

MIRROR
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Mirror Neurons based Object Recognition

Deliverable Item 4.5
Final Results of the Biological Experiments:
Monkey data, TMS and Behavioural Development

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Short Description: This deliverable item describes the data achieved within the MIRROR project. Single neuron recordings in behaving monkeys have been carried out during the three years, aiming to demonstrate that premotor neurons of area F5 (the ventral premotor cortex where mirror neurons have been located) apparently devoid of any visual property, indeed respond to the vision of one's own acting hand. This hypothesis, which has been largely confirmed by the experiments presented here, may explain the ontogenetic development of the mirror-neuron system. In addition, TMS experiments have been carried out in humans guided by the hypothesis that a mirror-neurons system, similar to that described in monkeys, is at work and may form the neural substrate for interindividual communication.

The first section of this deliverable (Monkey experiments) first describes the technique we set up to record neurons in behaving monkeys, to isolate single neurons from multispikes recordings and to analyze the recorded data. Second, it concentrates on the results achieved by the formal testing of about 200 neurons out of 500 recorded in area F5 and in the primary motor cortex (area F1). The second section of this deliverable describes some experiments we performed in humans with transcranial magnetic stimulation, aiming to investigate a possible role of the mirror-neuron system in speech perception.

The final part of the deliverable describes experiments regarding behavioural development, particularly, the basic neuronal processes, the development of predictive action and the development of manipulation.



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PART A: MONKEY EXPERIMENTS

1. Introduction

Background

It's well known that the frontal cortex is strongly involved in action programming and motor control. Histologically, it is characterized by an almost complete absence of granular cells in its fourth layer (agranular frontal cortex). The classical map of Brodmann (Brodmann, 1909) subdivides this agranular cortex in two areas, a caudal area 4 (the primary motor cortex, almost entirely buried inside the central sulcus) and a rostral area 6 (the premotor cortex). Anatomical studies (von Bonin and Bailey, 1947; Von Economo, 1927; Vogt and Vogt, 1919) have successively revealed that area 6 is not unitary, but is formed by a mosaic of distinct areas. According to the classification by Matelli et al. (1985), these areas have been named by adding a numerical suffix (from 1 to 7) to the letter 'F' (frontal, see figure 1).

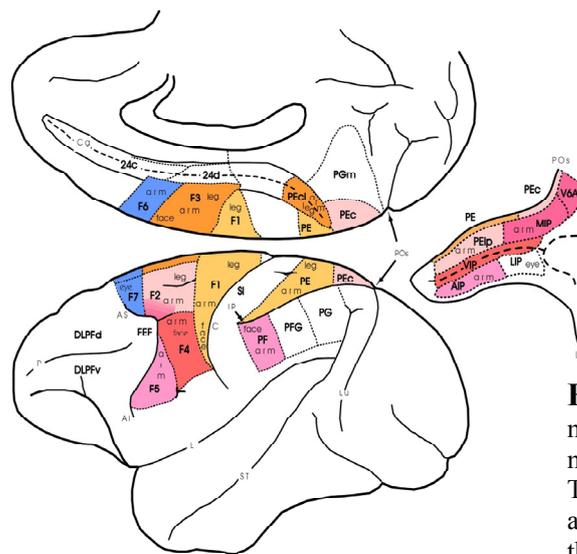


Figure 1. Mesial and lateral views of the monkey brain, showing the parcellation of the motor, posterior parietal and cingulate cortices. The areas located within the intraparietal sulcus are shown in an unfolded view of the sulcus in the right part of the figure

