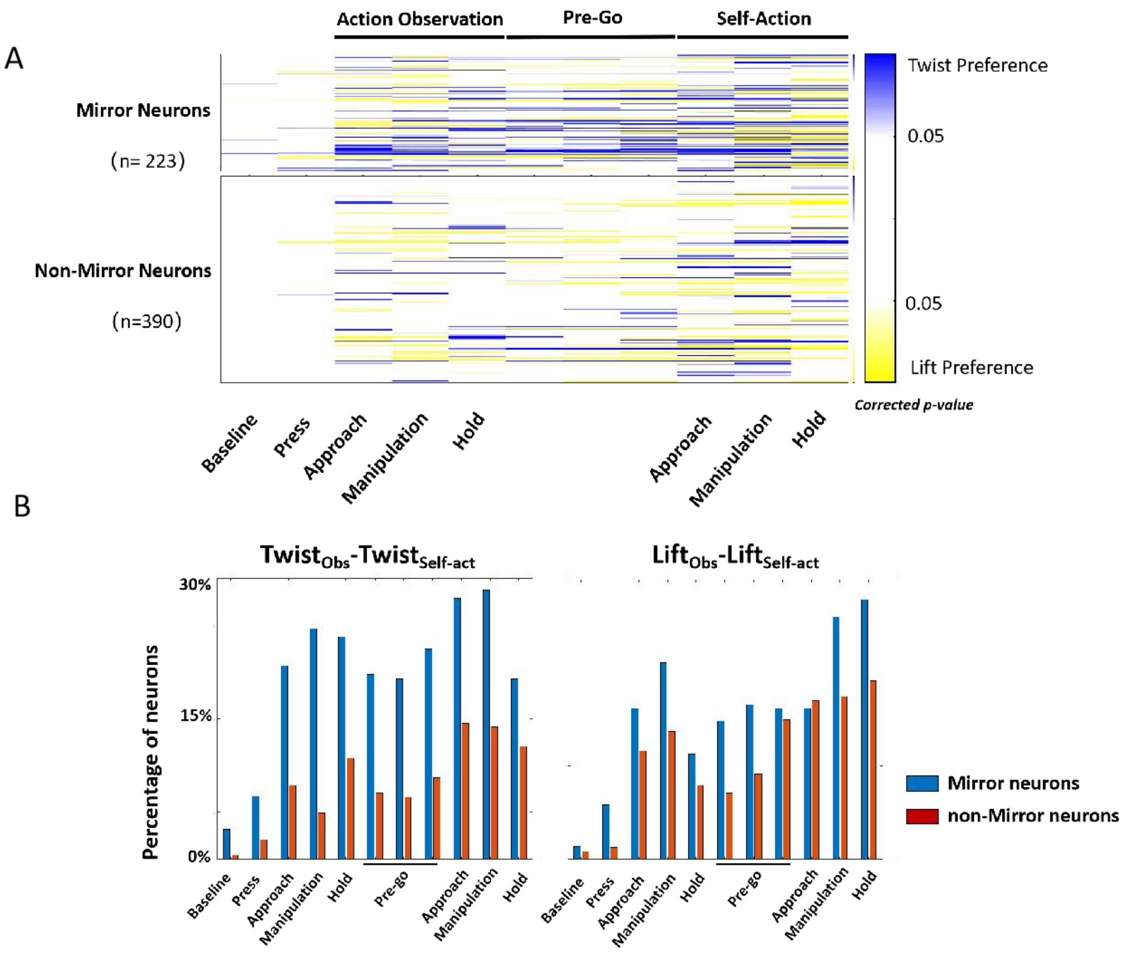

Figure 4 Analysis of action type preferences of mirror and non-mirror neurons as function of trial phase. Mean discharge rates were calculated for each trial phase for lift and twist trials and compared resorting to Wilcoxon-tests with Benjami-Hochberg corrections to reduce the false discovery rate. A Heat plot conveying the p-value of trial phase dependent action type preferences for the one or the other action for mirror and non-mirror neurons. B Percentages of neurons that showed a significant (i.e. $p< 0.05$) preference for one of the two action types ('percentage of neurons') as function of trial phase.

