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Monkey gaze behaviour during action observation and its relationship to mirror neuron activity

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Abstract

Mirror neurons (MNs) of the monkey ventral premotor cortex (area F5) are a class of cells that match the visual descriptions of others' actions with correspondent motor representations in the observer's brain. Several human studies suggest that one's own motor representations activated during action observation play a role in directing proactive eye movements to the site of the upcoming hand–target interaction. However, there are no data on the possible relationship between gaze behaviour and MN activity. Here we addressed this issue by simultaneously recording eye position and F5 MN activity in two macaques during free observation of a grasping action. More than half of the recorded neurons discharged stronger when the monkey looked at the action than when it did not look at it, but their firing rate was better predicted by 'when' rather than by 'how long' the monkey gazed at the location of the upcoming hand–target interaction. Interestingly, the onset of MN response was linked to the onset of the experimenter's movement, thus making motor representations potentially exploitable to drive eye movements. Furthermore, MNs discharged stronger and earlier when the gaze was 'proactive' compared with 'reactive', indicating that gaze behaviour influences MN activity. We propose that feedforward, automatic representations of other's actions could lead eye movements that, in turn, would provide the motor system with feedback information that enhances the neural representations of the ongoing action.

Introduction

Biological goal-directed actions are among the most complex visual stimuli that animals have to deal with (Gibson, 1979). Studies from the last two decades have shown that their processing relies not only on visual brain regions but also on cortical motor areas (see Bonini & Ferrari, 2011). In particular, the discovery of mirror neurons (MNs) in the ventral premotor cortex (area F5) of the monkey has demonstrated that the visual descriptions of others' actions are matched with correspondent motor representations in the observer's brain (Gallese *et al.*, 1996; Rizzolatti *et al.*, 1996; Rizzolatti & Sinigaglia, 2010). A number of different tasks and experimental conditions have greatly contributed to clarifying the functional role of MN in relation to the perception of others' actions and the recognition of the underlying motor goals (Umiltà *et al.*, 2001; Kohler *et al.*, 2002; Ferrari *et al.*, 2005; Caggiano *et al.*, 2009, 2011, 2012; Rochat *et al.*, 2010) and intentions (Fogassi *et al.*, 2005; Bonini *et al.*, 2010, 2013).

The activation of one's own motor representations during the observation of manual actions performed by others has been suggested to direct proactive eye movements to the site of the upcoming hand–target interaction (Flanagan & Johansson, 2003; Falck-Ytter *et al.*, 2006; Rosander & von Hofsten, 2011; Elsner *et al.*, 2012, 2013), thus assigning a crucial role in this function to MNs. However, previous studies on MNs either implicitly assumed that monkeys looked at the experimenter's action and did not use any instrumental measure of eye position (Umiltà *et al.*, 2001; Kohler *et al.*, 2002; Ferrari *et al.*, 2005; Fogassi *et al.*, 2005; Rochat *et al.*, 2010), or they monitored monkeys' eye movements only to ensure that they paid attention to the presented action (Caggiano *et al.*, 2009, 2011, 2012; Kraskov *et al.*, 2009; Bonini *et al.*, 2010; Vigneswaran *et al.*, 2013). Therefore, in spite of the considerable interest engendered by gaze behaviour as a means to understand how we predict others' action goal, no direct data are available on the possible relationship between MN activity and eye movement pattern during action observation.

Here we will describe gaze behaviour of two free-gazing monkeys during grasping execution and observation, and then we will provide an account of the functional relationship between gaze behaviour and MN visual response.

Materials and methods

The study was carried out on two 8-year-old macaque monkeys (*Macaca nemestrina*), one female (M1) and one male (M2). Before recordings, each monkey was habituated to comfortably sit in a primate chair, to interact with the experimenters and to become familiarized with the experimental setup. Then, each monkey was trained to execute a simple grasping motor task using the hand

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contralateral to the hemisphere to be recorded (see Bonini *et al.*, 2010). At the end of training, a head fixation system and a plastic recording chamber (AlphaOmega Engineering, Nazareth, Israel; inner diameter 18 mm) were implanted, based on stereotaxic coordinates of the cortical regions to be recorded. All the surgeries were performed under general anaesthesia (ketamine hydrocloride, 5 mg/kg i.m. and medetomidine hydrocloride, 0.1 mg/kg i.m.) followed by post-surgical pain medications (Rozzi *et al.*, 2006; Bonini *et al.*, 2010). All the experimental protocols were approved by the Veterinarian Animal Care and Use Committee of the University of Parma, and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

After chamber implantation, the functional region of interest (hand region with mirror properties of the ventral premotor area F5) was identified by studying single neurons and multiunit activity, and through intracortical microstimulation, as previously described elsewhere (Raos *et al.*, 2006; Rozzi *et al.*, 2008; Maranesi *et al.*, 2012).

Behavioural tasks and apparatus

Grasping execution. We employed the same grasping task described in previous studies (see Bonini *et al.*, 2010). In brief, each monkey held its hand (contralateral to the hemisphere to be recorded) on a fixed starting position while the experimenter positioned a 1-cm cube of food as target in a rectangular groove (40 × 12 mm, depth 10 mm) located in front of it. This was necessary in order to force the monkey to always adopt the same type of grip (precision grip). During these operations, a transparent screen was interposed between the monkey and the target, preventing it from reaching for the food. After screen removal (go signal), the monkey reached and grasped the target, brought it to the mouth and ate it. Eye movements were monitored during the entire duration of the trial, but the monkey was free to move its eyes and no-fixation was required. Importantly, none of the monkeys was previously trained to fixate, neither for the present nor for previous studies: thus, their gaze behaviour could not have been influenced by previous experiences within the laboratory setting.

Grasping observation. Grasping actions performed by the experimenter were presented in front of the monkeys in their extrapersonal space. As during action execution, monkeys were freely gazing and the only requirement was that they kept their hand contralateral to the recorded hemisphere still on the starting position.

The experimenter's actions were self-initiated. A metal plate (5 × 6 cm) used as a starting point was located on the table edge near the experimenter, along the monkey body midline, at a distance of 124 cm from its chest. The target of the experimenter's action (a 2-cm cube of food) was located 20 cm laterally with respect to the monkey body midline, in the field (left) contralateral to the recorded hemisphere (right), at a distance of 90 cm from the monkey (34 cm from the experimenter's hand starting position). A metal plate of 3 × 3 cm fixed to the table served as a support for the target of the experimenter's action. Once grasped, the target was slightly lifted and held until the end of the trial (2 s after the detachment of the experimenter's hand from the starting position).

If the monkey erroneously detached its hand from the starting position, the trial was discarded and not included in the data set. Because of the considerable variability of monkeys' gaze behaviour during action observation, we aimed at collecting the highest possible number of correct trials while simultaneously recording eye position and single neuron activity. Monkeys did not receive any reward during the inter-trial periods. However, to maintain their level of attention constant, the acquisition was randomly paused for some time (20–40 s) after a variable number of trials and, only during these periods, monkeys were given a piece of food by an experimenter different from the one that was performing the action. Therefore, no identifiable reward contingency was related to action observation trials.

Preliminary testing of neuronal activity

Before acquisition of neuronal activity, single and multiunit activity in each site was systematically tested as previously described elsewhere (Rozzi *et al.*, 2008; Maranesi *et al.*, 2012). In brief, monkeys were first required to grasp food items with their eyes closed, and with the arm and the wrist blocked. The food was brought towards the monkey's hand by the experimenter so that no reaching was required, enabling us to verify the presence of motor activity specifically related to the hand alone. Then, to exclude the possible presence of mouth-related responses, we studied neuronal activity with the monkeys' eyes closed by giving them small pieces of food directly into their mouth. Finally, visual properties were studied by presenting monkeys with 3D-objects (e.g. food items and solids) of different shape, size and orientation, moved in various space locations, direction and distances from the monkey, as well as with different manual actions performed by the experimenter. In particular, we focused our study on those cortical sites showing neuronal activity specifically related to the execution and observation of hand-grasping actions.