The complete picture is the following: in addition to the primary motor cortex (area F1) there are three pairs of areas: F3 (caudal, SMA proper) and F6 (rostral, pre-SMA) lay on the mesial wall of the frontal lobe; F2 (caudal) and F7 (rostral) form the dorsal premotor cortex and F4 (caudal) and F5 (rostral) form the ventral premotor cortex. Particularly interesting are the ventral premotor areas because of the strong visual input they receive from the inferior parietal lobule. These inputs subserve a series of visuomotor transformations for reaching (area F4, Fogassi et al., 1996) and grasping (area F5, Rizzolatti et al, 1988; Murata et al., 1997). In

addition, area F5 contains neurons forming an observation/execution matching system, which maps observed actions on the observer's internal motor representations (**mirror neurons**). As briefly described above, area F5 is located in the rostral part of the ventral premotor cortex and consists of two main sectors: F5c, located on the cortical convexity and F5ab, forming the posterior bank of the inferior arcuate sulcus. Both sectors receive a strong input from the secondary somatosensory cortex (SII) and from the rostral part of the inferior parietal cortex (PF) (Matelli et al., 1986). In addition, sector F5ab receives input from the anterior intraparietal area (AIP) (Luppino et al., 1999).

Electrical stimulation studies revealed that area F5 contains extensively overlapping representations of hand and mouth movements (Rizzolatti et al., 1988; Hepp-Reymond, et al., 1994). Single neurons studies have shown that most F5 neurons code specific actions, rather than the single movements that form them (Rizzolatti et al. 1988, Fadiga et al. 2000). It has been therefore proposed that, in area F5, a vocabulary of goals more than a set of individual movements, is stored. This goal-directed encoding, typical of area F5, is demonstrated by the discriminative behavior of F5 neurons when an action, motorically similar to the one effective in triggering neuron response, is executed in a different context. For instance, a neuron responding during grasping with the hand doesn't respond when similar finger movements are performed with different purposes, e.g., for scratching (Rizzolatti et al., 1988). The motor responses of the F5 neurons vary in their degree of abstraction. From the general encoding of an action goal (e.g., grasping, holding), to more specific responses related to particular aspects of the same goal (e.g., precision grip, whole hand grasping). Finally, there are neurons responding to different phases of these actions (e.g., during opening or closing the fingers while executing a specific grasping).

Several F5 neurons, in addition to their motor properties, respond also to visual stimuli. According to their visual responses, two classes of visuomotor neurons can be distinguished within area F5: canonical neurons and mirror neurons (Rizzolatti and Fadiga, 1998). The canonical neurons are mainly found in F5ab, which is the main target of parietal projections coming from area AIP. These neurons respond to visual presentation of three-dimensional objects (Murata et al., 1997). About one quarter of F5 neurons show object-related visual responses, which are, in the majority of cases, selective for objects of certain size, shape and orientation and congruent with the motor specificity of these neurons. They are thought to take part in a sensorimotor transformation process dedicated to select the goal-directed action, which most properly fits to the particular physical characteristics of the to-be-grasped object.

The mirror neurons form the second class of visuomotor neurons of area F5. This name was coined because of their property to "reflect" with their visual response an action executed

by another individual, if the seen action is similar to that motorically coded by them (di Pellegrino, et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996). In contrast to the canonical neurons, mirror neurons do not respond to the mere presentation of objects. Thus, the vision of a real action, performed by a biological agent (the experimenter or another monkey) is essential for their activation. A mimed action, not interacting with an object, or an action executed by a tool (e.g. pliers) are ineffective in triggering most of F5 mirror neurons. Almost all mirror neurons show a certain degree of congruence between the effective observed and executed action. This congruence is very strict in about one third of F5 mirror neurons. The remaining mirror neurons are characterized by a broader congruence, ranging from the very general aim of the action (e.g. ‘to grasp’: visual response to grasping with the hand and with the mouth; motor response to grasping with the hand, only) to an effector-specific broad congruence (e.g. ‘to grasp with the hand’: visual response to whole hand prehension, finger prehension and precision grip; motor response to precision grip, only). It has been proposed that these broadly congruent mirror neurons may generalize the goal of the observed action (Gallese et al., 1996; Rizzolatti et al., 1996a). Very recently, it has been reported that a fraction of mirror neurons, in addition to their visual response, become also active when the monkey listens to an action-related sound (e.g. breaking of a peanut) (Kohler et al., 2002). It is tempting therefore to conclude that mirror neurons may form a multimodal representation of goal directed actions, possibly involved in action recognition. The recent finding that mirror neurons become also active when the effective observed action is partially hidden to the monkey (Umiltà et al., 2001), suggests that they may represent actions in a rather abstract and cognitive way.