3.3.3 Exemplary F5 mirror neurons tested in the color mapping task

Out of the total of 613 F5 neurons tested in the action simulation task, 298 (245 from monkey, P, 53 from monkey F) could also be tested in the simplified color mapping task. Fig. 5 presents two exemplary F5 neurons that give a flavor of the responses of F5 neurons in this task. The two had been classified as mirror neurons and the figure compares their respective response patterns in the action simulation task with those in the color mapping task.

Neuron 1 (Fig. 5 A) exhibited a tiny, yet significant response to the observation of the lifting action in the approach phase that changed to the twisting action in the later object holding phase. During self-action the response pattern was much clearer and less complex, characterized by a burst-tonic discharge evoked by the twisting action. Also, in the color mapping task this neuron discharged in conjunction with the twisting self-action, yet less. On the other hand, it did not respond to the red light that cued the preferred twisting self-action but to the other (the green) cue that prompted the twisting self-action. Neuron 2 (Fig. 5B) was a mirror neuron that exhibited a strong response to the observation of the twist and a likewise strong response to the execution of a lift, in other words a complete reversal of action preferences from observation to execution. This neuron maintained its phasic discharge in association with the lifting self-action in the color mapping task without exhibiting a response to any of the two-color cues. However, quite surprisingly the twisting self-action that was ignored in the action-simulation task was associated with early, sluggish activity, largely preceding the self-action. According to the classical notion of a mirror neuron one might have expected stable preferences for self-action and a lack of responses to simple color cues. The two examples clearly deviate from this expectation, a conclusion that receives further support from a quantitative analysis of phase specific responses of mirror and non-mirror neurons, formally similar to the one underlying figure 5.