Recording techniques

Neuronal recordings were performed using single glass-coated tungsten microelectrodes (impedance 0.5–1 MΩ; AlphaOmega Engineering) inserted through the intact dura, perpendicularly to the cortical surface. Recording procedures were the same as previously described (Bonini *et al.*, 2010, 2012). Single spike shapes were further extracted and sorted off-line using dedicated software (Wave-Clus; Quiroga *et al.*, 2004). Eye position was recorded in parallel with neuronal activity by means of an eye-tracking system composed of a 50-Hz CCD camera provided with an infrared filter and two spots of infrared light (Ganz, F11CH4). At the beginning of each session a calibration procedure was applied using a square panel (60 × 60 cm) with four equidistant holes at the four corners delimiting the space of the scene, and one in the centre. A piece of food was repeatedly introduced into each hole in order to induce the monkey to gaze at it, enabling to record the relative position of the pupil. Contact-detecting electric circuits were used during both monkey and experimenter's actions to signal the main behavioural events, necessary for subsequent alignment of neuronal activity and eye position data. The recorded events were: (a) the detachment of the hand from the starting position; and (b) the contact of the hand with the target. Furthermore, during action observation trials, we also monitored that the monkey's hand remained steadily in contact with the starting position for the entire duration of the trial.

Analogue signals related to eye position and neuronal activity, isolated action potentials, and the digital events related to the behavioural paradigm were acquired and stored in parallel by means of LabVIEW-based software.

Definition of grasping epochs and behavioural data analyses

All data concerning monkeys' eye position were first checked in order to discard trials in which eye position could not be recorded for the entire trial duration (because of blinking, artefacts or other technical problems). We then analysed gaze behaviour in three epochs of interest: baseline, corresponding to a period of 500 ms and starting 2 s before the detachment of the experimenter's hand from the starting position; pre-contact, the 500-ms period before hand–target contact; post-contact, the 500-ms period after hand–target contact.

Data concerning monkeys' gaze behaviour during grasping execution were first analysed by considering a single spatial area of interest (AOI) of 4.4 ° centred on the location of the target, large enough to entirely include both the groove in which the target was positioned and the monkey's hand during grasping. We first computed the total amount of looking time in this AOI during the three epochs described above. Then, we identified the onset time of the first 'fixation' in the AOI during the pre-contact epoch: to this purpose, we considered as 'fixation' a period of at least 100 ms during which the gaze remained constantly within the AOI. Note that the term 'fixation', in this definition, is relative to the AOI and does not imply at all that monkeys did not produce small saccades or smooth eye movements 'within' the AOI. This broad definition of 'fixation' has been necessary to reduce the extreme variability of gaze behaviour in free-gazing monkeys and enabled us to subsequently apply the same criteria to correlate different gaze behaviours with neuronal firing pattern. In addition, we also looked for the arm-reaching onset, defined as the time of detachment of the hand from the starting position relative to hand–target contact.

Data concerning monkey's gaze behaviour during action observation were analysed by considering three 9.5 ° AOIs centred on the experimenter's: (a) face ('face AOI'); (b) hand starting position ('hand AOI'); and (c) target ('target AOI'). As for grasping execution, we first computed the total amount of looking time in these AOIs, with particular attention to the 'target AOI'. Then, the onset timing of fixation was identified as described above in relation to the 'target AOI' and with respect to the experimenter's hand–target contact. Similarly, the experimenter's reaching onset was defined by considering the detachment of the hand from the starting position relative to hand–target contact.

Analyses of neuronal data

For all recorded action observation trials we collected in parallel two sets of data: one for eye position; the other for neuronal activity. Eye position and neuronal activity were recorded and stored from 2 s before until 2 s after the detachment of the experimenter's hand from the starting position (4 s for each trial). Neuronal activity during action observation was analysed in the same epochs (baseline, pre-contact and post-contact) described above for gaze behaviour.

We first identified those grasping neurons responding significantly during action observation in at least one of the grasping epochs compared with baseline (repeated-measures ANOVA with significance criterion of $P < 0.05$) without taking into account the monkey's eye position. Then, we aimed at addressing the issue of whether looking or not at the presented action could influence MN visual response. Because the number of trials in which free-gazing monkeys looked or not at the 'target AOI' was highly variable, we adopted a bootstrap randomized permutation test ($N = 10^4$ permutations, $P < 0.05$). This procedure randomly selects sets of data from both groups to be compared, and provides the exact probability that the distribution within the two groups of data overlap. This enabled us to reliably compare the activity of trials in which monkeys looked with those in which they did not look at the 'target AOI' during pre- and post-contact epochs, even though the two data sets could differ in terms of number of observations. Based on the results of this analysis, we classified each neuron as either gaze dependent ($P < 0.05$) or gaze independent ($P > 0.05$).

In order to verify whether gaze-dependent and gaze-independent MNs constitute distinct neuronal populations, gaze dependency was expressed in terms of gaze preference index (PI), calculated for each neuron as follows: $PI = (R_{max} - R_0)/(R_{max} + R_0)$, where R_0 is the average neuronal response associated with trials in which the looking time was equal to 0 ms, and R_{max} is the average neuronal response associated with the same number of trials with the highest looking times obtained for that neuron. We then assessed whether the distribution of gaze PI values differed between the two neuronal populations (independent samples *t*-test).

To better understand the relationship between fixation onset and MN visual response, we first subdivided all the recorded trials of each neuron based on the onset timing of fixation in the 'target AOI' during grasping observation. We classified the trials as 'proactive' (when fixation onset occurred before hand–target contact) and 'reactive' (when fixation onset occurred after hand–target contact). We also considered trials in which the monkey never looked at the 'target AOI' during the whole action observation period ('no-fixation').

Population analyses were carried out in order to compare the time course and intensity of activity associated with proactive, reactive and no-fixation trials (repeated-measures ANOVA, $P < 0.01$). For each neuron, we calculated the mean time course of activity of proactive, reactive and no-fixation trials, aligned to the experimenter's reaching onset or hand–target contact. We employed a time window of 60 ms slit forward in steps of 20 ms, according to previous studies (Bonini *et al.*, 2010). Then, the time courses associated with proactive, reactive and no-fixation trials were normalized so that their values ranged from 0 to 1. Finally, the normalized time courses of each group of trials were averaged to obtain the population vectors. In order to calculate, for each neuron, the timing of peak of activity and discharge onset, we employed the same procedure described elsewhere (Bonini *et al.*, 2010).

A further population analysis has been carried out in order to better scrutinize the possible link between neurons discharge profile and the timing of monkeys' fixation onset. In order to reduce the impact of the extreme variability of monkey's eye behaviour during free gaze action observation, we first ordered all the available trials based on their timing of fixation onset. Then, we subdivided them into seven groups, with an equal number of trials per group. Of course, the use of a lower number of groups (higher number of trials per group) would have reduced the variability of neuronal discharge within each group, but would have increased the range of fixation onset timing; on the contrary, a higher number of groups (lower number of trials per group) would have shrunk the range of fixation onset timing of each group, determining at the same time an increased variability of neuronal discharge within each group. Seven groups ($N = 86$ trials per group) appeared to be the best compromise to fulfil both these requirements.