Aim

The goal of monkey experiments was to investigate the nature of the visuomotor coupling at the basis of the “mirror” response. Our hypothesis was that mirror discharge could be initially generated by the observation of one’s own acting effector, seen from different perspectives, performing repetitively the same action. We assumed that these different visual information could be associated by the brain as “common signals”, having in common the same motor goal. Following this learning phase, the system could become therefore capable to extract motor invariance also during observation of actions made by others. Although the learning process described above should mainly occur during development, we postulated that also in adult animals some vestigial residuals of this visuomotor coupling could have resisted in F5 motor neurons (generally considered as devoid of any visual property). To investigate this hypothesis, we programmed a series of single neuron recordings in monkey premotor area F5 while the animal was executing a grasping movement with normal and manipulated visual

information (e.g.: complete dark, brief flash of light during different phases of the movement). As a control, primary motor cortex neurons (area F1) have been recorded too.

2. Methods

2.1 General procedure

The experiments were carried out in two awake, partially restrained, macaque monkeys (*Macaca fascicularis*). Neuronal activity was recorded in two hemispheres. All experimental protocols were approved by the Veterinarian Animal Care and Use Committee of the University of Ferrara, Italian Ministry of Health and complied with the European law on the humane care and use of laboratory animals. Before starting the experiments, the monkey was habituated to the experimenters and the experimental conditions. The monkey was seated in a primate chair and trained to receive food, perform goal-directed task and to pay attention to the experimenter while making various hand and mouth actions.

2.2 Head implants and surgery

Modeling the chamber for neuron recordings

A titanium cylindrical chamber (height 20 mm, inner \varnothing 20 mm, outer \varnothing 24 mm) was selected because of the possibility to safely closed it with an O-Ring gasket mounted on a plastic cover (figure 2, left).



Figure 2. Left, the titanium chamber before modeling; the sealing cap used to close the chamber. Right, computer modeled surface, complementary to the to-be-implanted bone.

To obtain a perfect adhesion between the chamber and the bone (figure 2, right; figure 3), the inferior surface of the chamber was modeled by using the Rhinoceros 3D modeling software to replicate the skull surface, calculated by reconstructing the 3D shape of the bone with the public domain ETDIPS software (figure 3) on previously acquired computerized

tomography images (CT) (figure 4).