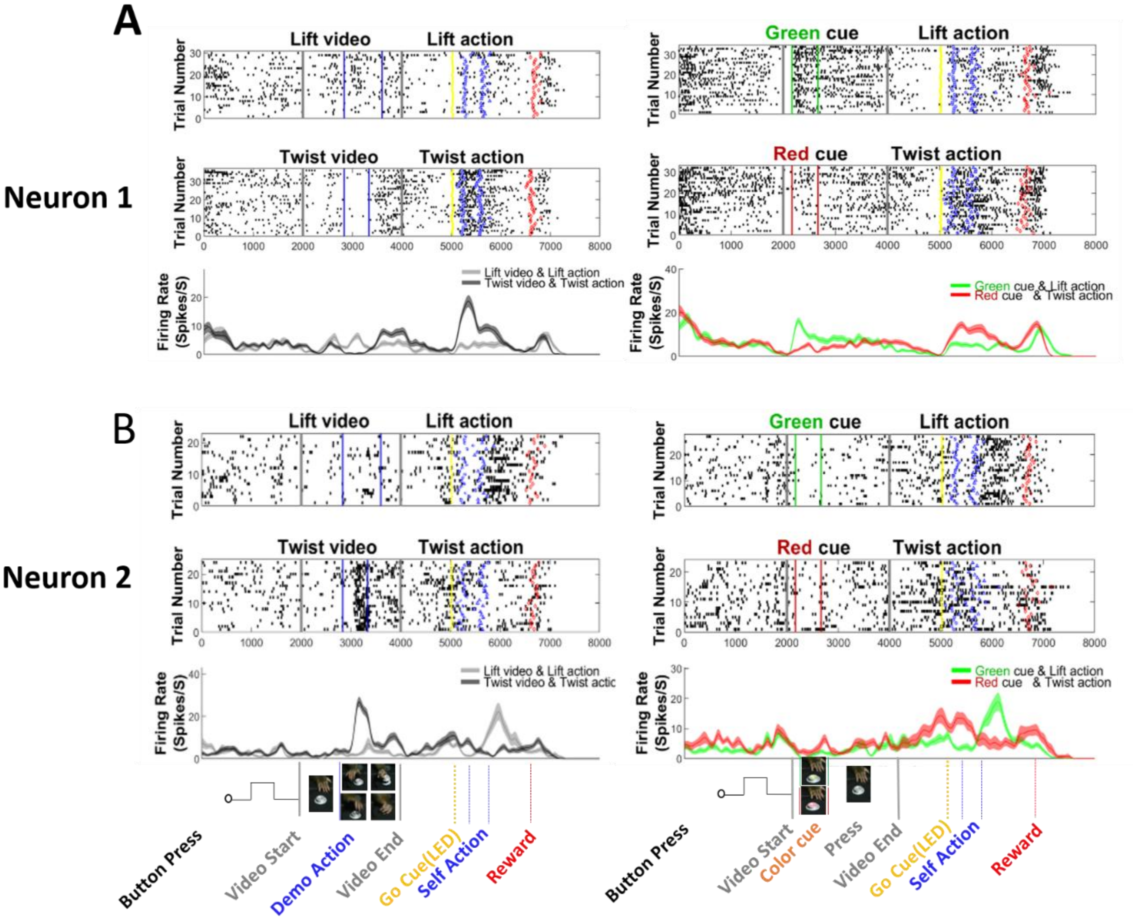

Figure 5 Two examples of mirror neurons tested in the color task. Each neuron is represented by a raster plot (top panels) and spike density functions (kernel 15 ms; bottom panels, plots of means and standard deviation). See the main test for a discussion of the discharge patterns.

3.3.4 Towards a quantitative analysis of the preferences of F5 mirror and non-mirror neurons in the color mapping task

In the preceding chapter I had tried to get a handle on the pronounced diversity of individual discharge profiles of F5 neurons tested in the action-simulation task by testing for phase specific action-type preferences, resorting to non-parametric Kruskall Wallis comparisons with Benjamin Hochberg corrections. Here I used a similar approach in order to test for phase specific discharge differences between the action simulation and the color mapping task, separately for the two action types. In the color mapping task, the self-action part is identical to the self-action part of the action simulation task. The same holds for the period separating the end of visual stimulus –i.e., the offset of the color cue in the color mapping task and the end of the action video in the action simulation task – and the go cue. Hence, when testing for phase specific preferences for particular action types, the phase architecture of the action simulation paradigm for these parts could also be applied to the color mapping paradigm, allowing a direct comparison of preferences in the two tasks. However, in order to compare the observation parts of the two tasks, adjustments were needed.

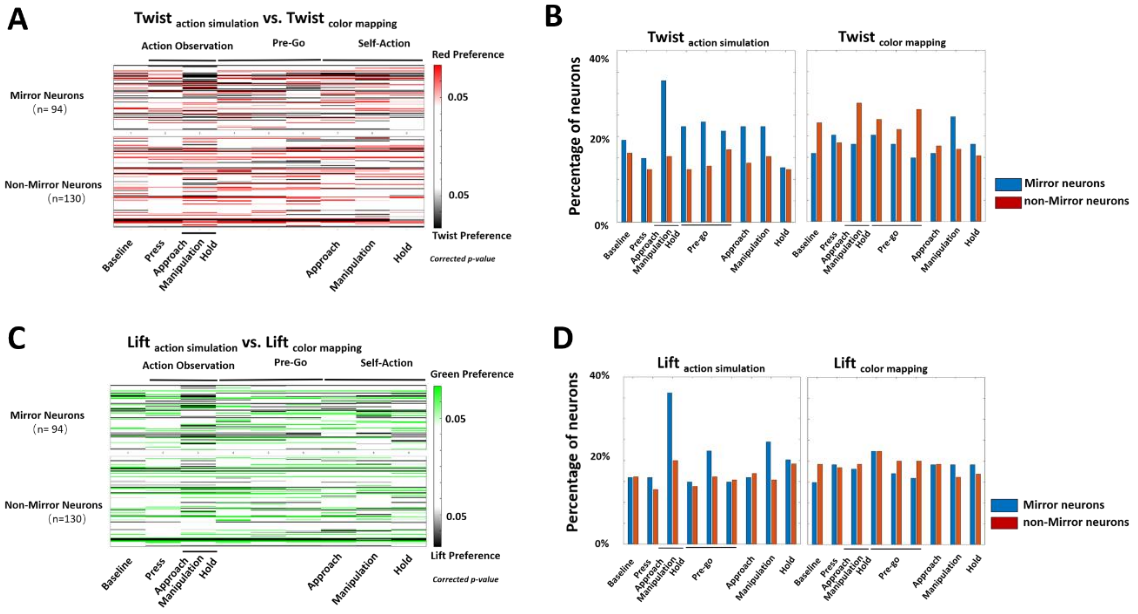

Figure 6 (A) (C) Heatmaps of preferences for action observation-based action selection or color cue based selection. A shows data for the twist action, C for the lift action. Note that in the color mapping task the red dot cues the twist action and the green dot the lift action. Mean discharge rates were calculated for each trial phase for lift and twist trials and compared, resorting to Wilcoxon tests with Benjami-Hochberg corrections to reduce the false discovery rate. (B) (D) Plot of the percentages of neurons that showed a significant (i.e., $p< 0.05$) action-type preference in one of the phases for action simulation compared to color mapping (left) or, conversely, for color mapping vs. action simulation (right). The upper two histograms represent these comparisons for the twist action, the lower two for the lift action.