Results

Monkey gaze behaviour during grasping execution and observation

Figure 1A shows the normalized looking time in the AOI centred on the target location ('target AOI', see Materials and methods). The data of the two monkeys have been pooled together because eye–hand coordination during grasping execution appeared to be extremely stereotyped in both animals. In particular, one-way repeated-measures ANOVA was used to compare the looking time

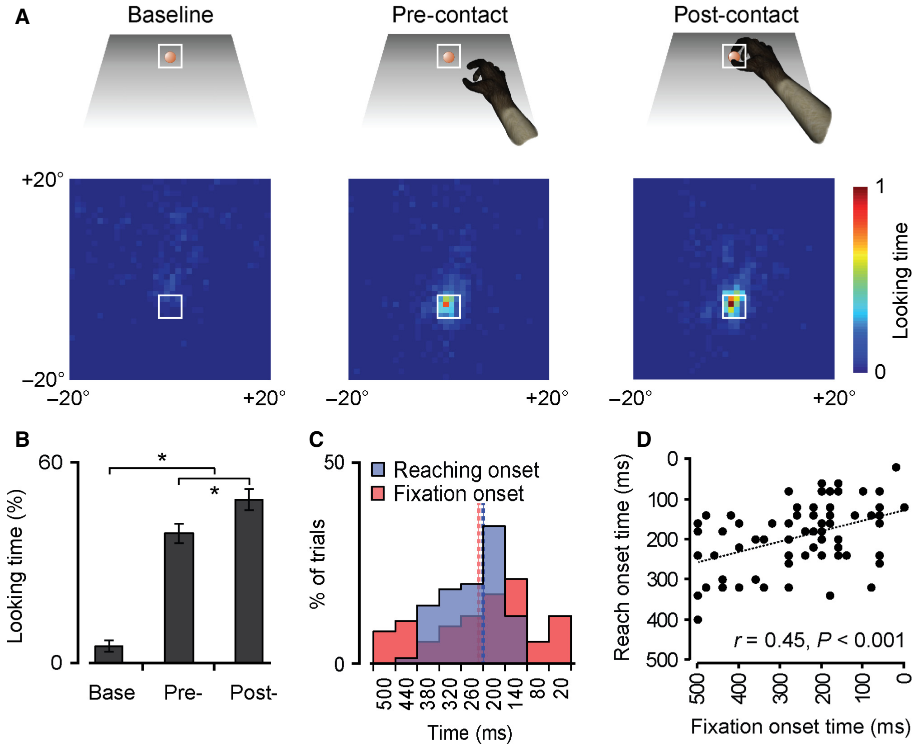

FIG. 1. Monkeys' gaze behaviour during grasping execution. (A) Schematic drawing of the different stages of grasping performed by the monkey and corresponding spatial distributions of normalized looking time. White squares indicate the 4.4° AOI centred on the target location. (B) Percentage of looking time within the AOI during baseline, pre- and post-contact epochs. Asterisks indicate significant comparisons ($P < 0.001$). (C) Frequency distribution of the timing of fixation and monkeys' reaching onset relative to hand–target contact. Dashed lines indicate the average time of reaching (blue) and fixation (red) onset. (D) Scatterplots of the fixation onset time as a function of the reaching onset time during proactive gaze trials. Time is expressed relative to the monkeys' hand–target contact (time 0).

among baseline, pre- and post-contact epochs ($F_{2,172} = 113.86$, $P = 0.000$). Bonferroni *post hoc* tests showed that the looking time was greater during both pre- and post-contact epochs than during baseline ($P = 0.000$ for both comparisons; Fig. 1B), and significantly increased during the post- compared with the pre-contact epoch ($P = 0.003$). Interestingly, monkeys looked at the 'target AOI' in all the recorded trials, and in the great majority of them (92% in M1 and 95% in M2) the gaze entered in the AOI already during the pre-contact epoch ('proactive' gaze), while in the remaining it entered in the AOI during post-contact epoch ('reactive' gaze). More specifically, we identified for each trial the onset time of the fixation (see Materials and methods), showing that monkeys directed their gaze to the target on average (± SD) 254 ± 142 ms before hand–target contact. Figure 1C shows that in trials characterized by proactive gaze, the distribution of fixation onset timing widely overlaps with that of reaching onset timing (270 ± 81 ms before hand–target contact, two-tailed paired sample *t*-test, $P = 0.31$). A linear regression analysis (Fig. 1D) revealed that fixation onset is positively correlated with arm-reaching onset ($r_{74} = 0.45$, $P = 0.000$). Taken together, these data indicate that monkeys' proactive eye movements guide object-directed hand actions, with the eyes staring at the target well in advance of hand–target contact.

Figure 2A shows monkeys' gaze behaviour during action observation. It is clear that there is a considerable inter-individual variability. Both animals looked longer at the experimenter's face and target AOIs, although in different epochs, rather than looking at the experimenter's hand starting position or following the reaching trajectory. To quantitatively compare the monkey's looking time in the three AOIs (face, hand and target; see Materials and methods) during different epochs of grasping action (baseline, pre-contact and post-contact), we carried out a 3 × 3 repeated-measures ANOVA (factors: AOI and Epoch), including Monkey as an additional grouping factor. The results are summarized in Fig. 2B. We found significant main effects of all factors (AOI: $F_{2,4010} = 312.57$, $P = 0.000$; Epoch: $F_{2,4010} = 870.04$, $P = 0.000$). In particular, the interaction between the factors Monkey and AOI was highly significant ($F_{2,4010} = 249.69$, $P = 0.000$), and Bonferroni *post hoc* tests revealed that, in general, M2 looked longer at the 'face AOI' ($P = 0.000$) compared with M1 which, in turn, looked longer at the 'target AOI' ($P = 0.000$). ANOVA also revealed a significant interaction among all factors ($F_{4,8020} = 28.412$, $P = 0.000$), and Bonferroni *post hoc* tests indicated that looking time generally increases with respect to baseline during both epochs of action observation. More specifically, during the pre-contact epoch both monkeys looked significantly longer at the experimenter's face compared with the other AOIs (M1: $P = 0.000$ for both comparisons; M2: $P = 0.000$ for both comparisons). Furthermore, in spite of the considerable inter-individual variability, a further interesting trend is evident in both animals: considering the 'target AOI', the looking time, particularly in M1, is greater during the pre-contact epoch as compared with baseline and further increases during post-contact epoch (M1: $P = 0.000$ for both comparisons; M2: $P = 0.000$ for the comparison between pre- and post-contact epoch). Note that this pattern of gaze behaviour is similar to the one found during action execution, although during action observation proactive gaze is less frequently observed (M1: 45.6% of all trials, on average 200.6 ± 163.3 ms before experimenter's hand–target contact; M2: 16% of all trials, on average 125.8 ± 99.4 ms before experimenter's hand–target contact).