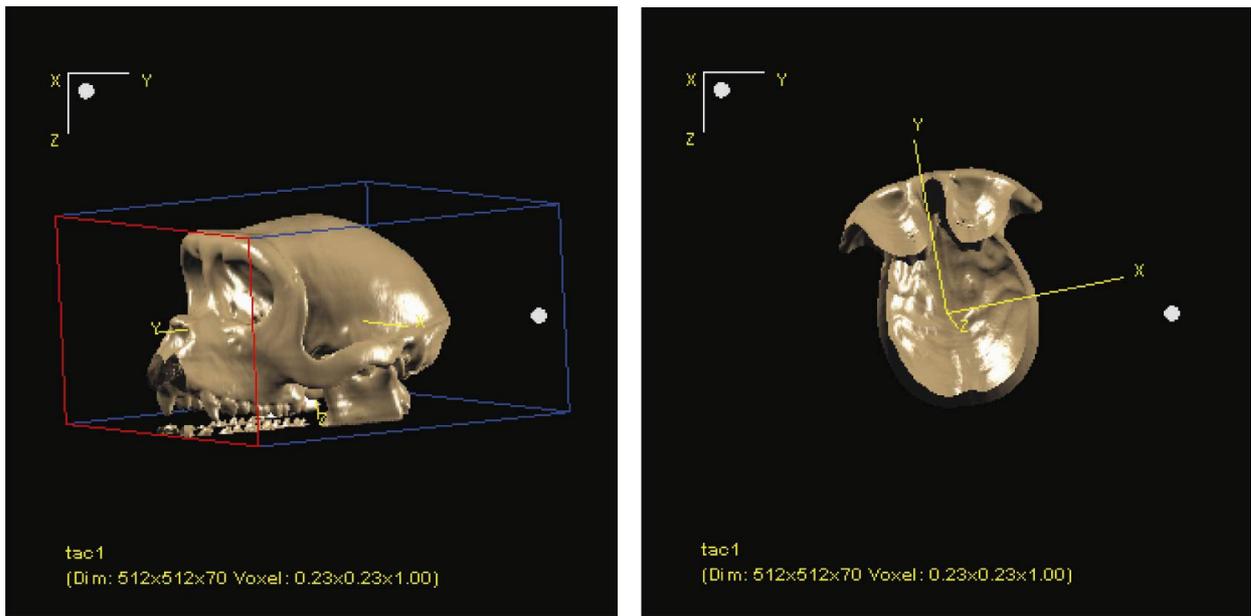


Figure 3. The 3D reconstructed skull of monkey MK1.



Figure 4. Complete series of CT slices acquired from monkey MK1. The slice thickness is 1 mm. The slice pixel resolution is 0.24 mm. Note the high contrast between bone and soft tissues.

The chamber was then milled from the titanium cylinder by a path-generating program (Mill Wizard, Delcam, UK) generating the correct G-code for driving a computer-driven 3D milling machine (figure 5).

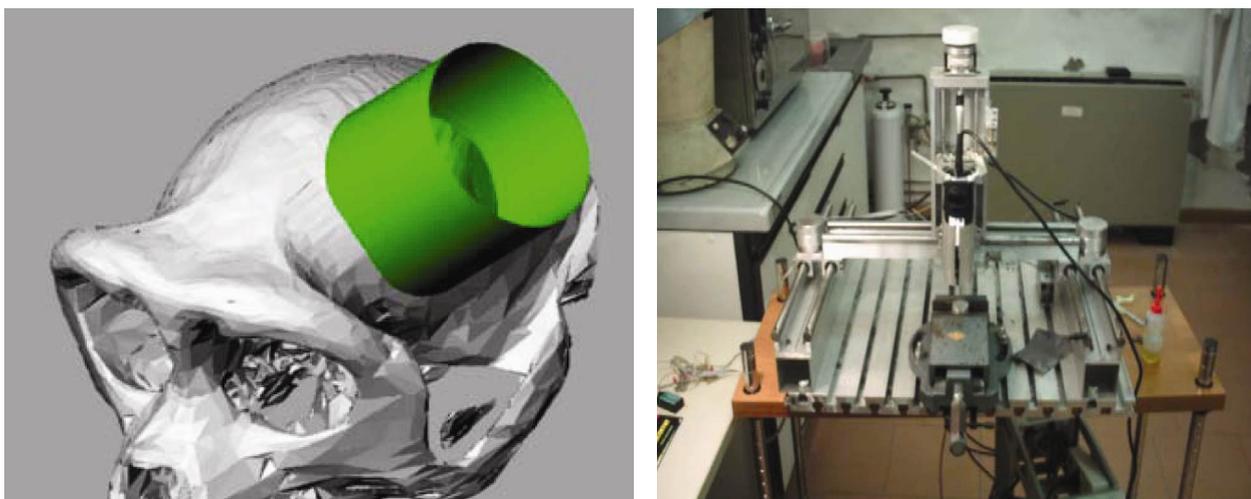


Figure 5. On the left, the head-chamber assembly, as modeled by Rhinoceros 3D software. On the right, the computer-driven 3D milling machine.

Determination of skull region under which premotor area F5 is located.

An important problem to solve before surgically implanting the chamber, was to determine the position of the target (frontal areas F5 and F1) on the monkey skull. After submitting the monkey to a CT scan which provided us with a series of horizontal slices (thickness, 1 mm) (figure 4) of the monkey head, the external and internal 3D surfaces of the skull were reconstructed by using the ETDIPS software. The system of coordinates of these 3D images was then adjusted according to the standard stereotaxic system (orbitomeatal plane). With a specifically designed software (created in our laboratory) we determined the position of the target cortices by using as references both the sulcal pattern impressed on the internal surface of the skull (see figure 3, right) and the stereotaxic atlas by Szabo and Cowan (1984) (Szabo, J. and Cowan, W. M., 1984). Figure 6 shows a screen shot of this program (Virtual Stereotaxic) that allows to navigate through the 3D reconstruction of the monkey's head in order to preoperatively select the target region.

