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ORIGINAL ARTICLE

Orienting movements in area 9 identified by long-train ICMS

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Abstract The effect of intracortical microstimulation has been studied in several cortical areas from motor to sensory areas. The frontal pole has received particular attention, and several microstimulation studies have been conducted in the frontal eye field, supplementary eye field, and the premotor ear–eye field, but no microstimulation studies concerning area 9 are currently available in the literature. In the present study, to fill up this gap, electrical microstimulation was applied to area 9 in two macaque monkeys using long-train pulses of 500–700–800 and 1,000 ms, during two different experimental conditions: a spontaneous condition, while the animals were not actively fixating on a visual target, and during a visual fixation task. In these experiments, we identified backward ear movements, goal-directed eye movements, and the development of head forces. Kinematic parameters for ear and eye movements overlapped in the spontaneous condition, but they were different during the visual fixation task. In this condition, ear and eye kinematics have an opposite behavior: movement amplitude, duration, and maximal and mean velocities increase during a visual fixation task for the ear, while they decrease for the eye. Therefore, a top-down visual attention engagement could modify the kinematic parameters for these two effectors. Stimulation with the longest train durations, i.e., 800/1,000 ms, evokes not only the highest eye amplitude, but also a significant development of head forces. In this research article, we propose a new vision of the frontal oculomotor fields, speculating a role for area 9 in the control of goal-directed orienting behaviors and gaze shift control.

Keywords Area 9 · Microstimulation · Ear–eye movements · Orienting movements · Monkeys

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Introduction

> *There is no reason to suppose that a part of the brain is excitable and another not. The question is how the stimulation manifests itself*, Ferrier (1876).

In his book *The Functions of the Brain*, David Ferrier discussed the phenomena of "electrical irritation of the cerebral cortex", comparing different animal models, from monkeys to fish (Ferrier 1876). He described a frontal region in monkeys, dogs, and jackals, which he numbered "12", as follows:

> Situated on the posterior half of the superior and middle frontal convolutions. The eyes open widely, the pupils dilate, and head and eyes turn toward the opposite side.

In humans, orienting movements are carried out by the eyes, head, and/or body operating alone or in various combinations depending on the behavioral situation. However, in non-human primates, such as macaque monkeys, head-orienting movements and, more generally, gaze shift are accompanied by ear-orienting movements, which

allow the shifting of auditory attention toward a sound of interest (Bon and Lucchetti 1994, 2006; Lanzilotto et al. 2013b; Lucchetti et al. 2008; Yin 2013). Although many neural circuits may participate in orienting processes, the frontal cortical regions, which in monkeys contribute prominently to this phenomenon, are the rostral area F7 or supplementary eye field (SEF), area 8A or frontal eye field (FEF) (Tehovnik et al. 2000), and area 8B or the premotor ear–eye field (Lanzilotto et al. 2013b).

Area 8B, a transitional area between the rostral area 6 and area 9, has recently been proposed as a new frontal field, the premotor ear–eye field (PEEF) (Bon et al. 2009; Lanzilotto et al. 2013b; Lucchetti et al. 2008). This field has an important role in controlling ear and eye movements with the purpose of detecting complex auditory stimuli in the environment (Bon and Lucchetti 2006; Lucchetti et al. 2008). Auditory properties were also found in the neighboring prearcuate and peri-principalis areas, such as areas 8A, 46, and 9 (Azuma and Suzuki 1984; Fuster et al. 2000; Plakke et al. 2013). This phenomenon could be a consequence of the connections of these areas with the caudal auditory cortex, through the dorsal auditory stream, which is thought to have a role in spatial sound localization (Rauschecker and Romanski 2011; Romanski et al. 1999a, b). Thus, this set of prefrontal areas could have an important role in the transformation of auditory stimuli in ear/eye motor commands to detect auditory stimuli in the space.

The same prefrontal areas that receive auditory information also receive visual information from visual areas and in particular from the pre-occipital cortex, parietal cortex, and temporal cortex (Chapman et al. 2012; Yeterian and Pandya 2010; Yeterian et al. 2012). In fact, experimental evidence—involving area 9—shows the presence of neurons that discharged for both auditory and visual stimuli and play an important role in cross-modal and cross-temporal association, depending on the goal of the action (Fuster et al. 2000). Interestingly, the injection of the rabies virus into the ocular lateral rectus muscle of the macaque monkey showed the presence of labeled neurons in areas 9, 8B, 46, as well as in the frontal eye field (FEF), supplementary eye field (SEF), and pre-supplementary motor area (pre-SMA) (Moschovakis et al. 2004).

Moreover, further experimental results have revealed the involvement of area 9 and other prefrontal areas in working memory. The prefrontal cortex, and especially the dorsolateral prefrontal cortex, has been shown to participate in spatial information processes. This has been demonstrated by lesion studies in which monkeys with bilateral lesions in the dorsolateral prefrontal cortex exhibited severe impairments in the performance of a delayed-response task and a spatial delayed alternation task (Funahashi 2013; Fuster 2008). Considering this evidence, it is reasonable to conclude that area 9 and, more widely, other dorsal prefrontal areas could have an important role in eye and ear motor control. In accordance with Funahashi (2013), we believe that the visual space is represented in the dorsal prefrontal cortex, and can be detected by visually guided movements, particularly through manual and ocular movements. This point of view is also supported by experimental evidence showing that a temporary dysfunction caused by a local injection of muscimol into areas 9 and 46 in monkeys is impairment of the visuospatial working memory; moreover, a clear memory map representation was found in this region (Sawaguchi and Iba 2001).

As with visual field representation, we believe there is also a representation of the surrounding auditory space in the same prefrontal regions, which can be detected by auditory-guided movements, particularly through ear and eye movements.

At this point, some questions arise spontaneously: what is the role of the gaze shift controlled by the dorsolateral prefrontal cortex? Is the ear motor control limited to PEEF, or does it involve, in a different way, other dorsolateral areas? Is the eye movement limited to FEF and SEF or is it more widely controlled by the dorsolateral prefrontal cortex? To answer these questions, we designed the following experiment. We stimulated a large part of the dorsolateral frontal cortex, including area 9, area 8B, and rostral F7 in two macaque monkeys. Given the complexity of the findings obtained, and the necessity to clearly argue each single result, we have presented data on area 9 in this paper.

We microstimulated area 9 by long-train intracortical microstimulation (LT-ICMS), during two different experimental conditions: a spontaneous condition, while the animals were not actively fixating on a visual target, and during a visual fixation task. Herein, we describe backward ear movements, goal-directed eye movements, and head forces development. We also show the kinematic properties for these effectors, advancing a possible hypothesis that area 9 is involved in regulating goal-directed orienting behaviors and gaze shift control.

Materials and methods

Two adult female macaque monkeys (*Macaca fascicularis*) (3–4 kg, 4–5 years) were used for these experiments. All phases of the experimental procedure were approved by the local Ethics Committee and we followed the standards established by the European Community and Italian law (D.L. 116/92). Finally, the project was approved by the Italian National Superior Institute of Health and received authorization from the Italian National Ministry of Health.

Behavioral methods

The monkeys were preliminary trained through an apparatus mounted on the monkey's home cage. Each monkey learned to press a bar to illuminate a bicolored (red/green), light-emitting diode (LED, SIEMENS LS110). The LED, with a diameter of 0.05°, was placed in front of the monkey. After a random time period (500–5,000 ms), the LED turned from red to green for a fixed period of time (500 ms). Since the animal had to release the bar during the green period to receive a liquid reward, the task required fixation on the red LED. After the monkey learned to perform the fixation task in the cage, it was taught to sit in a primate chair inside a Faraday's cage and to perform the same task in this new situation. When its performance reached a very good level (80–90 % of correct responses), it was prepared for the eye position measurement and painless head restraint (see "Surgical methods" section). Then, after 1 week, the monkey was trained to complete a visual fixation task (VFT) with a restricted head.

The monkey sat in a primate chair in front of a panel at the distance of 114 cm, on which 49 bicolored light-emitting diodes (LED) with a diameter of 0.05° were located. The monkey's head was painlessly restricted by MUPRO (Bon et al. 2002) a homemade multipurpose neck robot, designed to record both the isometric forces exerted at head level and the head rotations in the horizontal plane in the behaving monkey.

The monkey performed the VFT in a darkened Faraday's cage, and a trial began with the ignition of a central red LED (red period). The monkey was required to fixate on this target within an electronic window ranging from 3° to 8°. After a varying red period time of 2,000–2,500 ms, the LED turned yellow for a period of 500 ms (yellow period). If the monkey's eye moved out of the window, the trial was stopped, and it received neither a reward nor punishment. After the yellow period, the animal received some drops of fruit juice or water as a reward. The red stimulus, representing an instructional pre-cue, required the monkey to maintain the fixation and wait; the yellow stimulus, representing an instructional cue, required it to maintain the fixation and prepare to receive the reward. A 2,000-ms intertrial period followed each trial.

The visual stimuli were presented by homemade software running on a personal computer. An acoustic cue, with an intensity of 40–50 decibels (dB), was switched on at the beginning of each session of the trial and switched off at the end, thus signaling to the monkey the beginning and the end of the working period.

Surgical methods

Using an aseptic technique and under general anesthesia (Zoletil 10 mg/kg i.m.), a stainless steel cylinder was attached to the animals' skull with three screws, using stereotaxic coordinates and cemented in place to permit a painless fixation of the head. A scleral search coil was implanted subconjunctivally for eye movement detection (Judge et al. 1980).