Figure 2C shows the distribution of fixation onset timing in trials characterized by proactive gaze, overlapped with the distribution of

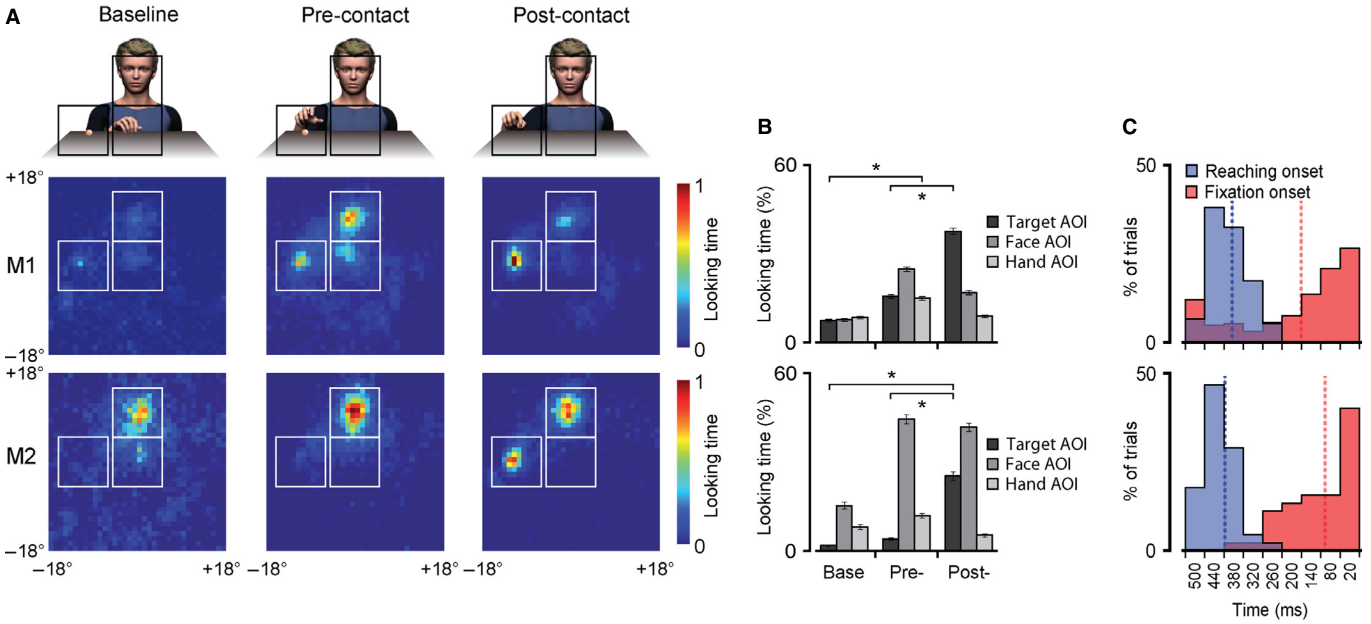

FIG. 2. Monkeys' gaze behaviour during action observation. (A) Schematic drawing of the different stages of grasping performed by the experimenter in front of the monkey and corresponding spatial distribution of normalized looking time. White squares indicate the 9.5° AOI centred on the experimenter's face, hand and target location. (B) Percentage of looking time within different AOIs during baseline, pre- and post-contact epochs. Conventions as in Fig. 1B. (C) Frequency distribution of the timing of monkey's fixation and experimenter's reaching onset relative to hand–target contact. Conventions as in Fig. 1C.

the timing of experimenter's reaching onset. Note that during proactive gaze trials the monkey's eye entered into the 'target AOI' 'in advance' with respect to the experimenter's hand–target contact, but 'after' experimenter's reaching onset (two-tailed independent sample *t*-test M1: $t_{818} = 24.93$, $P = 0.000$; M2: $t_{88} = 17.25$, $P = 0.000$), and the timing of fixation and experimenter's reaching onset were not significantly correlated during action observation (M1: $r_{410} = 0.03$, $P = 0.54$; M2: $r_{45} = 0.04$, $P = 0.78$). These findings strongly suggest that during action observation, the monkey's proactive gaze to the site of upcoming hand–target interaction requires the sight of at least the initial part of the experimenter's action.

Functional relationship between gaze behaviour and MN activity

We recorded the visual responses of 71 MNs while monkeys freely looked at the experimenter's action. Each neuron was recorded for as many trials as possible (see Materials and methods), from a minimum of 24 to a maximum of 67, on average (± SD) 45 ± 12 trials.

For each neuron, we first compared the discharge intensity of trials in which monkeys looked at the 'target AOI' with trials in which they never looked at it for the entire duration of the action (see Materials and methods). Results showed that the discharge of more than half of the recorded neurons ($n = 38$) was gaze dependent, being significantly stronger in trials in which the monkey looked compared with those in which it did not look at the action. The response of the remaining neurons ($n = 33$) was gaze independent. Figure 3 shows examples of gaze-dependent (Fig. 3A) and gaze-independent (Fig. 3B) neurons. It is clear that the discharge of the gaze-dependent neuron was weaker when the monkey did not look at the action than when it looked at it, while the response of the gaze-independent neuron remained unchanged. In order to verify whether gaze dependency is a normally distributed variable, we calculated for each neuron a PI (see Materials and methods) indicating its magnitude of gaze dependency. Then, we tested the PIs distribution by means of a Shapiro–Wilk normality test. Results indicate that PIs are not normally distributed ($W = 0.96$, $P = 0.048$). Indeed, their distribution of PI values shown in Fig. 3C strongly suggests that gaze-dependent and gaze-independent neurons form two distinct populations.

What aspects of monkey's gaze could account for MN gaze dependency? A likely possibility is that gaze-dependent MN visual response is influenced by 'how long' monkeys looked at the experimenter's action (looking time). In order to verify this hypothesis, for each gaze-dependent neuron we investigated the possible correlation between looking time and firing rate by means of a linear regression analysis. Surprisingly, results showed that only 42% ($n = 16$) of these neurons exhibited a positive and significant correlation. An example is shown in Fig. 4A. In contrast, the majority ($n = 22$) did not show any correlation (Fig. 4B). Furthermore, the strength of significant correlations was generally modest, with percentages of firing rate variation explained by the looking time ranging from 10% to 64%. Taken together, these results indicate that looking time appears to be a rather weak predictor of gaze-dependent MN firing rate.

An alternative possibility is that gaze dependency is better accounted for by 'when' (gaze timing) rather than 'how long' (looking time) the monkey's gaze is directed to the target during action observation. It is clear that the later the monkey looks at the 'target AOI', the shorter is the time available to look at the action. Thus, in order to avoid confounding possible effects due to gaze timing with those due to looking time, we analysed separately the gaze-dependent neurons with significant correlation between looking time and firing rate and those with no significant correlation. Figure 5 shows the discharge profile of gaze-dependent and gaze-independent neuronal populations during proactive, reactive and no-fixation trials (see Materials and methods). Results of 2 × 3 repeated-measures ANOVAS (factors: Epoch and Group of trials) demonstrate that all gaze-dependent neurons behave similarly, irrespectively of whether they showed significant correlation (Fig. 5A) or no correlation (Fig. 5B) between looking time and firing rate. More specifically, the main