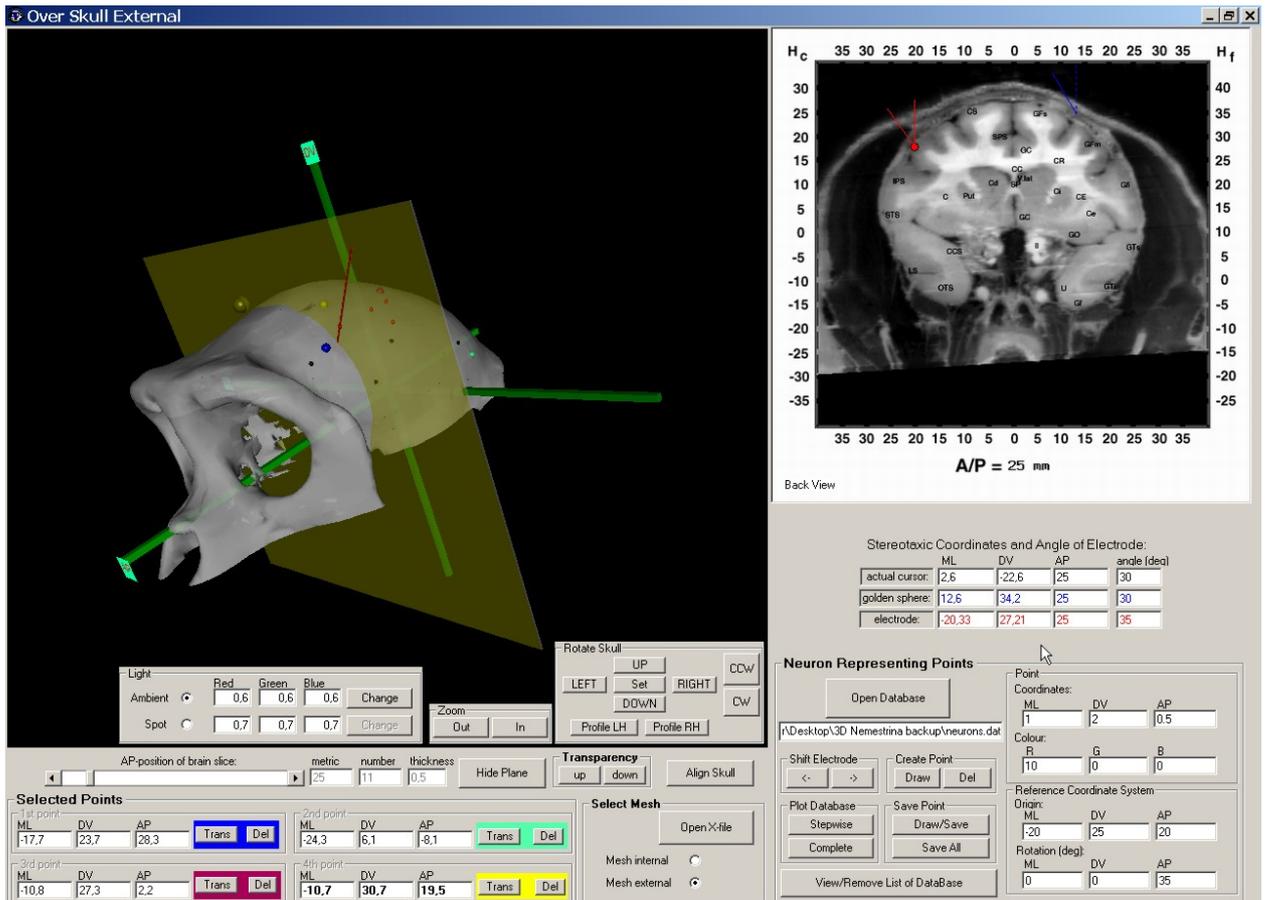


Figure 6. The 'Virtual Stereotaxic' software screenshot. It will be freely available on the web for downloading. The left panel depicts the 3D reconstructed skull of the to-be-implanted monkey, aligned on the orbitomeatal space (the green rods). The right panel shows the NMR image corresponding to the yellow plane cutting the skull, adapted to Szabo and Cowan atlas coordinates (see text).