After the training phase previously described, the monkeys underwent sterile surgery to record the chamber implant over one hemisphere using stereotaxic coordinates. The inner diameter of the recording chamber was 19 mm and it was vertically oriented to allow a perpendicular approach to the region of interest. During each experimental session, two stainless steel wires were inserted into the neck muscles to monitor the electromyogram (EMG). After each surgical intervention, treatment with antibiotics, cortisone, and analgesics were administered for up to one week.

Physiological methods

After the monkey had achieved about 90–95 % of correct trials in VFT, the experimental sessions began. Each monkey was placed in a primate chair with their head restricted by MUPRO. Quartz-platinum/tungsten microelectrodes were inserted through the dura using a Microelectrode Manipulator System (5-Channel Mini Matrix Thomas Recording). The unit activity was pre-amplified (Preamplifier DPA-4), amplified and filtered (5-channel Main Amplifier/Filter System MAF-05) to eliminate artifacts from 5 and 75 kHz. Amplified unit activity was monitored using an oscilloscope and was also audio-monitored.

The electrodes were advanced through the cortex for the entire depth of the cortex itself. Once the beginning and the end of the cortex were established, we proceeded to the microstimulation of at most two sites in the same column: one in the deep layers and the other in the superficial layers, with a distance between them of about 1,000 μm. We have not stimulated two sites in all penetrations, because due to the long trains and high current intensities used, the electrode impedance changed considerably from the beginning of the experimental session (0.5–1.0 MΩ). Only when the electrodes' properties were almost constant from the beginning to the end of the experimental session, we stimulated two sites in the same penetration. This procedure was used to achieve the most empirical experimental approach.

To identify evoked movements at each cortical site studied, stimulation was applied by an S88 stimulator and

two PSIU6 stimulus isolation units (Grass). Long trains of 500, 700, 800, and 1,000 ms of duration and 200 μs bipolar pulses were delivered at 300 Hz. Each stimulation pulse was obtained using a biphasic current where a negative phase was followed by a positive phase to minimize damage that could occur during long-duration stimulation (Graziano et al. 2002a, b). The current was measured by the voltage drop across a 1-kΩ resistor in series with return of the stimulus isolation units. At each cortical site, the stimulating current was injected starting from 20 μA and increased gradually in 10 μA steps until it reached 50 μA, and then gradually until 150 μA if movements were not evoked before 50 μA. The threshold, i.e., the current at which the movement was evoked 50 % of the times at 500 ms of train duration, was determined by two experimenters and then confirmed by offline analysis. If no movements were elicited at 150 μA, the site was defined as nonresponsive. The mean current threshold, described in the "General observation" section, was calculated considering the lowest current value even for the electrode penetrations with two microstimulated sites. Stimulation was applied in the spontaneous condition, i.e., outside the task performance, and during the execution of the visual fixation task (for detail see "Behavioral methods" section) (Fig. 1a–c). In this latter condition, the fixed train duration of 500 ms was used during the red period of the visual fixation task. Stimulation during a VFT was performed to verify if the kinematic parameters of the evoked movements changed when the visual attention was engaged.

The monkey's behavior was monitored by an infrared video camera placed in front/above the animal. The experimenters remained outside the Faraday's cage to provide quieter conditions for the animals.

Recording of evoked movements and data analysis

Eye movements were recorded by the search coil technique using the phase detection method (Remmel 1984). A coil was chronically implanted subconjunctivally, as previous described in the surgical methods. The same technique was used for the detection of ear movements (Bon and Lucchetti 1994): a coil was placed on the ear on a daily basis

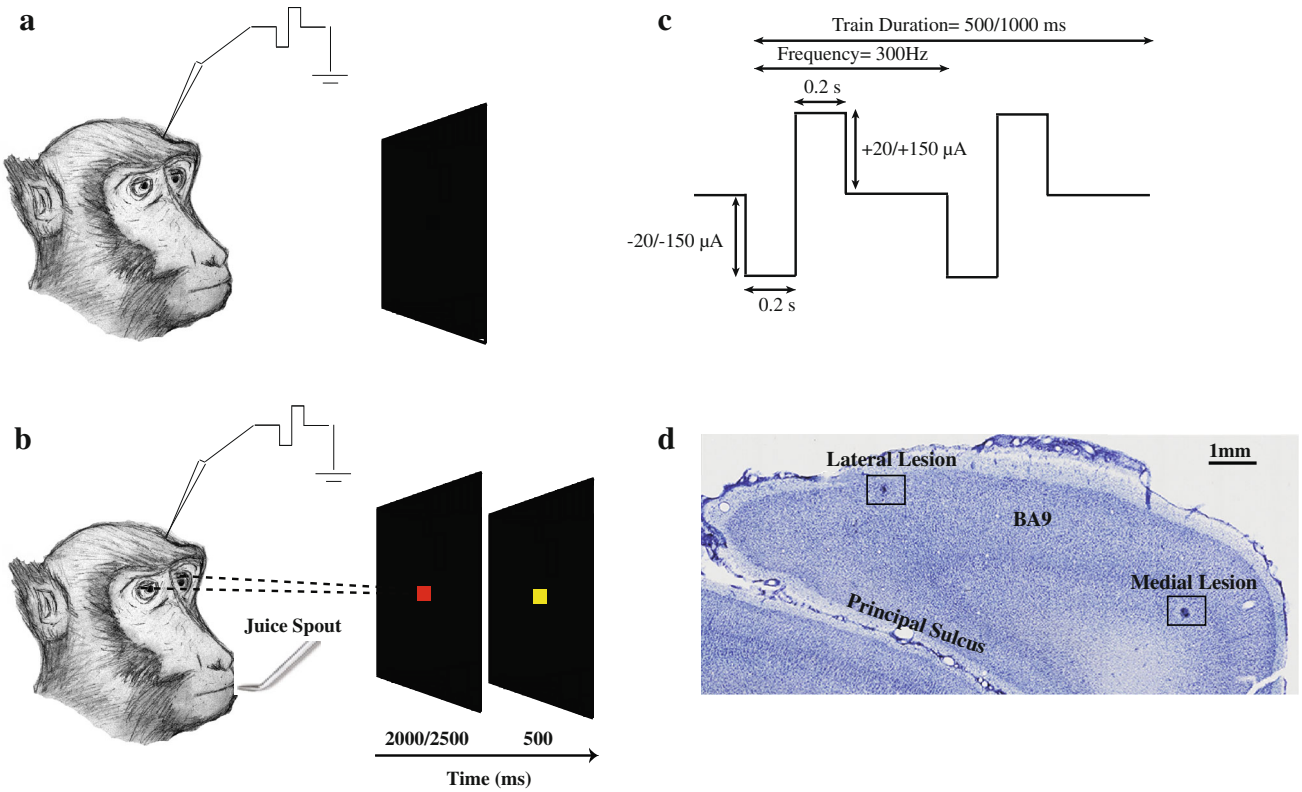

Fig. 1 Experimental design and histological control. **a** Long-train intracortical microstimualtion (LT-ICMS) during spontaneous condition, i.e., outside the execution of the visual fixation task. The electrical stimulation is randomly generated with train duration of 500, 700, 800 and 1,000 ms. **b** Stimulation during the execution of a visual fixation task. The electrical stimulation with fixed train duration of 500 ms is generated during the *red period* while the monkey is fixating. **c** Schematic representation of the microstimulation parameters. Bipolar pulses, characterized by a negative wave followed by a positive one, were generated. Pulse duration is 200 μs; frequency 300 Hz; current intensity ranged between 20 and 150 μA. **d** Photomicrograph showing the location of the electrolytic lesions in area 9. Medial lesion is at ~2 mm from inter-hemispheric line, while lateral lesion is at ~8 mm from inter-hemispheric line

by the same operator to minimize the variability in terms of positioning. This system allowed us to define the beginning and the end of the ear movement. A magnetic field was generated around the monkey's head and a current proportional to the sin θ (movement amplitude) was induced to both coils. Our search coil system defines a movement in two dimensions (x–y). As for eye movements, positive values on the y axis represent upper eye positions, while negative values represent lower eye positions. Positive values on the x axis represent eye rightmost positions, while negative values represent the leftmost eye position. As for ear movements, positive values on the y axis represent upper ear positions, while negative values represent lower ear positions. Positive values on the x axis represent rostral ear positions, while negative values represent caudal ear positions.

Finally, forces and/or rotation applied by the monkey's head in the horizontal plane were detected by MUPRO (Bon et al. 2002), a homemade multipurpose neck robot. MUPRO consists of a mechanical device, comprising a cardan joint, a potentiometer, an electromagnetic brake, and four flexion load cells (which identify the isometric forces applied in four direction of the space, i.e., forward, backward, right, and left), plus an oleo dynamic system that allows head rotation in the horizontal plane between ±20°. These components are assembled on a column bolted to the primate's chair. An electrical device provides DC power for the potentiometer and the brake.

Eye and ear movements, LED levels, unit activity, auditory marker, head forces, rotation signals, and the stimulation marker were sampled at 1 kHz and stored by SuperScope II (GWI) software for data acquisition. Movements were recorded continuously during the experimental session and kinematic features were analyzed offline using custom MATLAB programs (The MathWorks).