In the color mapping task, the observation period involves four phases, the baseline period, the press phase, characterized by the onset of the video showing the object and the demonstrator´s hand pressing the home button, followed by the cue phase in which either the red or the green object LED, determining the action type is switched on. Hence, in order to accommodate the comparison with action observation related preferences in the action simulation task, I adjusted the phase architecture of the latter by merging the various phases of action observation (i.e. approach, touch, manipulate and hold; overall duration 1166 msec) to arrive at a mean discharge rate that could be compared with the mean discharge rate evoked by the color cue, thereby allowing a comparison of phase specific preferences for the color cued action vs. action simulation cued action, separately for the twist and the lift actions, in both the visual and the self-action parts of the two tasks. Figure parts 6 A and C show this comparison based on heatmaps. Here red and green respectively indicate a significant preference for color cue and the associated action type relative to action-simulation and its observation, whereas black identifies phases in which the mean discharge seen was stronger in the action simulation task. And finally, white signifies the absence of a difference. The histograms on the left side of Fig. 6 B, D plot the percentages of neurons that showed a significant (i.e. $p < 0.05$) preference in one of the phases of the action simulation condition whereas the ones on the right plot the percentages of neurons preferring color mapping.

The heatmaps document patterns of bewildering complexity with many neurons exhibiting a preference for color in specific phases and others for action simulation. Moreover, preferences for the one or the other are not constant during a trial but may change. A closer look suggests that in the pool of mirror neurons the preference for action simulation seems to dominate. Yet, also in this pool, many neurons can be found that prefer color mapping. The same conclusion can be drawn from the histograms shown in figures 6 B D that do not show the expected dominance of mirror neurons preferring action simulation.

Finally, as noted earlier (see 3.4.2), a few neurons exhibited an action type preference in the action simulation task already in the baseline period, arguably reflecting the reverberation of the action type preference in the preceding trial. The same could be seen in the color mapping task and as discussed earlier, also here most probably reflects a spilling over of the action type specific influence on the discharge in the preceding trial.

3.4 Discussion

The study, presented in this chapter, was carried out in an attempt to test the hypothesis that mirror neurons might be involved in selecting a re-action in response to the action carried out by another agent. This proposal could be understood as a specific variant of the general picture of the role of premotor cortex in the sensory guidance of movements by associating visual and other sensory cues with appropriate movement responses (Weinrich, 1984). I reasoned that the peculiarity of F5, distinguishing it from other parts of premotor cortex might be twofold: first, the ability to process a particular class of sensory stimuli, namely observed actions and second the ability to use the information on the observed action enable a movement of the hand allowing the agent to get hold of an object of interest and to use it in a purposeful manner rather than to guide a simple reaching-out movement towards a distinct spatial location. And importantly, which motor act might be chosen by the observer in response to the other´s action would dependent on a number of factors like the perspective taken or estimates of the benefits of the observed action for the other, its relevance for the observer and the expected value of the observer´s re-action. As chapter 2 showed, observation and self-action related discharge profiles of mirror neurons typically lack the similarity one might demand if these neurons should underpin action understanding. However, arguably a role in binding information on the other´s action with appropriate re-actions of the observer would not necessarily require a congruency of discharge profiles, these neurons obviously do not offer.