Implanting chamber and spheres for head fixation: Hydroxyapatite coating and surgical procedure.

All head implants, including head holders and screws, were custom designed and fabricated in titanium (a biocompatible material). To further improve the adhesion to the bone, titanium surfaces were coated with hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) slightly modifying in our laboratory a very recent low-temperature sol/gel coating procedure (Liu et al., 2001). In brief, after drying at 80°C , the HA coated titanium is calcinated at 450°C in order to obtain crystalline HA and to induce its adhesion to the substrate. The chemical similarity between hydroxyapatite and mineralized bone increases the affinity of coated implants to host hard tissues. It is known that the growing of bone's cells is stimulated by the HA coating and that HA forms a substrate for optimal osteointegration that guarantees a high stability, because of the

hard linkage between the bone and the implant. Furthermore, the implant-bone adhesion reduces the risk of fluid leakage from the chamber, thus reducing the risk of infections. The electron microscopy of one of our templates (figure 7) shows the hydroxyapatite matrix, coating the titanium implant.

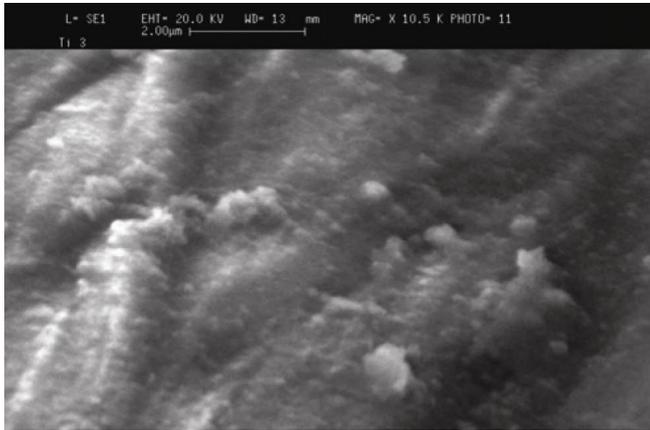


Figure 7. The hydroxyapatite layer, coating the titanium implants. Calibration bar, 2 µm.

At the present, in order to reduce as much as possible surgery invasivity, we are also testing a new procedure of implant fixation, based on a commercial implant system used in maxillofacial surgery as support for secondary mounted prosthesis (MID-PLANT®, HDC-Health Development Company, Italy). The main advantages of this new implant are: (1) easier and faster insertion into the bone by its self-screwing properties; (2) reduction to the minimum of the number of screws; (3) possibility to guide the trepanation drill by using the holding frame of the head fixation bars as guiding system (see figure 8); (4) minimized dimensions.

In order to implant the chamber and the head fixation system, the monkey was submitted to **three surgical sessions** that were developed according to standard protocols. During each session the monkey head was kept fixed by a stereotaxic apparatus that allows measuring in stereotaxic coordinates the appropriate location of the different implant components. The stereotaxic coordinate system was the same of that used in our Virtual Stereotaxic software.

The surgical implantation of the recording chamber was carried out under general anesthesia by tiletamine-zolazepam (10-20 mg/kg, IM), after atropine sulfate (0,1 mg/kg, IM) premedication, and followed by isoflurane anesthesia for the whole duration of surgery. All vital parameters (heart rate, body temperature, blood oxygen saturation, respiratory function) were monitored continuously during operation. The most critical aspect during surgery is the respect of the thermal equilibrium of the exposed bone in order to maintain its vitality and to allow its successive growth on the HA-titanium substrate. To this purpose, during the whole surgery, and during skull drilling in particular, care was taken in order to prevent bone overheating. With this goal in mind we modified the illumination source of an operative

microscope from the traditional bulb lamp to a fiber optic cold-source-system and we continuously irrigated the operative field with cold saline during bone trepanation. An important advantage of our newly developed procedure was the absence of cement for implant fixation.