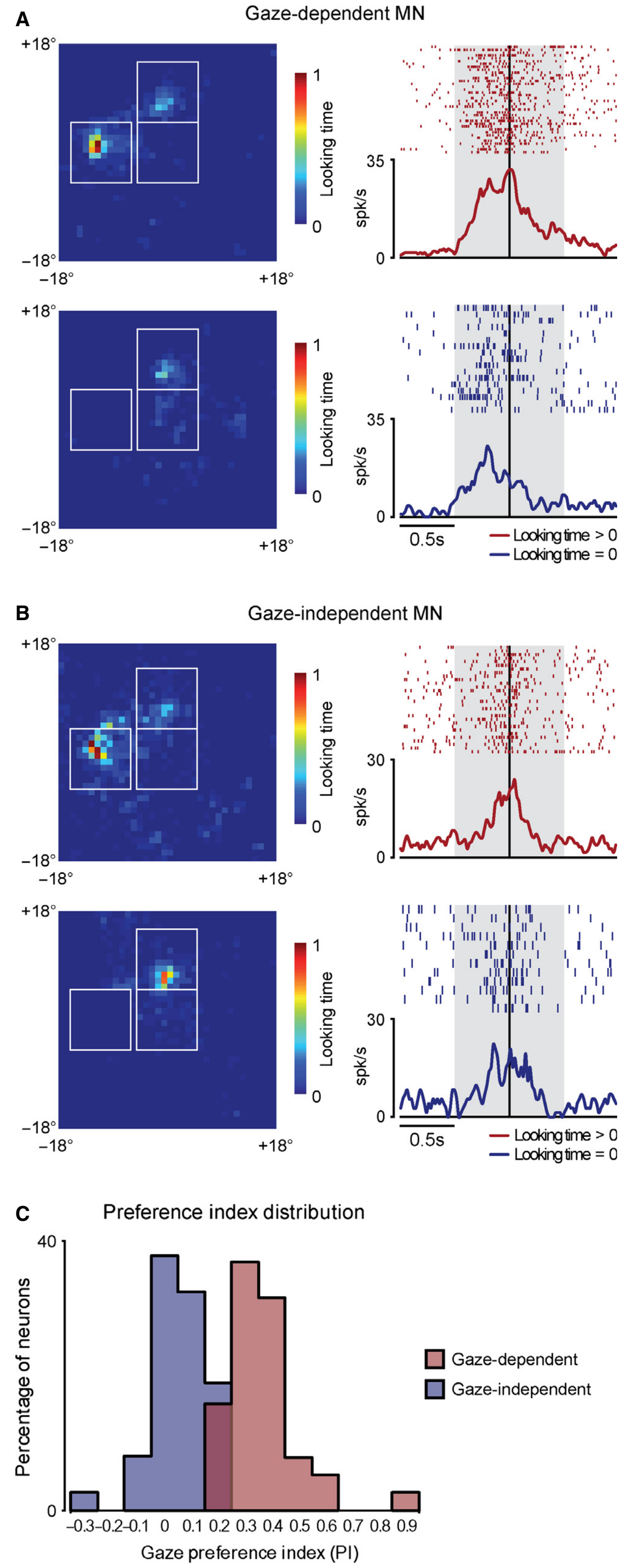

FIG. 3. Examples of gaze-dependent and gaze-independent MN visual response during action observation. (A) Gaze-dependent MN discharging stronger during trials in which the monkey looked (upper panel) compared with those in which it did not look (lower panel) at the target AOI. Rasters and histograms have been compiled using trials associated with looking time higher than (red) or equal to (blue) zero, calculated on the whole grasping period (grey shaded area). Histograms have been computed in 20-ms bins smoothed with a 60-ms sliding window, and aligned with the experimenter's hand–target contact. (B) Gaze-independent MN discharging similarly during trials in which the monkey looked compared with those in which it did not look at the target AOI. Conventions as in (A). (C) Distribution of PI values for gaze-dependent (red) and gaze-independent (blue) neurons.

effect of the factor Epoch was not significant ($F_{1,15} = 2.14$, $P = 0.16$ for population analysis shown in Fig. 5A; $F_{1,21} = 1.08$, $P = 0.31$ for population analysis shown in Fig. 5B), indicating that the overall discharge was similar during pre- and post-contact epoch.

Furthermore, Bonferroni *post hoc* tests carried out on the significant interaction effects ($F_{2,30} = 14.57$, $P = 0.000$ for the population shown in Fig. 5A; $F_{2,42} = 15.96$, $P = 0.000$ for the population shown in Fig. 5B) showed that, during the pre-contact epoch, the discharge was stronger in proactive compared with both reactive ($P = 0.000$ for both populations of Fig. 5A and B) and no-fixation trials ($P = 0.000$ for both populations), which did not differ from each other ($P = 1.00$ for both populations). During post-contact epoch, the discharge intensity associated with proactive and reactive trials did not differ significantly ($P = 1.00$ for both populations), but they both displayed a stronger activity as compared with no-fixation trials ($P = 0.000$ for both comparisons and populations). In contrast, the same 2 × 3 repeated-measures ANOVA carried out on gaze-independent neuronal population (Fig. 5C) showed a significant main effect of the factor Epoch ($F_{1,36} = 18.11$, $P = 0.000$), indicating that the discharge was generally higher during post-contact compared with pre-contact epoch, but no significant difference among proactive, reactive and even no-fixation trials ($F_{2,72} = 4.70$, $P = 0.012$).

In order to better clarify the functional relationship between the timing of monkey's gaze behaviour and the visual response of gaze-dependent MNs, we subdivided all the proactive and reactive trials into seven groups, based on the timing of fixation in the 'target AOI' (see Materials and methods). Figure 6 shows the results concerning gaze-dependent neurons with no significant correlation between looking time and firing rate. The onset of MN visual response (Fig. 6A) is tightly linked to the onset of the experimenter's hand movement, raising within 120 ms (median and mode 60 ms) after the detachment of the experimenter's hand from the starting position, and it is not influenced by when (and whether) the monkey's gaze is directed to the AOI ($r_8 = 0.17$, $P = 0.68$). In contrast, the peaks of activity show a greater variability, ranging from 120 ms before to 320 ms after the experimenter's hand–target contact (Fig. 6B). Importantly, we found that the timings of peaks of activity were strongly and positively correlated with the timing of fixation onset ($r_7 = 0.98$, $P = 0.000$; Fig. 6C). Thus, gaze-dependent MN peak of activity is shifted in time depending on whether, and when, the monkey gazes at the target of the experimenter's action. The same analyses carried out on those gaze-dependent neurons that showed a significant correlation between looking time and firing rate provide similar results ($r_7 = 0.78$, $P = 0.000$), supporting the claim that gaze-dependent MNs firing rate and peak of activity are influenced by whether and when the monkey gazes at the site of hand–target interaction.

Discussion

Proactive and reactive gaze in monkeys during action execution and observation

Previous studies (Flanagan & Johansson, 2003; Falck-Ytter *et al.*, 2006; Rosander & von Hofsten, 2011; Elsner *et al.*, 2012) have

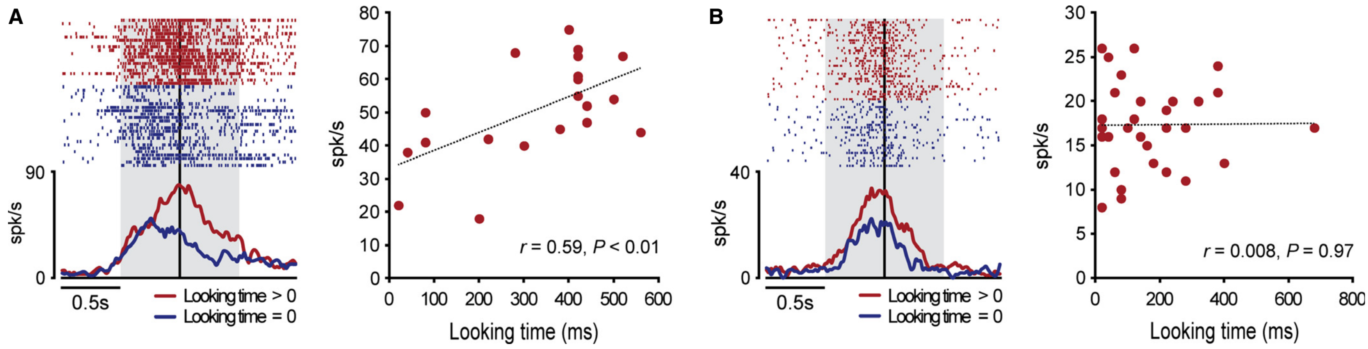

FIG. 4. Relationship between looking time and gaze-dependent MN firing rate. (A) Example of a gaze-dependent MN showing firing rate positively correlated with the looking time during action observation. (B) Example of a gaze-dependent MN in which the firing rate is not significantly correlated with the looking time. Rasters and histograms have been compiled as described in Fig. 3A. Scatterplots show, for each neuron, the average activity (during the whole grasping period) of all trials associated with a looking time higher than 0 as a function of the looking time.