In the main task used to test the re-action hypothesis that this chapter addresses, the experimental monkey had to mimic an object-directed action of a demonstrator monkey. Two actions could be demonstrated, either the lifting or the twisting of a given cylindric object. In accordance with previous work on F5 reviewed in chapter 1, many of the neurons studied in this task could be categorized as motor neurons, presenting action-related discharge yet lacking observation related responses and mirror neurons, combining sensitivity for the observed action with sensitivity to the self-action, both with specificity of varying degree to the one or the other action type. Further variants like visual neurons responding to the observed action but not to the self-action and canonical neurons responding to the visual appearance of the object were not differentiated from non-responsive neurons, although the statistical analysis that tested for phase specific discharge preferences would have made this possible. Whether a more fine-grained categorization could be productive seems doubtful, considering the bewildering complexity of the discharge profiles of F5 neurons in all my experiments, i.e. those that underpin chapters 2 and 3. Hence, rather than trying to squeeze F5 neurons into a procrustean bed of a few distinct categories, it may be much more fruitful to assume that these neurons combine various streams of visual and motor-related information with weights that differ between neurons and phases. Not unexpectedly in view of the heterogeneity of discharge profiles and the results reported in chapter 2, the observation and self-action related discharge profiles of mirror neurons lacked similarity in the majority of cases. While these findings are by and large corroborations of previous observations, the observation of conspicuous activity in the 'memory' period linking action observation and the start of the self-action is new. It is reminiscent of the 'set-related' activity of dorsal pre-motor cortex neurons (e.g. Weinrich, 1984） in the interval between the presentation of a spatial cue and the delayed execution of a reach towards the spatial location or 'memory' activity of saccade-related neurons in the frontal eye field and parietal area LIP (Shadlen 1996,2001, Roitman 2002), varyingly discussed as reflections of shift of attention or intentions and the implementation of a motor plan. In the case of the F5 mirror neurons tested in the action simulation task, the activity profiles in the memory period in which the monkey had to wait for the go signal to reproduce the observed action were quite diverse, anything from a more or less gradual reverberation of the earlier visual response to a building up of activity towards a peak at or after movement onset. Hence, memory activity might reflect different functions such as a memory trace of the observed action or, alternatively, the development of a specific motor plan. In order to distinguish between these two options, a control paradigm would have been needed, asking the monkey not to reproduce the observed action but to choose the other action. Activity reflecting the memory of the observed action should be independent of the type of action chosen by the demonstrator, whereas, conversely, activity indicating the chosen motor plan should be independent of the observed action but yoked to a particular motor plan. But of course, also combinations of both might be conceivable with possibly changing contributions from observation end to movement onset. Unfortunately, in the interest of time I had to refrain from embarking on an experiment comparing the two possible consequences of an observed action, namely simulating the observed action or deciding to perform the other action. Whereas this important control experiment could not be realized, I could at least realize a second control experiment – a color mapping experiment – albeit in a simplified and non-optimal f that deviated from the original plan. In the color mapping experiment, the monkey was asked to rely on a simple dot cue to select one of the two possible self-actions. The goal of this experiment was the critical investigation of the justification of the assumption that the visual sensitivity of mirror neurons is largely confined to the observation of actions. The results summarized in Fig. 6 that show that quite a few F5 neurons – mirror-neurons and non-mirror-neurons alike – respond to the presentation of the color cues, representing the two action types. This is in accordance with reports of earlier preliminary observations_(Gallese 1996, Caggiano 2009, 2011, 2012, 2016). In fact, the percentage of mirror neurons that exhibit stronger responses to the observed action is not clearly higher than the percentage of those that prefer the dot cues. In fact, a major difference between the impact of action observation and dot cueing pertains to motor neurons. Whereas motor neurons by definition do not respond to action observation, about 20% of them discharged in response to the dot cue. Interestingly, my preliminary analysis of the data suggests that in mirror neurons, the action that is preferred during observation does not seem to predict which of the two dot cues, calling for a particular self-action would be effective. This may not be surprising considering that – as said before –mirror neurons´ preferences for a particular observed action are on the whole independent of the preferred self-action. In the original variant of the color mapping task that had to be given up as the behavioral performance of the monkeys remained insufficient despite extended training efforts, the color information for the later selection of a self-action was embedded in the action context that characterized the action-simulation task. However, in the color mapping variant realized, this action context was absent. Differences in context may be relevant and possibly modulate the sensitivity to color cues, calling for particular actions. It is tempting to speculate that the presence of an object-directed hand action in the visual scene presented might further decrease the sensitivity to the dot cue, although the latter would be task-relevant and therefore receiving the observer´s attention. Both the at best weak relationship between responses to observed actions and subsequent self-actions and the limited preference for observed actions might question current hypotheses on the role of these neurons. This holds not only for the popular role in action understanding but also for a possible role in the generation of pantomimed action or response selection, the latter guiding the experiments reported in this chapter. A necessary qualification is that the presented analysis of the data collected must be considered preliminary. More sophisticated and also more powerful approaches may lead to insights that may be more compatible with the response-selection idea or lead to novel interpretations, as yet not considered. Another concern that needs to be taken into account is that the two monkeys trained on the action simulation task may have actually used the observed action as a cue much like the color dots in the color mapping experiments, rather than to resort to a semantic categorization of the grasp. Neither the behavioral nor the electrophysiological data obtained allow me to exclude this possibility. However, future work deploying causal manipulations, able to compromise the link between action observation and the subsequent selection of a self-action should allow one to critically test it. Muscimol injections blocking neuronal activity for many minutes would not only compromise this link but also interfere with the processing of information on the other´s action. Nevertheless, they would be very valuable as they would reveal if F5 is needed at all for the selection of appropriate re-actions. However, in order to break the link between observation and observation dependent action selection, electrical micro-stimulation, disrupting the natural neural code, would be the method of choice, easily adaptable to both the action simulation and the color mapping paradigm. One might hope that micro-stimulation restricted to a few hundred milliseconds at different times during the observation periods and the subsequent memory period of the two paradigms should elicit specific disturbances of the translation of visual information into self-action choices. For instance, stimulating during the action observation period might disrupt the self-action choice but leave the selection of the action based on a color cue unaffected, in accordance with the hypothesis of F5 playing a specific role in processing observed action. This would be the outcome that would allow one to sweep aside the aforementioned concern. Unfortunately, the suggested causal manipulations must be left to future work.