- (1) During the **first session** four spheres held by a mounting flange were implanted by means of specially designed titanium screws. By this way, the head can be kept still by a frame device, mounted on the primate chair, consisting of four fixating rods with internal conic holes perfectly hosting the four spheres.
- (2) During the **second session** the chamber was placed in the correct location on the skull to cover the cortical surface from the central sulcus to the arcuate one. The chamber was then fixated with titanium screws to the bone.
- (3) During the **third surgical session**, after a recovery period (6-8 weeks), the bone inside the chamber was removed and the dura mater exposed. The chamber was then covered by a plastic cap internally holding an O-Ring gasket that can be placed and removed simply by means of three screws.

The following figure (figure 8) shows the result of chamber and head fixating spheres implantation, after three months from surgery.



Figure 8. Posterior, lateral and frontal views of the implanted monkey, sitting on the chair. Note the absence of infective reactions around the implants.

2.3 Neuron recordings

During recordings, the behaving monkey was sitting on a restraining chair with the head kept fixed by the holding frame shown in figure 8. Arms and legs were allowed to freely move. Special micromanipulators were firstly used to calibrate the electrode tip position by using as a reference point the center of an aluminum cap fitting the top of the chamber. The electrode was then moved to the desired location, according to the stereotaxic coordinates of the target region. Recordings were made by using varnished (Sivamid, Altana, Germany) tungsten microelectrodes with impedance 0.15–1.5M Ω (measured at 1 kHz). The electrode penetrated with an angle of 32-40° (with respect to the sagittal plane) in the premotor cortex, pushed by a hydraulic advancer (Trent Wells, CA, USA; step resolution, 10 μ m).

Recorded signal was amplified $\times 10,000$ (BAK Electronics, Germantown MD, USA), filtered by a dual variable filter VBF-8 (KEMO Ltd., Backenham, UK) (bandwidth 300-6000 Hz), digitized (PCI-6071E, National Instruments, USA) at 10 kHz of sampling rate and stored for further off-line analysis. The acquisition program (see figure 12) was made in our laboratory by using the LabView 7 Express software (National Instruments, USA). The electrical activity as well as the action potentials isolated online with a dual voltage-time window discriminator (BAK Electronics, Germantown MD, USA) was acoustically amplified by an Audio Monitor (Grass Instruments, USA) to give to the experimenter an auditory feedback on the discharge during neuron's testing. The experimental acquisition was preceded by a preliminary mapping of the exposed cortex. This was done by recording the neural activity and by correlating neurons' responses with visual and somatosensory stimulation and during monkey's motor behavior (e.g. by giving food of different dimensions to the animal and by exploring grasping performed in different spatial locations). Stimuli and procedures were used as described in our previous studies (Rizzolatti et al., 1988; Rizzolatti et al., 1990; Gallese et al., 1996). Criteria and functional characteristics described by Umiltà et al., (2001) were used to distinguish F1, F4 and F5 areas as well as regions in F1 and F5 characterized by a high density of neurons exhibiting hand-related activity during goal-directed actions (Umiltà et al., 2001). In addition, intracortical microstimulation (train duration, 50-100 ms; pulse duration, 0.2 ms; frequency, 330 Hz; current intensity, 3–40 μ A) was administered on every 500 μ m along the electrode track in order to establish the motor threshold and the motor somatotopy of the recorded region. The current intensity was controlled by measuring the voltage drop across a 10 k Ω resistor in series with the stimulating electrode and by displaying it onto an HM 507 oscilloscope (HAMEG Instruments, Germany).

2.4 Experimental device and paradigm

To standardize the grasping movement, a specially designed apparatus has been used. It consists of a box that was mounted at reaching distance (30 cm) in front of the monkey, with little pieces of food hidden inside (figure 9).