In both animals, the microstimulation trials were performed during two different experimental conditions: a spontaneous condition, i.e., outside a task, versus during a visual fixation task. The starting positions for both the eye and ear effectors were rarely the same except for the eye during the visual fixation task. This was an optimal situation to test the relationship between the starting and final position and the relationship between the starting position and amplitude of the evoked movement.

To avoid interference between the spontaneous and evoked movements, and for analysis purposes, displacement of $\geq 1°$ in the x and/or y components were considered. The analysis sought to define the classes of movements and their topography across the cortical surface. We synchronized the stimulation markers with x and y components of the ear and eye movement and head forces signals for the duration of stimulation period. We plotted the x and y components bi-dimensionally. For each stimulated site, the kinematic variables were obtained by averaging the values obtained in at least five microstimulation trials.

As for evoked eye movements, they were included for analysis if the peak eye velocity was higher than 30°/s, while the ear movements were included if the peak velocity was higher than 20°/s. The maximal velocity was determined for each evoked movement. Eye onset and offset were then defined as the last points on either side of the peak velocity before which the tangential velocity fell below 30°/s (Stanford et al. 1996). The onset and offset of the ear's movements were calculated using the same method considering a tangential velocity of 20°/s. This was done because, in general terms, the ear movement is slower than eye movement (see "Results") and because it better represented the onset and offset of the ear movements, studied trial by trial. The time range between stimulation and movement onsets of the fastest movement was defined as movement latency (in ms). The total time spent during movement was defined as the movement duration (in ms). Moreover, for each eye and ear movement, we determined the amplitude of the movement (°), the maximal velocity (°/s), and the mean velocity (°/s).

Finally, even though no head rotation movements were observed during the stimulation period, to establish if there was a relationship between current intensity and/or train duration and head forces detected, we constructed averaged histograms that calculated the maximal and averaged forces applied by the monkey's head in the horizontal plane during the stimulation period. Data are presented as mean ± standard error of the mean (SEM) of n determinations. To analyze the differences in current threshold between the ear and eye in the same monkey, a t Student test was performed. Moreover, we performed the Wilcoxon–Mann–Whitney test to observe for differences between the monkeys.

In addition, to analyze differences in kinematic means between spontaneous and VFT conditions, the Wilcoxon–Mann–Whitney test (WMW test) was performed. The same test was used to identify significant differences between different train durations. It was applied for latency, movement amplitude, duration, maximal, and mean velocities.

A Pearson correlation (r) was used to assess the relationship between kinematic variables, in particular between maximal velocity and movement amplitude/movement duration/mean velocity/latency. Moreover, a non-linear correlation (Kendall correlation) was also used to compare, and eventually to better estimate, the fit between kinematic variables. Values of $p < 0.05$ were considered statistically significant.

Histological reconstruction

At the end of the experiments, marking lesions (D.C., 10 μA, 15 s) were made in the stimulated area,

respectively, medially (~2 mm) and laterally (~8 mm) with respect to the inter-hemispheric line. The animals were then perfused through the left ventricle with 0.9 % NaCl physiological saline, followed by 4 % formalin. The brains were removed and stored for 3 days in a 10 % glycerol and 2 % dimethylsulfoxide solution. The brains were stored for 3 further days in 20 % glycerol and 2 % dimethylsulfoxide solution. Later, the brains were frozen in pentane at −80 °C, serially sectioned at 60 µm, mounted on slides, and stained with thionin. Slides were examined under light microscopy to identify the marking lesions (Fig. 1d). Sections presenting the marking lesions were plotted and the maps were reconstructed.

Results

General observations

Short-train intracortical microstimulation (ST-ICMS) evokes short single effector twitches. In this study, we used long-train intracortical microstimulation (LT-ICMS), duration range 500/1,000 ms and current intensity until 150 µA, in an attempt to evoke complex movements. Altogether, 37 electrode penetrations (18 monkey L; 19 monkey S) were performed in area 9 of the two left hemispheres of the macaque monkeys (Fig. 2a, b).

We distinguished area 9 from the neighboring areas 8B and rostral F7 for both cytoarchitectonic and functional features. In particular, we observed a different representation of the direction of the ear movement and a different location of the end-points. Moreover, there was also a different segregation in the visual field of the end-points regarding the evoked saccades. The results from area 8B and rostral F7 are now under further analysis.

All the microstimulated sites were considered in the analysis. Ear movements, eye movements, and head forces were elicited by stimulation in both monkeys. The mean current threshold to evoke movements was different for the ear and eye in both monkeys. In monkey L, ear movements were evoked with a current threshold of 52.77 ± 17.42 µA while eye movements were evoked with a current threshold of 64.71 ± 20.04 µA. The eye current threshold was significantly higher by 11.93 ± 2.61 µA than the ear

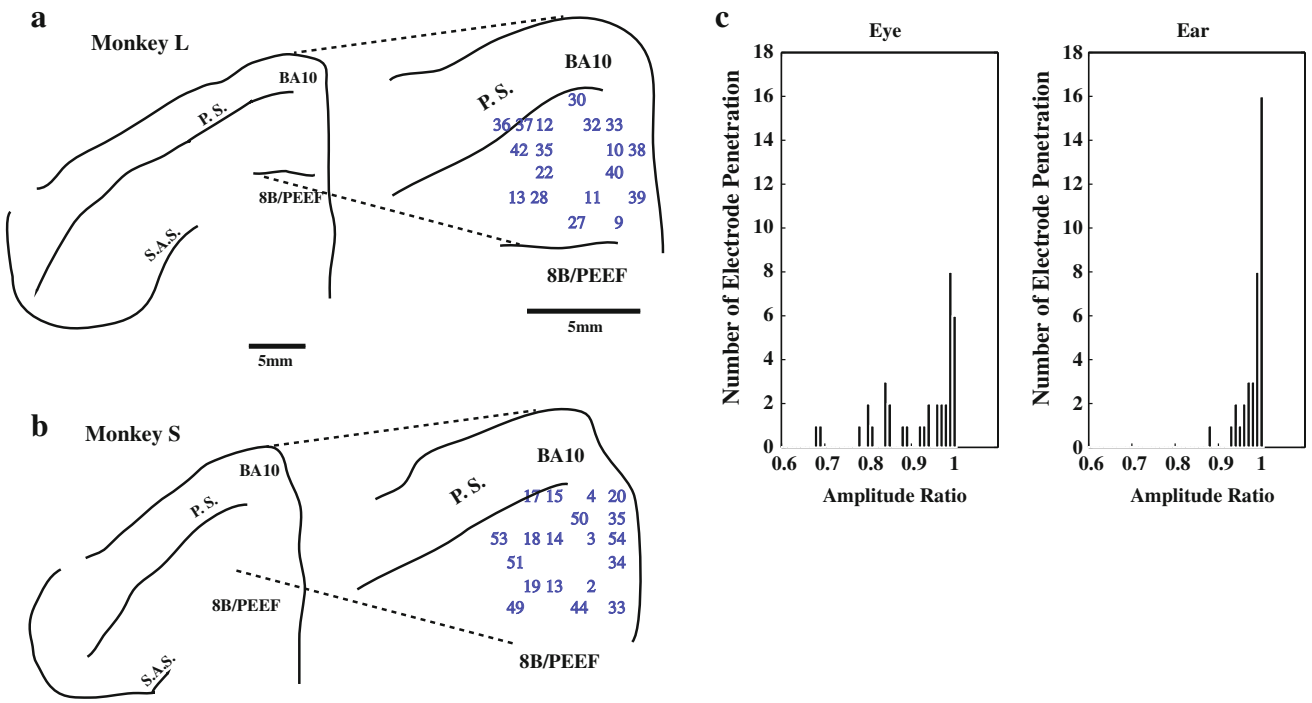

Fig. 2 Maps of the electrode penetrations and amplitude ratio histograms. **a** Maps of monkey L electrode penetrations. *Each number* represents the electrode penetration number. For detail regarding current intensity thresholds, the depth of the microstimulated sites from the beginning of the cortex, see Table 1A. *SAS* superior arcuate sulcus, *PS* principal sulcus, *BA 10* Broadmann area 10, *PEEF* premotor ear–eye field. **b** Maps of monkey S electrode penetrations. For details regarding current intensity thresholds, the depth of the microstimulated sites from the beginning of the cortex, see Table 1B. **c** Amplitude ratio is quantified by dividing the difference between maximal evoked movement amplitude and minimal evoked movement amplitude by the maximal evoked movement amplitude. This ratio varies between 0 and 1. It is 0 if the amplitude movement does not change with start position, yielding the same amplitude for maximal and minimal evoked movement amplitude. Conversely, it takes up 1 if the minimal amplitude becomes 0

Table 1 **A** The current intensity thresholds for eye and ear evoked movements in monkey L. **B** The same properties for monkey S