Final discussion and conclusion

Mirror neurons are a type of brain cell in the premotor cortex of macaque monkeys that were first discovered in the 1990s by a group of scientists led by Giacomo Rizzolatti in premotor area F5 (di Pellegrino et al., 1992) and later also found in posterior parietal cortex of these animals (Fogassi et al., 2005). Both the premotor cortex and the posterior parietal cortex are regions involved in planning and coordinating of movements. According to classical descriptions, what makes mirror neurons special is that they fire both when an individual performs an action and when she or he observes someone else performing the same action. In other words, they 'mirror' the behavior of others, hence the name 'mirror neuron'. This phenomenon suggests a direct link between observing and performing actions. Mirror neurons have been the subject of extensive research and in view of their intriguing properties have been implicated in a range of functions related to social cognition, empathy, imitation, and understanding the intentions and emotions of others and, more generally, to play a crucial role in our ability to learn from others, to understand their behaviors, and engage in viable social interactions (Gallese and Goldman, 1998). Many of these interpretations of the role of F5 mirror neurons are variants of the idea that mirror neurons underpin action understanding by mapping the other´s action onto the observer´s motor repertoire. In other words, mirror neurons are interpreted as a motor representation, accommodating a motor vocabulary whose elements are set in resonance by the observation of another agent performing a particular action. This hypothesis hinges on mirror neurons displaying an action tuning that aligns between action observation and execution. However, the existing evidence for this proposition is insufficient, primarily due to prior studies lacking a comprehensive exploration of action space tuning and the problem that responses to action observation may be confound by object vision.