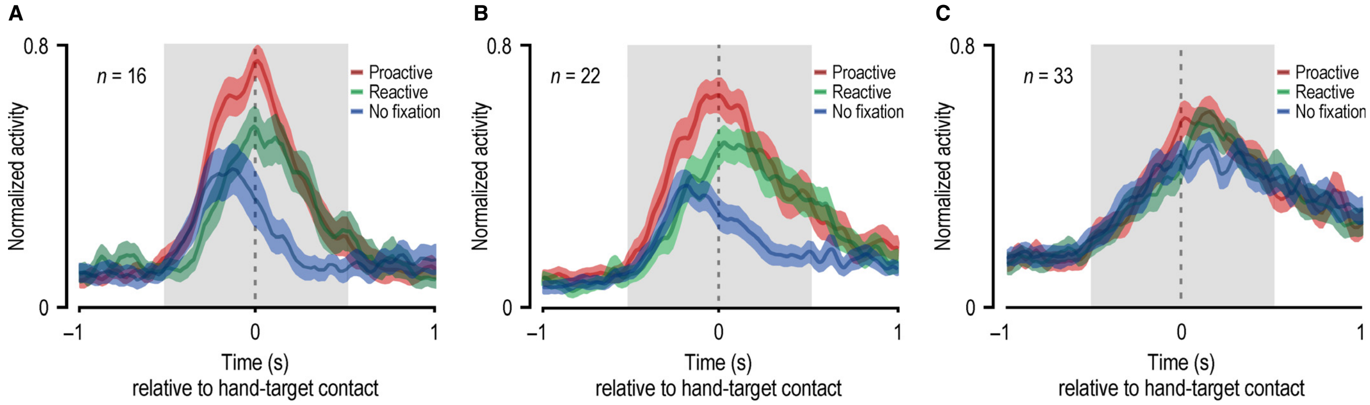

FIG. 5. Population activity of gaze-dependent and gaze-independent MNs. (A) Time course of activity of gaze-dependent neurons with significant correlation between looking time and firing rate. (B) Time course of activity of gaze-dependent neurons with no correlation between looking time and firing rate. (C) Time course of activity of gaze-independent neurons. For each neuron included in these analyses, trials have been subdivided based on the timing of fixation onset in the 'target AOI'. Grey shaded regions indicate the grasping period.

shown that proactive gaze is a hallmark of human oculomotor behaviour during both action execution and observation. Our data demonstrate that, as previously shown in humans (Johansson *et al.*, 2001), monkey's proactive eye movements guide object-directed hand actions, with the eyes staring at the target well in advance of hand–target contact and in tight relation with arm-reaching onset.

Our findings also provide the first evidence that proactive gaze occurs even during action observation, although in this condition it is less consistent and more subject to inter-individual variability than in humans. This might be not surprising as in the human studies cited above subjects are typically presented with a relatively low number of trials in a single session, so that the observed action maintains its salience across trials. In contrast, in our experiments each monkey was daily presented with several identical trials of action observation for many consecutive sessions, in order to collect neuronal activity in a sufficient number of trials. This certainly had the drawback of reducing the salience of the observed action, which is known to be a crucial factor in determining proactive gaze in humans (Henrichs *et al.*, 2012). A further possibility is that the greater variability of monkey's gaze behaviour compared with human gaze behaviour is due to different species-specific strategies or to a different relevance attributed by monkeys to the presented stimuli, as suggested by previous studies (Berg *et al.*, 2009; Shepherd *et al.*, 2010).

Taken together, our behavioural data indicate that monkeys, similarly to humans, strongly rely on eye–hand coordination for the visual guidance and monitoring of hand actions execution. In spite of a considerable inter-individual variability and a high number of trials characterized by reactive gaze, monkeys can proactively look at the site of the upcoming hand–target interaction even during action observation.

The relationship between monkey's gaze behaviour and MN activity

The recording of MN activity during free-gazing observation of grasping actions has enabled us to show that part of the recorded neurons were gaze independent, in that their firing rate did not vary depending on whether or not the monkey looked at the action, while others could be classified as gaze dependent. Interestingly, the distribution of gaze PIs of the recorded neurons does not fit a normal distribution, with gaze-dependent and gaze-independent neurons forming the two tails of a continuum. Rather, it appears that two distinct sets of MNs do exist, and this distinction is also supported by the results of population analyses.

As far as gaze-independent neuronal population is concerned, its activity was maximal during the post-contact epoch of the observed grasping, regardless of whether proactive, reactive or no-fixation trials were considered. Gaze-independent neurons appear therefore to code the interaction between the agent's hand and the target largely irrespectively of the monkey's focus of attention, providing a highly

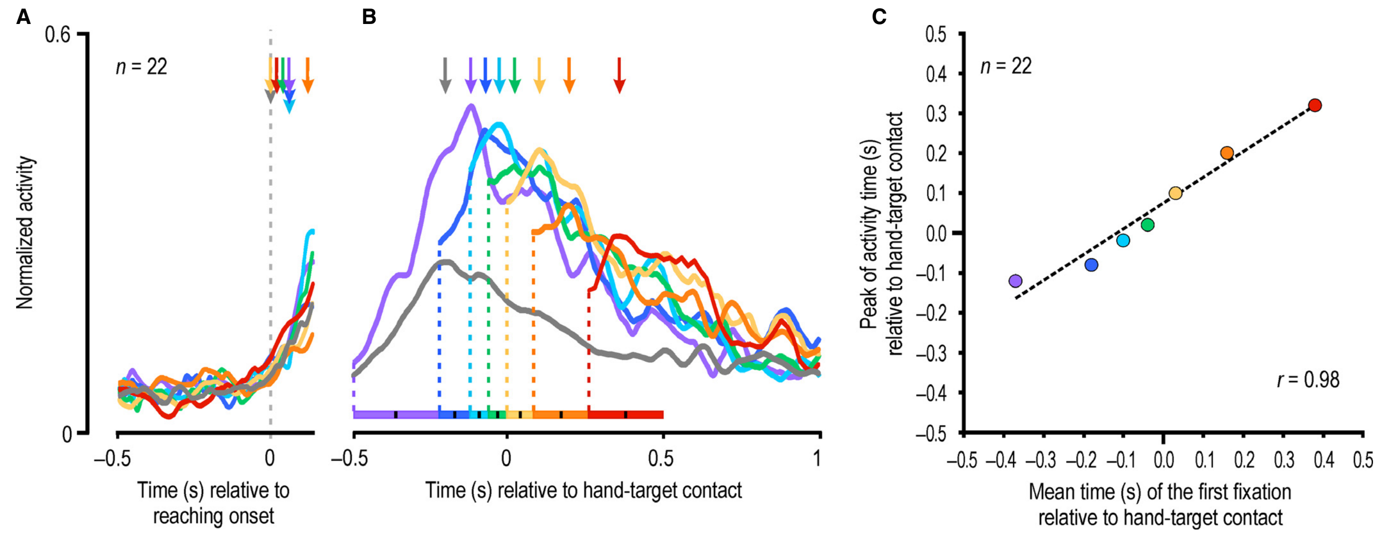

FIG. 6. Population analyses of the relationship between the discharge profile of gaze-dependent neurons and fixation onset timing during action observation. (A) Discharge profile relative to the detachment of the experimenter's hand from the starting position (reaching onset). Coloured lines indicate groups of trials (n = 86) in which fixation onset relative to hand–target contact (see B) fell in the range of: −500/−240 ms (violet); −239/−140 ms (blue); −139/−80 ms (light blue); −79/−20 ms (green); −19/60 ms (yellow); 61/240 ms (orange); 241/500 ms (red). The grey line represents no-fixation trials. Coloured arrows indicate, for each population vector, the first of a series of five consecutive 60-ms bins in which the activity significantly differed ($P < 0.05$) from that during an earlier period (200 ms long, taken from 500 to 300 ms before reaching onset). (B) Discharge profiles of the same groups of trials described in (A), aligned to the experimenter's hand–target contact. The coloured bars below the lines indicate the intervals and average time (small black bars) of fixation onset for each group of trials (colour code as in A). The grey line represents no-fixation trials. (C) Scatterplot of the peak of activity timing of the seven groups of trials described in (A) and (B), as a function of fixation onset time.

automatic form of recognition of other's action. These observations are in line with previous studies showing that MNs can activate even when the agent's hand–object interaction is concealed behind a screen, provided that the monkey is aware of the presence of the target object (Umiltà *et al.*, 2001). Other studies also showed that some MNs (audio-visual MNs), although activated during action observation, can code noisy actions (e.g. peanut breaking) even when simply listening to the specific sound they produced (Kohler *et al.*, 2002), enabling an automatic activation of a motor representation in the absence of any visible action.