Electrode penetration	Depth from the beginning of the cortex (mm)	Current threshold eye (μA)	Current threshold ear (μA)
A			
9	0.493	70	70
10	1.705	50	90
	0.330	70	70
11	0.190	50	50
13	1.750	/	70
	0.725	/	70
21	0.955	90	70
22	1.988	/	70
	0.988	90	90
27	0.815	50	50
28	1.330	70	40
30	1.263	110	70
32	0.380	70	50
33	2.000	40	30
35	0.970	90	30
36	1.288	70	50
	0.588	/	90
37	0.970	50	50
38	1.058	50	30
39	0.928	50	30
40	1.515	50	50
42	1.750	50	50
	0.250	50	50
B			
2	0.590	70	70
3	0.518	50	40
4	2.190	40	70
13	1.483	50	20
	0.233	50	50
14	1.170	70	50
	0.268	90	90
15	1.365	30	30
17	0.940	70	50
18	1.333	50	30
19	1.308	70	70
	0.398	/	/
20	0.660	110	50
33	1.383	50	70
	0.488	50	50
34	0.613	90	80
35	0.140	90	70
44	0.875	70	50
49	0.430	50	50
50	0.295	90	70
51	0.573	30	30
53	1.375	90	70
54	1.300	70	50

Moreover, the depth from the beginning of the cortex is presented for each stimulated site. Each number represents the electrode penetration number. Values in italics represent electrode penetrations where twice stimulations were done

[$t(16) = 2.2069$, $p = 0.0423$]. In monkey S, ear movements were evoked with a current threshold of 53.68 ± 17.70 μA while eye movements were evoked with a current threshold of 65.26 ± 22.20 μA. The current threshold of the eye movements was significantly higher by 11.58 ± 4.49 μA than the ear [$t(18) = 3.45$, $p = 0.003$]. No significant differences were found between monkeys for the current threshold (ear: WMW test, $z = -0.1318$, $p = 0.895$; eye: $z = -0.2698$, $p = 0.7873$). We stimulated some sites twice, one site in the deep layers and the other in the superficial layers, and as was predictable in some cases, we needed higher current intensities to evoke a movement. Sometimes, we were not able to evoke movements in the superficial layers until the current intensity reached 150 μA. For details regarding the anatomical location of the electrode penetration, the sites' depth from the beginning of the cortex, and the current thresholds see Table 1(A, B).

The amplitude of the evoked ear and eye movements was strictly dependent on the starting position. For example, if the monkey's ear position was rostral at the time of stimulation, we obtained larger backward movements. In contrast, if the monkey's ear position was caudal at the time of stimulation, we obtained smaller backward movements. Thus, the action of microstimulation depended on the initial state of the ear. Similarly, if the monkey maintained its eyes around or at the end-point, we obtained smaller evoked saccades. Again, consistent with the results from the ear movements, if the monkey's eye was located away from the end-point, we obtained a larger evoked saccade. Figure 2 presents the histograms of an index computed for the ear and eye. The index quantifies the change of amplitude by dividing the difference between maximal movement amplitude and minimal movement amplitude, by the maximal movement amplitude. This ratio varies between 0 and 1. It would have been 0 if the movement amplitude did not change with the starting position, yielding the same values for maximal and minimal movement amplitude. Conversely, the ratio would have been 1 in cases where the minimal amplitude became 0. We observed that for both ear and eye movements, the

amplitude ratios were distributed for the greatest number of sites toward 1, highlighting the dependence on the starting position (Fig. 2c).

Overall, it was possible to evoke backward ear movements and goal-directed eye movements in all sites approximately in the central part of the visual field. Only one site was declared unresponsive for eye movements.

Evoked ear movements

Stimulation elicited ear movements in both animals. All evoked ear movements were classified as backward movements (Fig. 3a). In all penetration sites, it was possible to evoke principally contralateral ear movements and in some cases bilateral ear movements. We defined the direction of movements during and after the experiment by reconstruction of the horizontal and vertical components of ear movement. The direction of movement was accepted when two researchers agreed on it, and confirmed by the offline analysis. Since the animal moved its ear spontaneously, it was difficult to stimulate repetitively with the ear in exactly the same spatial position. Otherwise, it was in an optimal condition for testing if the movement amplitude was dependent on the starting position. All evoked ear movements were plotted for each electrode penetration for both the monkeys (Fig. 4a).

In order to assess the effect of the long-train stimulation on the evoked ear movements, we studied the relationship

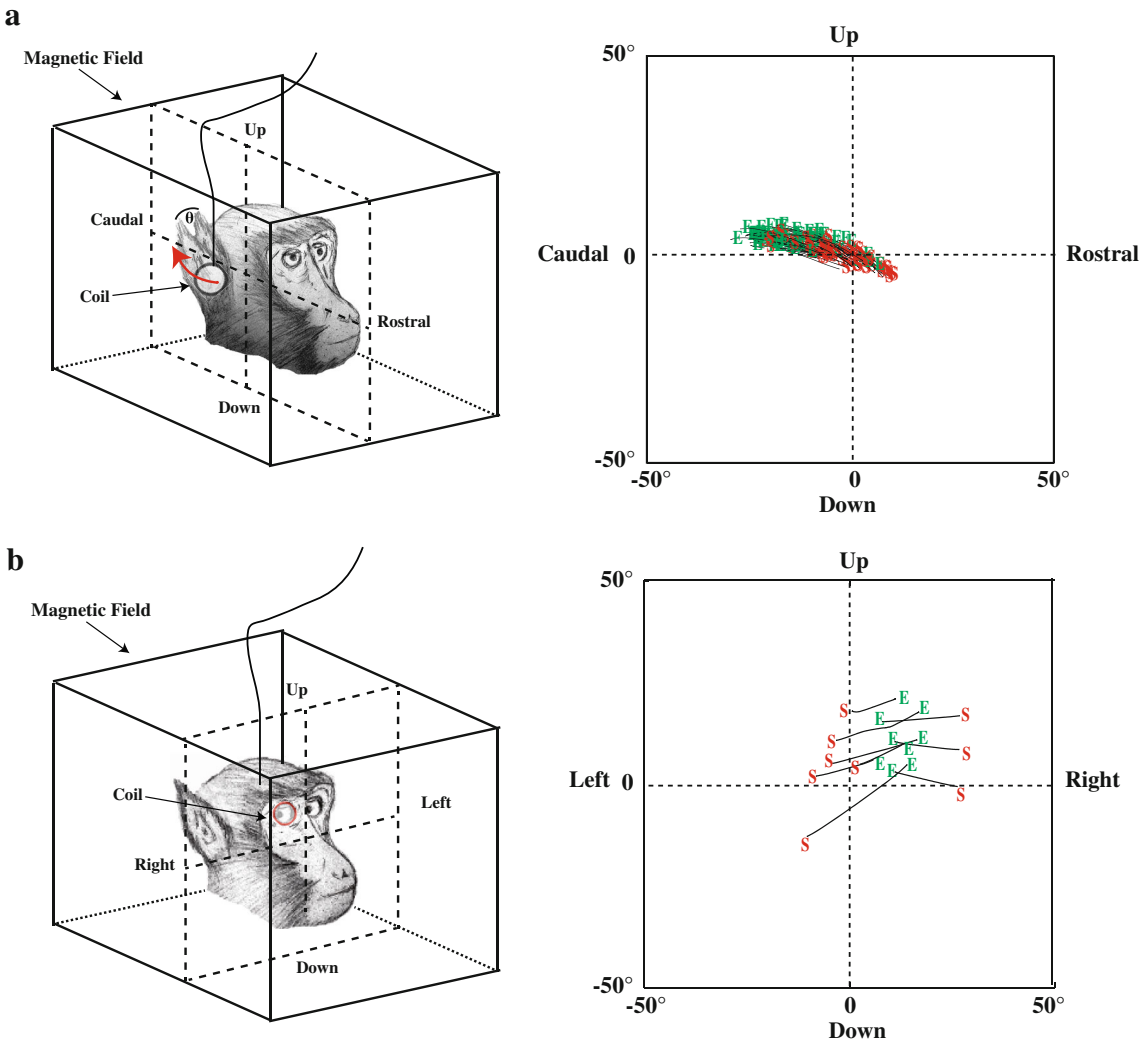

Fig. 3 Examples of evoked ear and eye movements. **a** *Left* the draft schematizes a magnetic field generated around the monkey's head. The coil is placed on the monkey's ear and detects the movement in its *x* and *y* components. θ, angle of movement amplitude. *Right* bidimensional plot of evoked backward ear movements. **b** *Left* the draft schematizes a magnetic field generated around the monkey's head. The coil is placed under the monkey's eye conjunctiva and detects the movement in its *x* and *y* components. *Right* bidimensional plot of evoked eye movements. *S* represents starting positions, *E* represents final positions. *Each line* represents the trajectory of the evoked movement