To surmount these limitations, we conducted an experiment involving identical objects that were manipulated in three distinct ways to serve distinct action goals. Our results demonstrate that a population of F5 mirror neurons can adeptly distinguish between the three actions, both during observation and execution. However, the neural code for action execution displayed only a weak correlation with the code for observation. Only a small subset of neurons exhibited completely matched codes, and even they were limited to brief, variable segments of the overall action. These findings, reported in chapter 2 of the thesis, clearly challenge the notion that an observer comprehends the actions of others through the activation of motor representations mirroring the observed actions. And by same token they are clearly also at odds with the notion that mirror neurons might serve imitation, an assumption which would in any case have been in conflict with the lack of behavioral evidence for imitation in adult monkeys. However, although macaque monkeys probably do not imitate, they must take the other´s behavior into account when planning their own actions as the resultant of a convergence of internal needs and information on others and the prevailing context. If mirror neurons served this convergence, sensitivity to self-actions and the others´ actions would be expected. However, in contrast to a role in action understanding or imitation, such a more general role in shaping behavioral reactions would not require a matching of preferences for observed and executed actions. These were the considerations that led we to ask if F5 mirror neurons might employ information on observed actions to guide the selection of behavioral responses. This idea could be understood as a specific variant of the general picture of the role of premotor cortex in the sensory guidance of movements by associating visual and other sensory cues with appropriate movement responses (Weinrich, 1984). The distinctiveness of F5, setting it apart from other regions of the premotor cortex, might stem from two key abilities: first, its capacity to process a specific class of sensory stimuli—observed actions. Second, its capability to utilize this information to execute a purposeful hand movement, enabling the agent to grasp and employ an object of interest, rather than simply directing a reach towards a specific spatial location. While a functional role of F5 mirror neurons in response selection to others' actions has been theorized, it has never been empirically tested. In an attempt to close this gap, we embarked on the study, reported in chapter 3. The experiment involved training two adult macaque monkeys to discern the grip type used by a demonstrator monkey in manipulating an object, and to mimic this action accordingly. As in the first study, mirror neurons in F5 were identified based on established criteria, focusing on their discharge modulation in response to both action observation and execution. The majority of mirror neurons recorded in this ‘action simulation study’ experiment exhibited standard responses to action observation and execution, along with distinct discharge modulation—either suppression or excitation—during the pre-go period. Again, the responses to action observation and execution exhibited very little correspondence. Rather than showing matching, preferences were non-congruent in most cases, in full accordance with the results of experiment 1, reported in chapter 2 and, therefore in principle in line with the action selection concept. Despite the lack of congruence in discharge profiles, a connection between action observation and execution was evident in the presence of ‘memory activity’. This activity was characterized by discharge during the phase in which the monkey must remember the observed action to replicate it upon receiving a go-signal. The diversity in memory activity profiles suggests potential functions, such as the formation of a memory trace for the observed action or the development of a motor plan. Surprisingly, and in contrast to the traditional depiction of mirror neurons, a noteworthy number of recorded mirror neurons also responded to simple color cues that indicated a specific action. Moreover, quite a few even displayed a preference for dot cues. Although at odds with the action understanding and action imitation ideas, also this feature would be in line with the general notion of action selection, at least when assuming that the action must not be necessarily related to an observed action in the sense of a re-action. Rather like neurons in other parts of premotor cortex also mirror neurons might be sensitive to more elementary sensory cues prompting behavioral responses. In any case, Causal manipulations are indispensable to test the action selection hypothesis by breaking the link between observation and the subsequent action of the observer will clarify the role of F5 in shaping behavior that depends on sensory information. Yet, in order to decide whether this role is specific to sensory information on the other´s actions, monkeys will be needed that are naïve to the meaning of the elementary cues, used in the color cue experiment in order to prompt specific actions. The reason is that the observed sensitivity to the color cues might have been a reflection of long-term training on associations between the color cues and actions, not found under natural conditions. However, even a causal manipulation in naïve monkeys, able to break the link between an observed action and a subsequent re-action would not rule out a role of the F5 mirror neuron system in choosing actions that depend on a broad spectrum of sensory input, including simple elementary cues. After all, this system might be needed for learning new sensory-motor associations, no matter what the visual input may be. Long-term causal interference experiments, trying to block learning, would be needed in order to rule this possibility out.

In summary this thesis has been able to provide clear evidence against influential concepts on the role of the F5 mirror neurons in critical experiments designed to challenge the action understanding and action imitation hypotheses. Based on the results, these hypotheses can be refuted. On the other hand, convincing evidence on a role in action and/ or response selection has been much harder to obtain. Although the data collected is in principle in line with such a role it is clearly not sufficient to firmly establish it, given the absence of causal evidence. And, moreover, at least from the current vantage point, the causal experiments needed seem very challenging and may in the end not lead to definite answers. Hence after 30 years of research on mirror neurons, are still far from being able to give a definitive answer to the question what their functional role might be.

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Statement of Contribution

Chapter 1

Peter Thier suggested the content. Shengjun Wen wrote the texts, Peter Thier revised the texts.

Chapter 2

Joern Pomper, Peter Thier conceptualized the study and designed the paradigm. Joern Pomper, Shengjun Wen performed the experiments. Joern Pomper, Mohammad Shams-Ahmar analyzed the date and wrote original texts. Peter Thier revised the texts. Shengjun Wen participate the writing review.

Chapter 3

Peter Thier, Silvia Spadacenta, Shengjun Wen designed the paradigm. Shengjun Wen performed the experiments, analyzed the data, wrote the initial texts. Peter Their revised the texts.

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