Gaze-dependent neurons appear to have a more complex behaviour. On one hand, their response even during no-fixation trials strongly indicates that they can contribute to a fast and automatic coding of the observed action, as previously discussed for gaze-independent neurons. On the other hand, we showed that MN discharge is more influenced by 'when' rather than by 'how long' monkeys looked at the experimenter's action. Effectively, proactive gaze was associated with the strongest discharge during pre-contact epoch, while reactive gaze enhanced the activity in the post-contact epoch compared with no-fixation trials. This effect was extremely robust as it was found not only in gaze-dependent neurons showing significant correlation between looking time and firing rate, but also in those showing no correlation. Thus, it cannot be accounted for by the fact that the earlier the monkey gazed at the action, the longer it could observe it. The finding of a strong relationship between the timing of monkey's gaze behaviour and MN response to action observation raises the issue of whether MN activity mainly causes or is caused by monkey's gaze shift.

Previous human studies suggested that internal motor representations play a crucial role in driving eye movements during action observation because observers proactively gaze at a potential target object only when observing biological motions while they reactively follow non-biological stimuli (Flanagan & Johansson, 2003). However, neither the present nor the previous findings enable to infer a causal relationship between MN activity and gaze behaviour. Nevertheless, here we show for the first time that MN visual responses are triggered by the initial phase of the observed action, with their activity rising largely in advance of hand–target contact. Furthermore, the timing of MN discharge onset is completely unaffected by both 'whether' and 'when' the monkey will look at the target of the observed action during its unfolding, thus indicating that their activation is always highly automatic.

In line with this finding, several human transcranial magnetic stimulation (TMS) studies revealed that the stimulation of motor cortex produces a higher corticospinal facilitation when delivered during observation of 'implied' hand actions than during observation of static hand–object interactions (Urgesi *et al.*, 2006, 2010), suggesting that the frontal component of the mirror system plays a crucial role in anticipatory simulation of future phases of observed hand actions. This capacity of the motor system to generate predictive representations of the upcoming actions has also been supported by a recent study showing that gaze proactivity during action observation increases when the observer pre-activates motor representations of grip types compatible with those used in the observed action (Costantini *et al.*, 2012). Furthermore, another TMS experiment has directly shown that the stimulation of the motor hand area, but not of the leg area, slows proactive gaze behaviour during the observation of other's action (Elsner *et al.*, 2013). Altogether, the present and previous findings strongly support the idea that hand motor representations could play a causal role in driving gaze behaviour. This might occur through different anatomical pathways linking area F5c, in which MNs are located, with distinct oculomotor fields. First, cortical sites containing neurons involved in the control of eye movements have been reported in a ventral premotor sector (see Fujii *et al.*, 1998), likely corresponding to area F4, tightly linked with area F5c (Gerbella *et al.*, 2011). Second, area F5c is tightly connected with area F5a (Gerbella *et al.*, 2011). Although its functional properties are not very well known, F5a is deemed to be part of the cortical MNs system (Nelissen *et al.*, 2011), and has been considered a crucial gateway linking the other F5 sectors with prefrontal areas (mainly areas 46v and 12) involved in executive control of motor plans, including those for eye movements (Tanji & Hoshi, 2008). Furthermore, area F5a is also directly connected with the superior colliculus, a structure very well known for its role in the control of gaze behaviour (Borra *et al.*, 2012).

Our data further extend the knowledge about the mechanisms involved in the representation of others' action also showing that the timing of monkey's gaze behaviour, during both proactive and reactive gaze trials, is generally predictive of gaze-dependent MN magnitude of discharge and timing of peak of activity (Fig. 6). This clearly indicates that MN visual response can strongly benefit from the visual feedback derived from gazing at other's action, particularly when the gaze shift conveys information on hand–target interaction while it is occurring. Thus, the present findings suggest that during action observation feedforward representations of others' actions might lead eye movements that, in turn, provide the motor system with feedback visual information enhancing motor representations of the ongoing action.

Conclusion

Why should motor representations be necessary to guide eye movements during action observation? As motor representations of hand actions to be performed can trigger agent's proactive eye movements to monitor and control action unfolding, similarly these representations might activate during action observation to better analyse other's behaviour serving potential social interactions. Recent human experiments (Ambrosini *et al.*, 2012) have shown that proactive eye movements during action observation are abolished if the observers' hands are tied behind their backs, suggesting that the activation of motor representations of other's actions crucially depends on our possibility to interact with the observed agent, in line with single neuron data (Caggiano *et al.*, 2009). These considerations point to the idea that MNs might be part of a wider perception–action system in which the automaticity and predictive nature of one's own motor representations are exploited to anticipate and decode other's behaviour in complex social contexts.

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Abbreviations

AOI, area of interest; MN, mirror neuron; PI, preference index; TMS, transcranial magnetic stimulation.

References

Ambrosini, E., Sinigaglia, C. & Costantini, M. (2012) Tie my hands, tie my eyes. *J. Exp. Psychol. Hum. Percept. Perform.*, **38**, 263–266.

Berg, D.J., Boehnke, S.E., Marino, R.A., Munoz, D.P. & Itti, L. (2009) Free viewing of dynamic stimuli by humans and monkeys. *J. Vision*, **9**, 11–15.

Bonini, L. & Ferrari, P.F. (2011) Evolution of mirror systems: a simple mechanism for complex cognitive functions. *Ann. NY Acad. Sci.*, **1225**, 166–175.

Bonini, L., Rozzi, S., Serventi, F.U., Simone, L., Ferrari, P.F. & Fogassi, L. (2010) Ventral premotor and inferior parietal cortices make distinct contribution to action organization and intention understanding. *Cereb. Cortex*, **20**, 1372–1385.

Bonini, L., Ugolotti Serventi, F., Bruni, S., Maranesi, M., Bimbi, M., Simone, L., Rozzi, S., Ferrari, P.F. & Fogassi, L. (2012) Selectivity for grip type and action goal in macaque inferior parietal and ventral premotor grasping neurons. *J. Neurophysiol.*, **108**, 1607–1619.

Bonini, L., Ferrari, P.F. & Fogassi, L. (2013) Neurophysiological bases underlying the organization of intentional actions and the understanding of others' intention. *Conscious. Cogn.*, **22**, 1095–1104.

Borra, E., Gerbella, M., Rozzi, S., Tonelli, S. & Luppino, G. (2012) Projections to the Superior Colliculus from Inferior Parietal, Ventral Premotor, and Ventrolateral Prefrontal Areas Involved in Controlling Goal-Directed Hand Actions in the Macaque. *Cereb. Cortex*, doi: 10.1093/cercor/bhs392 [Epub ahead of print].