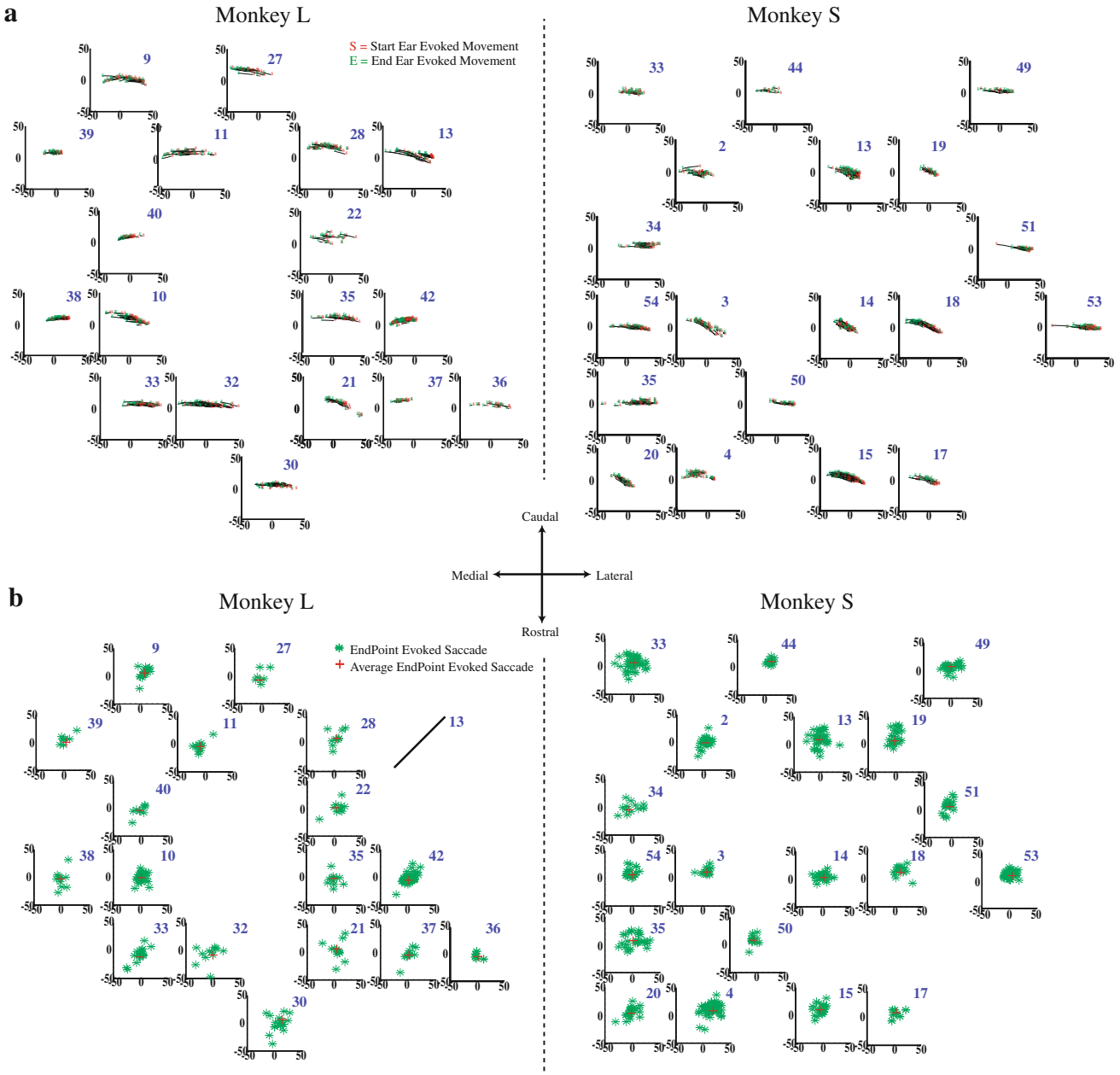

Fig. 4 Evoked ear and eye movements for each electrode penetration for both monkeys. **a** *Left* plots show evoked ear movements for each electrode penetration in monkey L. *S* represents starting positions, *E* represents final positions. *Each line* represents the trajectory of the evoked movement. In all electrode penetration it is possible to observe backward ear movements with caudal end-points. *Right* plots show evoked ear movements of each electrode penetration in monkey S. **b** *Left* plots represent the evoked eye end-points for each electrode penetration in monkey L. *Green point* represents each eye end-point. *Red cross* represents the average of the end-points. *Right* plots represent the evoked eye end-points for each electrode penetration in monkey S. The intermingled empty regions were not stimulated for troubles due to the artifacts. In those cases there was blood on the electrodes tip. *Each number* represents the electrode penetration's number. "*Solidus*" *bar* in 13 electrode penetration indicates no evoked eye movements

between the kinematic parameters for both monkeys, as well as for different train durations. We observed that the latency of the ear evoked movements ranged between 100 and 200 ms. More interestingly, the Wilcoxon–Mann–Whitney test that we performed on the movement amplitude, revealed that amplitude is greater during VFT than spontaneous conditions: VFT condition versus 500 ms (WMW, $p = 1.36e{-}07$), versus 700 ms (WMW, $p = 0.0058$), versus 800 ms (WMW, $p = 0.00021$) and versus 1,000 ms (WMW, $p = 6.36e{-}12$). The same test performed on movement duration shows that the evoked movement lasts longer during VFT than all other conditions: VFT condition versus 500 ms (WMW, $p = 0.0014$), versus 700 ms (WMW, $p = 0.012$), versus 800 ms

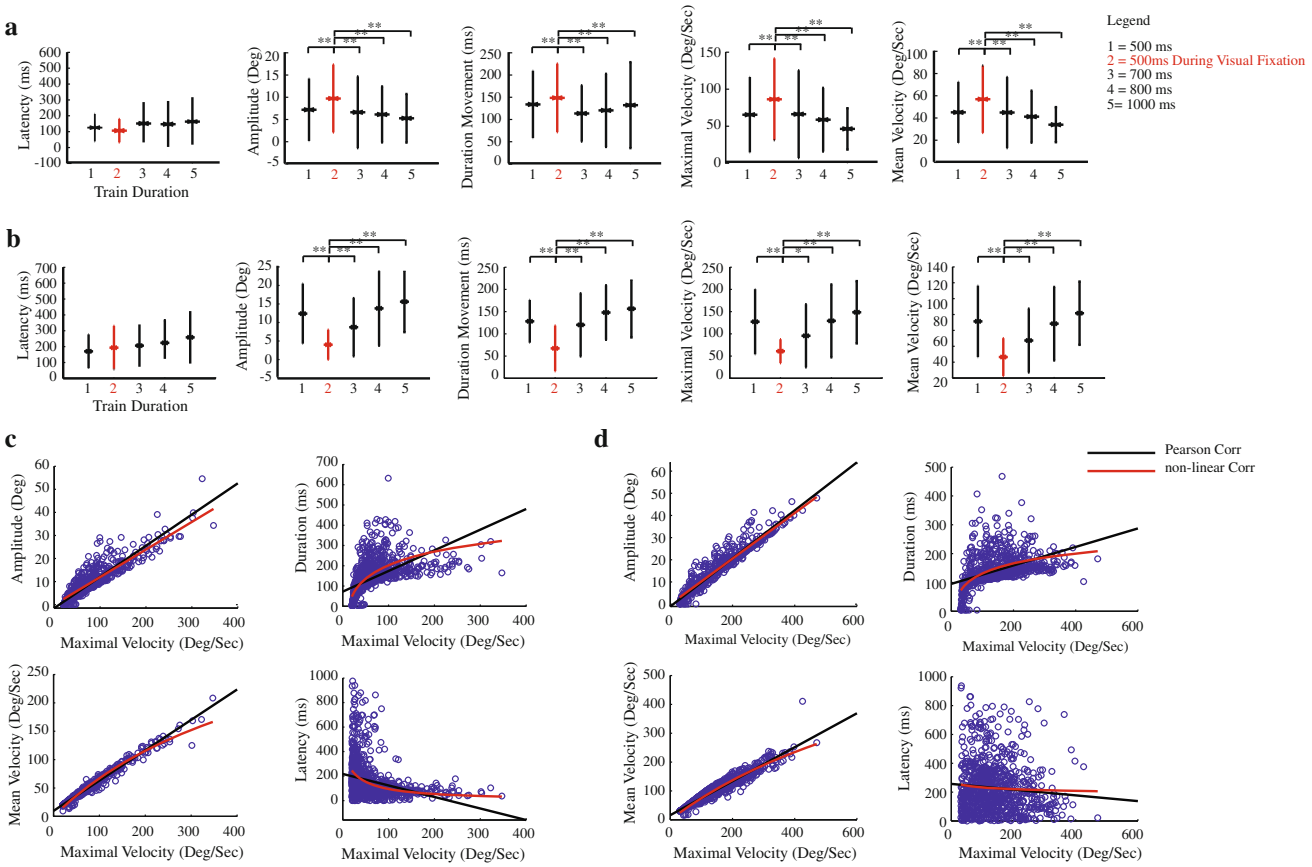

Fig. 5 Kinematic study for ear and eye evoked movements. **a** Plots put in relationship kinematic parameters of ear movements with different train durations (*numbers* on *x* axis), during visual fixation task (*red*), and spontaneous conditions (*black*). **b** Plots put in relationship kinematic parameters of eye movements with different train durations (*numbers* on *x* axis), during visual fixation task (*red*), and spontaneous conditions (*black*). Latency, amplitude movement, duration movement, maximal and mean velocities are studied. Data are mean ± SEM of *n* determinations; $*p < 0.05$; $**p < 0.01$, different from other. **c** Correlation studies for the evoked ear movements. *Black line* represents Pearson correlation, while *red line* represents non-linear regression (Kendall). Maximal velocity (*x* axis) is compared with remaining kinematic parameters. **d** Correlation studies for the evoked eye movements. *Black line* represents Pearson correlation, while *red line* represents non-linear regression (Kendall). Maximal velocity (*x* axis) is compared with remaining kinematic parameters

(WMW, $p = 0.0043$), versus 1,000 ms (WMW, $p = 0.00069$). As for maximal velocity, the WMW test also reveals a faster movement in the VFT condition than the spontaneous conditions: VFT versus 500 ms (WMW, $p = 1.85\text{e}{-10}$), versus 700 ms (WMW, $p = 0.0081$), versus 800 ms (WMW, $p = 5.38\text{e}{-05}$) and versus 1,000 ms (WMW, $p = 1.69\text{e}{-17}$). Finally, the same is observable for mean velocity: VFT versus 500 ms (WMW, $p = 1.79\text{e}{-10}$), versus 700 ms (WMW, $p = 0.0071$), versus 800 ms (WMW, $p = 3.77\text{e}{-05}$) and versus 1,000 ms (WMW, $p = 1.15\text{e}{-18}$) (Fig. 5a).

The Pearson correlation analysis was used to describe the relationship between maximal velocity and the other kinematic variables. The correlation between maximal velocity and movement amplitude was positive (Pearson, $r = 0.93$, $p < 0.001$). Even non-linear regression showed the same result (Kendall, $r1 = 0.83$, $p < 0.001$). The correlation was also positive between maximal velocity and movement duration (Pearson, $r = 0.56$, $p = 1.89\text{e}{-85}$), even though it is possible to observe a plateau at around 200 ms. In fact, a non-linear regression revealed a logarithmic trend (Kendall, $r1 = 0.67$, $p = 9.6808\text{e}{-228}$). The Pearson correlation between the maximal velocity and mean velocity was also positive (Pearson, $r = 0.99$, $p < 0.001$). The same was confirmed by non-linear regression (Kendall, $r1 = 0.94$, $p < 0.001$). On the contrary, the correlation between the maximal velocity and latency was negative (Pearson, $r = -0.28$, $p = 6.75\text{e}{-20}$). The non-linear regression demonstrated the best fit, showing a hyperbolic trend (Kendall, $r1 = -0.3$, $p = 8.9547\text{e}{-47}$) (Fig. 5c).

Evoked eye movements

Stimulation elicited eye movements in both animals. All evoked eye movements were classified as goal-directed

Table 2 **A** Table represents the eye coordinates of the average end-points for monkey L. **B** Table represents the eye coordinates of the average end-points for monkey S

Penetration site	*X*	*X*	*X* SD	*Y* SD
A				
9	6.14	6.23	6.48	11.97
27	−4.32	−6.9	5.98	4.96
39	4.41	0.95	10.87	17.91
11	−8.91	−3.93	11.37	11.74
28	4.8	10.08	10.87	22.26
40	−2.55	−3.02	10.02	10.87
22	2.23	1.5	14.32	13.59
38	−0.86	−2.57	8.79	8.91
10	0.83	−0.38	5.57	11.12
35	−0.48	−1.6	6.29	12.16
42	1.33	−4.91	10.18	7.56
33	−2.42	−11.08	11.84	11.39
32	−0.52	−7.71	11.49	17.93
21	3.81	6.16	13.41	14.83
37	1.55	−5.08	10.71	19.21
36	1.82	−3.96	7.11	6.65
30	14.01	6.91	8.72	9.26
B				
33	2.57	7.72	9.72	8.76
44	11.97	11.03	3.67	6.04
49	−0.22	7.29	10.6	6.22
2	2.47	−0.69	6.25	8.37
13	−4.19	8.98	9.14	12.39
19	−2.28	6.88	7.79	10.55
34	−6.07	−4.18	15.34	8.14
51	4.52	5.57	7.58	15.28
54	−0.64	6.84	8.35	6.82
3	7.21	11.81	4.8	4.96
14	1.19	3.1	9.46	3.88
18	9.77	12.68	10.3	9.33
53	4.26	9.41	8.46	9.35
35	1.27	8.35	12.04	14.39
50	−6.65	8.94	5.96	10.93
20	−1.81	4.01	8.06	10.18
4	17.22	8.54	12.16	13.79
15	−5.85	10.4	8.06	6.95
17	4.39	10.38	10.43	8.9

Each number in the leftmost column represents the electrode penetration number. Coordinates are represented in degrees. Negative numbers in *x* axis indicate ipsilateral end-points. SD represent standard deviation for both *x* and *y* components

saccades (Fig. 3b). We defined the direction of movements during and after the experiment by reconstruction of horizontal and vertical components of eye movements. Since the animal moved the eye itself in the spontaneous condition, it was difficult to stimulate repetitively with the eye exactly in the same spatial position, except during the visual fixation task. The amplitude of the evoked eye movement was strictly dependent on the starting position. All end-points of the evoked goal-directed saccades were plotted for each electrode penetration, for both monkey L and monkey S (Fig. 4b). In monkey L, in 59 % of sites, stimulation elicited goal-directed saccades localized in the contralateral hemifield. In the remaining 41 % of sites, stimulation evoked goal-directed saccades in the ipsilateral hemifield (Table 2A). In monkey S, in 53 % of sites, stimulation elicited goal-directed saccades localized in the contralateral hemifield. In the remaining 47 % of sites, stimulation evoked goal-directed saccades in the ipsilateral hemifield (Table 2B).

In order to assess the effect of the long-train stimulation on the evoked eye movements, for both monkeys we studied the relationship between the kinematic parameters and different train durations. The latency for the evoked movements ranged between 150 and 300 ms. More interestingly, the Wilcoxon–Mann–Whitney test, performed on the movement amplitude, revealed that the amplitude is smaller in the VFT condition than the spontaneous condition: in VFT condition versus 500 ms (WMW, $p = 1.19e{-}11$), versus 700 ms (WMW, $p = 0.015$), versus 800 ms (WMW, $p = 7.056e{-}10$) and versus 1,000 ms (WMW, $p = 3.46e{-}15$); the behavior was opposite to that seen in ear. The same test performed on movement duration shows that the evoked movement is shorter during VFT than all other conditions: VFT condition versus 500 ms (WMW, $p = 3.31e{-}12$), versus 700 ms (WMW, $p = 0.0024$), versus 800 ms (WMW, $p = 4.32e{-}11$), versus 1,000 ms (WMW, $p = 7.86e{-}15$). As for maximal velocity, the WMW test showed a slower movement in the VFT condition than the spontaneous condition: VFT versus 500 ms (WMW, $p = 1.001e{-}09$), versus 700 ms (WMW, $p = 0.011$), versus 800 ms (WMW, $p = 1.05e{-}07$) and versus 1,000 ms (WMW, $p = 3.78e{-}14$). Finally, the same was observable for mean velocity, VFT versus 500 ms (WMW, $p = 4.53e{-}10$), versus 700 ms (WMW, $p = 0.048$), versus 800 ms (WMW, $p = 8.44e{-}08$) and versus 1,000 ms (WMW, $p = 7.77e{-}14$) (Fig. 5b).

The Pearson correlation analysis was used to describe the relationship between maximal velocity and the other kinematic variables. The correlation between maximal velocity and movement amplitude was positive (*Pearson*, $r = 0.96$, $p < 0.001$). Even non-linear regression showed the same result (Kendall, $r1 = 0.87$, $p = 1.3289e{-}310$). The correlation was also positive between the maximal velocity and movement duration (Pearson, $r = 0.45$, $p < 0.001$), even though it is possible to observe a plateau at around 200 ms, such as for ear behavior. In fact, a non-linear regression showed a logarithmic trend (Kendall,

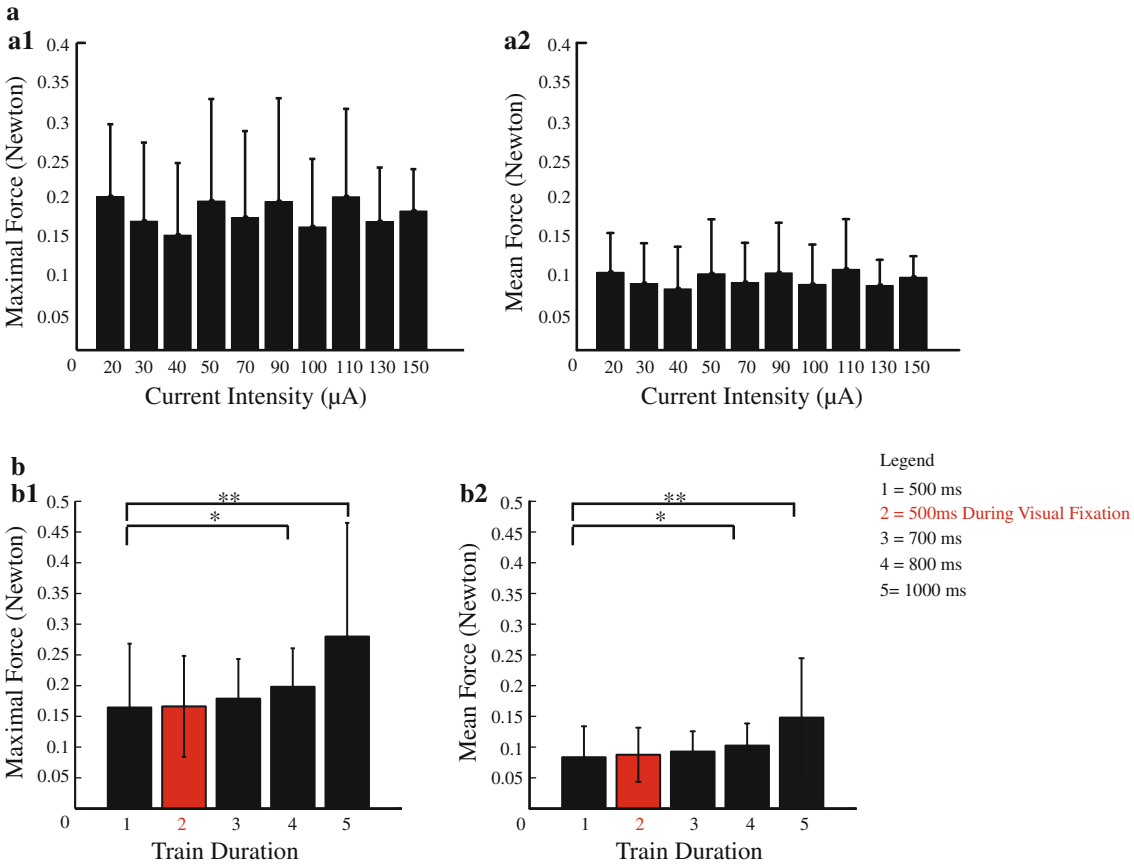

Fig. 6 Histograms showing the relationship between microstimulation parameters and evoked forces development. **a** Histograms do not show any kind of relationship between current intensity (*x* axis) and maximal (*a1*) and mean (*a2*) head forces detected during the stimulation period. The head forces are independent by the current intensity. **b** Histograms show a linear increasing of the maximal (*b1*) and mean (*b2*) head forces development depending by train duration (*x* axis). Significant variations are observable between 500 versus 800 and 1,000 ms train durations. Data are mean ± SEM of n determinations; $*p < 0.05$; $**p < 0.01$, different from other