Caggiano, V., Fogassi, L., Rizzolatti, G., Thier, P. & Casile, A. (2009) Mirror neurons differentially encode the peripersonal and extrapersonal space of monkeys. *Science*, **324**, 403–406.

Caggiano, V., Fogassi, L., Rizzolatti, G., Pomper, J.K., Thier, P., Giese, M.A. & Casile, A. (2011) View-based encoding of actions in mirror neurons of area f5 in macaque premotor cortex. *Curr. Biol.*, **21**, 144–148.

Caggiano, V., Fogassi, L., Rizzolatti, G., Casile, A., Giese, M.A. & Thier, P. (2012) Mirror neurons encode the subjective value of an observed action. *Proc. Natl. Acad. Sci. USA*, **109**, 11848–11853.

Costantini, M., Ambrosini, E. & Sinigaglia, C. (2012) Does how I look at what you're doing depend on what I'm doing? *Acta Psychol. (Amst)*, **141**, 199–204.

Elsner, C., Falck-Ytter, T. & Gredeback, G. (2012) Humans anticipate the goal of other people's point-light actions. *Front. Psychol.*, **3**, 120.

Elsner, C., D'Ausilio, A., Gredeback, G., Falck-Ytter, T. & Fadiga, L. (2013) The motor cortex is causally related to predictive eye movements during action observation. *Neuropsychologia*, **51**, 488–492.

Falck-Ytter, T., Gredeback, G. & von Hofsten, C. (2006) Infants predict other people's action goals. *Nat. Neurosci.*, **9**, 878–879.

Ferrari, P.F., Rozzi, S. & Fogassi, L. (2005) Mirror neurons responding to observation of actions made with tools in monkey ventral premotor cortex. *J. Cognitive Neurosci.*, **17**, 212–226.

Flanagan, J.R. & Johansson, R.S. (2003) Action plans used in action observation. *Nature*, **424**, 769–771.

Fogassi, L., Ferrari, P.F., Gesierich, B., Rozzi, S., Chersi, F. & Rizzolatti, G. (2005) Parietal lobe: from action organization to intention understanding. *Science*, **308**, 662–667.

Fujii, N., Mushiake, H. & Tanji, J. (1998) An oculomotor representation area within the ventral premotor cortex. *Proc. Natl. Acad. Sci. USA*, **95**, 12034–12037.

Gallese, V., Fadiga, L., Fogassi, L. & Rizzolatti, G. (1996) Action recognition in the premotor cortex. *Brain*, **119**(Pt 2), 593–609.

Gerbella, M., Belmalih, A., Borra, E., Rozzi, S. & Luppino, G. (2011) Cortical connections of the anterior (F5a) subdivision of the macaque ventral premotor area F5. *Brain Struct. Funct.*, **216**, 43–65.

Gibson, J. (1979) *The Ecological Approach to Visual Perception*. Houghton Mifflin, Boston.

Henrichs, I., Elsner, C., Elsner, B. & Gredeback, G. (2012) Goal salience affects infants' goal-directed gaze shifts. *Front. Psychol.*, **3**, 391.

Johansson, R.S., Westling, G., Backstrom, A. & Flanagan, J.R. (2001) Eye-hand coordination in object manipulation. *J. Neurosci.*, **21**, 6917–6932.

Kohler, E., Keysers, C., Umilta, M.A., Fogassi, L., Gallese, V. & Rizzolatti, G. (2002) Hearing sounds, understanding actions: action representation in mirror neurons. *Science*, **297**, 846–848.

Kraskov, A., Dancause, N., Quallo, M.M., Shepherd, S. & Lemon, R.N. (2009) Corticospinal neurons in macaque ventral premotor cortex with mirror properties: a potential mechanism for action suppression? *Neuron*, **64**, 922–930.

Maranesi, M., Rodà, F., Bonini, L., Rozzi, S., Ferrari, P.F., Fogassi, L. & Coude, G. (2012) Anatomo-functional organization of the ventral primary motor and premotor cortex in the macaque monkey. *Eur. J. Neurosci.*, **36**, 3376–3387.

Nelissen, K., Borra, E., Gerbella, M., Rozzi, S., Luppino, G., Vanduffel, W., Rizzolatti, G. & Orban, G.A. (2011) Action observation circuits in the macaque monkey cortex. *J. Neurosci.*, **31**, 3743–3756.

Quiroga, R.Q., Nadasdy, Z. & Ben-Shaul, Y. (2004) Unsupervised spike detection and sorting with wavelets and superparamagnetic clustering. *Neural Comput.*, **16**, 1661–1687.

Raos, V., Umiltà, M.A., Murata, A., Fogassi, L. & Gallese, V. (2006) Functional properties of grasping-related neurons in the ventral premotor area F5 of the macaque monkey. *J. Neurophysiol.*, **95**, 709–729.

Rizzolatti, G. & Sinigaglia, C. (2010) The functional role of the parieto-frontal mirror circuit: interpretations and misinterpretations. *Nat. Rev. Neurosci.*, **11**, 264–274.

Rizzolatti, G., Fadiga, L., Gallese, V. & Fogassi, L. (1996) Premotor cortex and the recognition of motor actions. *Brain Res. Cogn. Brain Res.*, **3**, 131–141.

Rochat, M.J., Caruana, F., Jezzini, A., Escola, L., Intskirveli, I., Grammont, F., Gallese, V., Rizzolatti, G. & Umilta, M.A. (2010) Responses of mirror neurons in area F5 to hand and tool grasping observation. *Exp. Brain Res.*, **204**, 605–616.

Rosander, K. & von Hofsten, C. (2011) Predictive gaze shifts elicited during observed and performed actions in 10-month-old infants and adults. *Neuropsychologia*, **49**, 2911–2917.

Rozzi, S., Calzavara, R., Belmalih, A., Borra, E., Gregoriou, G.G., Matelli, M. & Luppino, G. (2006) Cortical connections of the inferior parietal cortical convexity of the macaque monkey. *Cereb. Cortex*, **16**, 1389–1417.

Rozzi, S., Ferrari, P.F., Bonini, L., Rizzolatti, G. & Fogassi, L. (2008) Functional organization of inferior parietal lobule convexity in the macaque monkey: electrophysiological characterization of motor, sensory and mirror responses and their correlation with cytoarchitectonic areas. *Eur. J. Neurosci.*, **28**, 1569–1588.

Shepherd, S.V., Steckenfinger, S.A., Hasson, U. & Ghazanfar, A.A. (2010) Human-monkey gaze correlations reveal convergent and divergent patterns of movie viewing. *Curr. Biol.*, **20**, 649–656.

Tanji, J. & Hoshi, E. (2008) Role of the lateral prefrontal cortex in executive behavioral control. *Physiol. Rev.*, **88**, 37–57.

Umiltà, M.A., Kohler, E., Gallese, V., Fogassi, L., Fadiga, L., Keysers, C. & Rizzolatti, G. (2001) I know what you are doing: a neurophysiological study. *Neuron*, **31**, 155–165.

Urgesi, C., Moro, V., Candidi, M. & Aglioti, S.M. (2006) Mapping implied body actions in the human motor system. *J. Neurosci.*, **26**, 7942–7949.

Urgesi, C., Maieron, M., Avenanti, A., Tidoni, E., Fabbro, F. & Aglioti, S.M. (2010) Simulating the future of actions in the human corticospinal system. *Cereb. Cortex*, **20**, 2511–2521.

Vigneswaran, G., Philipp, R., Lemon, R.N. & Kraskov, A. (2013) M1 corticospinal mirror neurons and their role in movement suppression during action observation. *Curr. Biol.*, **23**, 236–243.