$r1 = 0.51$, $p = 6.0095\text{e}{-107}$). The Pearson correlation between the maximal velocity and mean velocity was also positive (Pearson, $r = 0.96$, $p < 0.001$). The same was confirmed by non-linear regression (*Kendall*, $r1 = 0.86$, $p = 5.0534\text{e}{-303}$). On the contrary, the correlation between maximal velocity and latency was negative (*Pearson*, $r = -0.0867$, $p = 0.0122$). The non-linear regression revealed a hyperbolic trend (*Kendall*, $r1 = -0.0437$, $p = 0.0589$), such as in the ear movements (Fig. 5d).

The development of evoked head forces

Finally, even though no head rotation movements were observed during the stimulation period, to establish if there was a relationship between current intensity and/or train duration and head forces development, we constructed averaged histograms to calculate the maximal and averaged forces applied by the monkey's head in the horizontal plane during the stimulation period. Data are presented as mean ± SEM of *n* determinations.

First, we built averaged histograms to compare the current intensity and head forces. The Wilcoxon–Mann–Whitney test, performed on all the range of current intensities (20–150 µA), did not show any significant correlation between the current intensity and peak of forces applied by the monkeys' head during the stimulation period (Fig. 6a1, a2). Next, considering the current intensity as an independent variable, we built histograms showing a correlation between the duration of the trains and maximal/mean head forces development. The Wilcoxon–Mann–Whitney test, performed on the forces peak, shows a significant difference between 500 versus 800 ms (WMW, $p = 0.019$) and versus 1,000 ms (WMW, $p = 8.65\text{e}{-06}$) (Fig. 6b1). The same is observable for the mean forces: 500 versus 800 ms (WMW, $p = 0.0201$) and versus 1,000 ms (WMW, $p = 3.69\text{e}{-06}$) (Fig. 6b2).

Discussion

In natural conditions, when a new object appears in the visual field an animal will direct its eyes and head toward it

(orienting movements). On the other hand, the same behavior is observable when an auditory stimulus appears either in or outside of the visual field. To detect an auditory stimulus, non-human primates can move their eyes, either alone or in coordination with the head and ears. Humans, unlike cats or monkeys, cannot usually move their ears even though electrical stimulation of the temporal lobe can evoke ear movements (Yu et al. 2010).

Area 9, and more generally, the dorsal prefrontal cortex is considered to be involved in higher-order cognitive functions related to the monitoring of goal-directed behaviors (Genovesio et al. 2012) as well as working memory and temporal monitoring of the action (Funahashi 2013; Fuster 2008). In the present paper, for the first time, orienting movements have been described by stimulation in area 9, one of the most rostral prefrontal cortical areas. Herein, we show three classes of orienting movements involving three different effectors: backward ear movements, goal-directed eye movements, and head force development. These results are not in contrast with previous notions: the cortical neuronal population cannot organize any motor program without a memory or temporal association. In support of this statement, areas, such as pre-supplementary motor area and supplementary motor area, involved in programming and executing arm movements (Fujii et al. 2002; Matsuzaka and Tanji 1996), show properties also related to working memory and temporal association (Akkal et al. 2004; Lucchetti and Bon 2001; Lucchetti et al. 2005, 2012).

Movement classes and the effect of microstimulation on kinematics

Evoked ear movements

In the present study, we have identified that in all cortical sites backward ear movements are elicited with a goal located caudally with respect to the head. Ear movements in non-human primates have been described in other cortical areas. Backward and forward ear movements were identified by microstimulation and unit activity recordings in the neighboring area 8B, which was recently renamed as a new premotor ear–eye field (PEEF) (Bon and Lucchetti 1994, 2006; Lanzilotto et al. 2013b; Lucchetti et al. 2008; Bon et al. 2009). Moreover, Preuss et al. (1996), showed that ear movements can be evoked in area 8B and even more rostrally in owl monkeys. Several lines of evidence have shown that other regions such as FEF and SEF are involved in the orienting processes and microstimulation, and unit activity recording studies confirm that these areas are related principally to eye but also to ear movements (Bruce and Goldberg 1985; Tehovnik et al. 2000). Long-train intracortical microstimulation in ventral intraparietal areas (Cooke et al. 2003), elicited in 88 % of stimulation sites complex facial movements including ear movement flattening against the head (backward) and rotating downward. The same behavior was visible by microstimulating the medial and dorsal posterior parietal cortex (Thier and Andersen 1998) and caudal part of the temporal and parietal lobe (Ferrier 1876). Moreover, recent findings in humans show that stimulation of a temporal region can evoke ear movements (Yu et al. 2010).

As regards kinematics, we have shown in our experiments that the latency of the evoked ear movements ranged between 100 and 200 ms. This is in accordance with previous findings in PEEF (Bon and Lucchetti 1994). Moreover, movement amplitude, duration, and maximal and mean velocities were significantly greater during VFT than in spontaneous conditions. Probably, during the visual fixation task, a top–down visual attention engagement occurs, causing an inhibition of eye movements and a facilitation of neuronal population assigned to the ear control. In other words, microstimulation has an additional effect on an excited neuronal substrate. This result reminds us of previous findings described in PEEF using single-unit activity recording because 50 % of auditory and auditory-motor neurons showed a modulation of the activity during the visual fixation task (Bon et al. 2009; Bon and Lucchetti 2006; Lanzilotto et al. 2013b; Lucchetti et al. 2008).

Moreover, as has been demonstrated for the eye and other effectors (Thier and Andersen 1998; Cheron et al. 1999), we also observed a positive correlation between maximal velocity and amplitude, duration movement, and mean velocity for ear movements. On the contrary, we found a negative correlation between maximal velocity and latency. Stanford et al. (1996), in a microstimulation study of superior colliculus, showed that eye latency depended on stimulation frequency. A higher frequency of stimulation produced movements with a shorter latency and higher velocity. This means that there is an inverse relationship between latency and velocity. In our experiments, we never modified the stimulation frequency, which was then a constant parameter. The finding that ear kinematic parameters overlap with the eye parameters suggests that the same brain regions involved in eye motor control could also be involved in ear motor control. Alternatively, a subpopulation of neurons situated in the same regions could be engaged depending on the goal of the action.

Evoked eye movements

In accordance with the hypothesis just reported, it was possible to elicit goal-directed eye movements in the same sites where stimulation evoked ear movements. The oculomotor system has been extensively studied in both cortical and subcortical brain regions. The principal areas

involved in the eye motor control as regards visual-guided saccades generators are the supplementary eye field (SEF), frontal eye field (FEF), lateral intraparietal region (LIP), and superior colliculus (SC). There is evidence that the dorsolateral prefrontal cortex (DLPFC) is also involved in saccade control, and in particular in the execution of anti-saccade tasks in short-term spatial memory and in decisional processes (Pierrot-Deseilligny et al. 2004).

In our experiments, we have shown that stimulation of area 9 evokes goal-directed saccades principally in the central part of the visual field. In 41 % of the stimulated sites in monkey L, and in 47 % in monkey S, stimulation elicited goal-directed saccades in the ipsilateral part of the visual field. As demonstrated in other brain regions, the probability of evoking eye movements with the end-point in the neuron's receptive field and/or motor field is higher than in other locations (Tehovnik et al. 2003; Tehovnik and Slocum 2004). The fact that microstimulation of area 9 evokes goal-directed saccades toward the ipsilateral visual field in about 40–50 % of stimulated sites could have an intrinsic explanation: the stimulated neuronal population could have a receptive field and/or motor field in the ipsilateral visual field. This could be in accordance with the observation that DLPFC has a role in action inhibition and anti-saccade generation (Funahashi et al. 1993). Otherwise, a representation of both ipsilateral and contralateral visual fields in area 9 could occur. However, further experiments are required to verify this observation.

In some aspects, kinematic parameters for the evoked eye movements follow a specular ear behavior. The eye movement latency is between 150 and 300 ms, assuming that ear evoked movements start faster than eye evoked movements. The Pearson and Kendall correlation analyses show a relationship between the maximal velocity and other kinematic parameters, which overlap with the ear parameters. Otherwise, the same regression tests revealed that evoked eye movements show a significant negative correlation between latency and maximal velocity. In this case, the r value of the evoked eye movement is clearly lower than the corresponding r value for the evoked ear movement. This could be due to the fact that, in general terms, the animals can better control their eyes than their ears. In fact, several times the monkeys spontaneously fixed regions of the visual field and did not allow eliciting of an eye movement.

An interesting result of the analysis is the association between the train duration and kinematic eye parameters in two different experimental conditions: a spontaneous condition versus VFT. Our results agree with Tehovnick and Slocum's observation (2004), showing that the effect of the stimulation in awakened monkeys depends on the monkey's behavioral state. In our experiments, during the VFT conditions all parameters, such as movement amplitude, duration, and maximal and mean velocities were significantly decreased, revealing an opposite behavior to that seen in the ear. As previously described for the ear, during the visual fixation task, a top-down visual attention engagement occurs, causing an inhibition of eye movements. In other words, microstimulation has its effect on an inhibited neuronal substrate. This finding is in line with other studies carried out on other cortical regions, in which the experimenters showed that when a monkey is required to actively fixate on a spot to receive a juice reward, the current threshold for the production of stimulation evoked saccades is increased threefold for the frontal eye field, 16-fold for the Dorso-Medial Frontal Cortex, and over 40-fold for V1. In fact, in the latter case, currents as high as 1,500 μA were ineffective in eliciting saccades from V1 (Tehovnik et al. 2003). The authors concluded that the behavioral state of an animal can therefore override the effects of electrical stimulation delivered to the cerebral cortex.

The development of evoked head forces

Surprisingly, although we never evoked head rotation movements coordinated with ear and eye movements, the analysis of the head forces recorded during the stimulation period showed involvement of the neck. The reasons why we never evoked head-orienting movements could be twofold. First of all, in our experimental approach there was a mechanical impediment. Although we always partially released the head during the experimental phase, the MUPRO inertia could resist the evoked movement. Secondly, we tested the penetration sites with current intensity until 150 μA to elicit ear and eye movements. Several other investigators were able to evoke in the FEF, SEF, parietal cortex, and superior colliculus gaze shift-induced eye–head orienting movements (Chen and Tehovnik 2007). However, in some cases, they evoked head-orienting movements with current intensities that exceeded 150 μA (Thier and Andersen 1998; Tu and Keating 2000) and in unrestricted head conditions (Chen and Walton 2005). Despite this, we were able to indirectly show involvement of the neck by analyzing the forces applied by the monkeys' head in the horizontal plane during the stimulation period. We observed that at 800 and 1,000 ms of train durations there was the highest amplitude movement for the eye (see Fig. 5b): the amplitude was on average 15.59°. Interestingly, at the same train durations we observed significant head forces development (see Fig. 6b). In accordance with this, Chen and Walton (2005) found that SEF microstimulation in head-unrestricted monkeys evokes eye alone, head alone, or eye–head movements. Chen and Walton (2005) agree with Sparks et al. (2001) regarding the *eye centering* hypothesis. They found that if

the eyes were already centered (or within the flexible oculomotor range), the head should move very little. Moreover, a lawful relationship must exist between the amount of eye deviation in the orbit and the amount of post-gaze shift head displacement.

Stryker and Schiller (1975) found that increasing the train duration made it easier to evoke head-orienting movements in the superior colliculus. Our result suggests that by using stimulation, a significant involvement of the neck in the orienting processes occurs when the eye movement amplitude is $\geq$15.59°. This corresponds with other studies in free head monkeys, which show that, when the eyes are centered in the orbits, gaze shifts smaller than 20° are usually completed without any head contribution (Gandhi and Sparks 2001). Otherwise, a gaze amplitude of $\geq$20° usually involves a significant contribution of the head (Freedman and Sparks 1997).

General discussion and conclusion

Hearing is especially important for most primate species as they live in habitats of dense vegetation that limits vision. Stebbins (1980) summed up the evolution of the auditory system by assuming that earliest mammals exploited nocturnal niches since they were relatively free of many of the large, diurnal, predacious reptiles. Therefore, hearing and smell were more useful at night than vision. Following this hypothesis, the auditory system in mammals evolved to compensate for the lack of a visual system. In other words, where we cannot see, we can hear.

Anatomical studies indicate that the hierarchical organization of the auditory cortical system is constituted by two different streams, termed dorsal and ventral, which project to the frontal cortex in non-human primates (Romanski et al. 1999a, b). The dorsal stream, originating from the caudal auditory belt, directly projects from the CL area (caudal lateral) and part of the ML (middle lateral) to the dorsolateral frontal areas 8A, 46d (dorsal), 9, 10, and 8B (Romanski et al. 1999a), to bring information about sound spatial localization. The ventral stream, originating from AL area (anterior lateral) and the RPB (rostral parabelt) projects to the ventral prefrontal areas 47/12, 46v (ventral), 45A, 45B (Gerbella et al. 2010; Rauschecker and Romanski 2011; Romanski and Averbeck 2009; Romanski et al. 1999a). These latter areas, in turn, are connected to area 9, dorsal prefrontal areas 10, 8B, 46d (dorsal), 8Ad (dorsal), and 24 in the medial surface (Borra et al. 2011; Gerbella et al. 2010; Yeterian et al. 2012). Thus, the dorsolateral prefrontal cortex could have an important role in linking the auditory and motor systems. This region in fact has an interesting medio-lateral gradient in its spatial selectivity and in particular regarding the proportion of neurons sensitive to visual or auditory stimuli. Azuma and Suzuki (1984) showed that in lateral portions of the dorsofrontal cortex, corresponding to the central part of the periarcuate region (FEF), auditory neurons have a directional preference in the visual field within ±10°. As one moves more medially, the directional tolerance increases to ±40° and above. Moreover, the amplitude of the evoked head movements increases in this region (Chen 2006). Finally, more neurons in this area are responsive to auditory stimuli and similar results have been described more medially, in PEEF, where auditory and auditory-motor neurons were found (Bon and Lucchetti 2006; Lucchetti et al. 2008). Recently, this field has been proposed as a new frontal field, involved in the spatial localization of complex auditory stimuli (Bon et al. 2009; Bon and Lucchetti 2006; Lanzilotto et al. 2013b; Lucchetti et al. 2008). This supports the hypothesis that the visual system and auditory systems could be organized in a central and a peripheral region showing an inverse gradient in their cortical representation.

As is well known, the frontal regions such as FEF, SEF, PEEF, and DLPFC, which receive information from the auditory system, also receive visual information from the visual system involving them in the transformation of visual signals into saccade motor commands (Schall 1997). Then, the cortical frontal regions, involved in the gaze shift control, can be activated by a visual and/or auditory stimulus.

In accordance with Mitz and Godschalk (1989), we think that eye movements, and in general gaze shifts, are more broadly represented in the frontal lobes than previously described, leading to the speculation that there are more than two frontal eye fields. Therefore, PEEF, SEF, FEF, and other surrounding areas in DLPFC, such as area 9, might be part of parallel oculomotor circuits with the purpose of detecting visual and/or auditory stimuli in different regions of the space (Lanzilotto et al. 2013a).

An interesting recent anatomical result (Gerbella et al. 2010) shows that two areas in the ventral prefrontal cortex, area 45A and 45B, are part of two different circuits. Area 45A is connected with the rostral and caudal auditory parabelt, and more interestingly with the dorsal part of FEF, where large fixed-vector saccades are represented, with the lateral part of PEEF/area 9 and with SEF. Area 45B, which is adjacent to area 45A, is connected with the ventral part of FEF, where small fixed-vector saccades are represented, the medial part of PEEF, area 9, and SEF. These two circuits could be involved, respectively, in peripheral vision and central vision suggesting the presence of at least two oculomotor circuits that detect stimuli in two different regions of the visual field. Our hypothesis could be supported by a recent finding (Borra et al. 2013), that both areas 45B and 45A, with more ventrolateral prefrontal areas, project to brainstem preoculomotor structures, basal

ganglia, and cerebellar oculomotor loops. However, further investigation is required to verify our hypothesis regarding the presence of at least two parallel oculomotor circuits; one for central vision and the other for peripheral vision.

Otherwise, following the functional hypothesis proposed by Gerbella et al. (2010) that has been confirmed by Borra et al. (2013), area 45A could be part of a communication circuit while area 45B could be part of an oculomotor circuit. Considering the anatomical connections between area 45 and area 9, in the light of our results, we can speculate on another and more interesting role for area 9. Non-human primates are characterized by flattening of the ears during social communication, such as lip-smacking. During such flattening, part of the auricular musculature somewhat retracts the posterior part of the scalp and this generally occurs together with elevation of the eyebrows. This behavior usually occurs when an animal's face comes in contact with another animal and it is most marked in macaques, mangabeys, and baboons (Andrew 1963). Thus, the fact that stimulation in area 9 evokes eye goal-directed saccades principally in the central part of the visual field and backward ear movements could support the hypothesis that area 9 is part of a social communication circuit.

Our findings could provide the basis for a new vision regarding the functional properties of area 9; however, more research is necessary to better explain the role of this region in goal-directed orienting behaviors and gaze shift control.

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Conflict of interest The authors declare the absence of any potential conflict of interest, financial or otherwise.

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