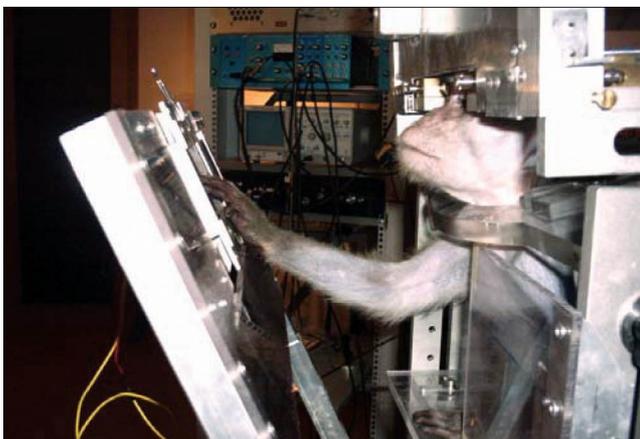


Figure 9. The experimental apparatus.

The box was covered by two doors. A more superficial one (see figure 10, left) whose opening at distance by the experimenter signaled to the monkey the beginning of the trial, and a second one (see figure 10, right), hosting a small plastic cube working as a handle. This plastic cube was translucent and back-illuminated from inside the box by a red LED in order to allow the monkey to fast reach it, also in the dark. The handle was buried inside a groove that forced the monkey to open the door by grasping the handle only by using a precision grip. When both thumb and index finger touched the handle, an electronic circuit (Schmitt's trigger) gave to the acquisition system the synchronization signal. Neuronal activity was recorded during the two seconds following handle grasping, with one second of pre-trigger acquisition.



Figure 10.

In order to test the experimental hypothesis, recorded neurons were submitted to four conditions:

- a. grasping in full vision*
- b. grasping in dark with no hand visual feedback*
- c. grasping in dark with instantaneous visual feedback before contact*
- d. grasping in dark with instantaneous visual feedback at object contact*

In the last two conditions a very brief (20 microseconds) xenon flash illuminated the scene at two different phases of the grasping action: during hand approaching (as triggered by a pyroelectric infrared sensor) (c) and at the moment of handle touch (d).

Apart from the aforementioned conditions, two additional conditions were tested in some neurons to force the nervous system to strongly use visual feedback control:

- e. grasping in vision through true prisms (10° 15° and 20° of horizontal or vertical deviation);*
- f. grasping in vision through fake prisms (created by superimposing two prisms with the same strength but with opposite direction).*

To test these conditions a special experimental device, holding a sliding mechanisms which could host the various prisms used in the experiment (Press-On™, 3M Health Care, USA), was mounted in front of monkey's eyes, see figure 11.

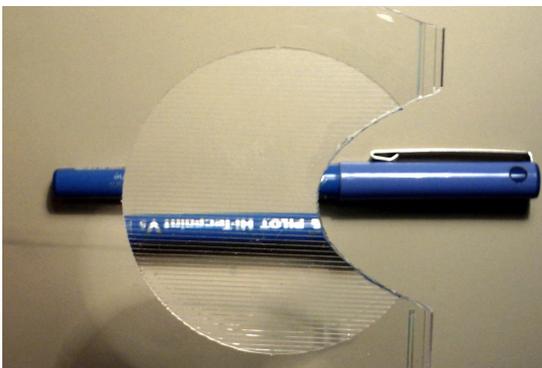


Figure 11. As the monkey sees the world through one of the 10° prisms used in condition e.

Conditions were recorded in blocks of twelve trials. Inter-trial period was randomly modified. The first condition was always repeated at the end, to confirm the stability of neuronal activity along the experimental testing.

The starting position of the monkey's hand was on the hip board of the primate chair near the monkey's body. The animal was continuously forced to maintain it before the start of each trial because the experimenter was keeping closed the sliding door if the monkey's hand was not correctly positioned. The uniformity of the trials and their correct execution was additionally controlled by another experimenter managing the acquisition program. This software, appositely designed during the project, allowed to acquire all data about neuronal activity (captured as raw samples and not as triggered signals, like many acquisition programs do), discriminated spikes coming from the hardware threshold discriminator, trigger occurrence and the infrared signals coming from the pyroelectric sensor (figure 12).

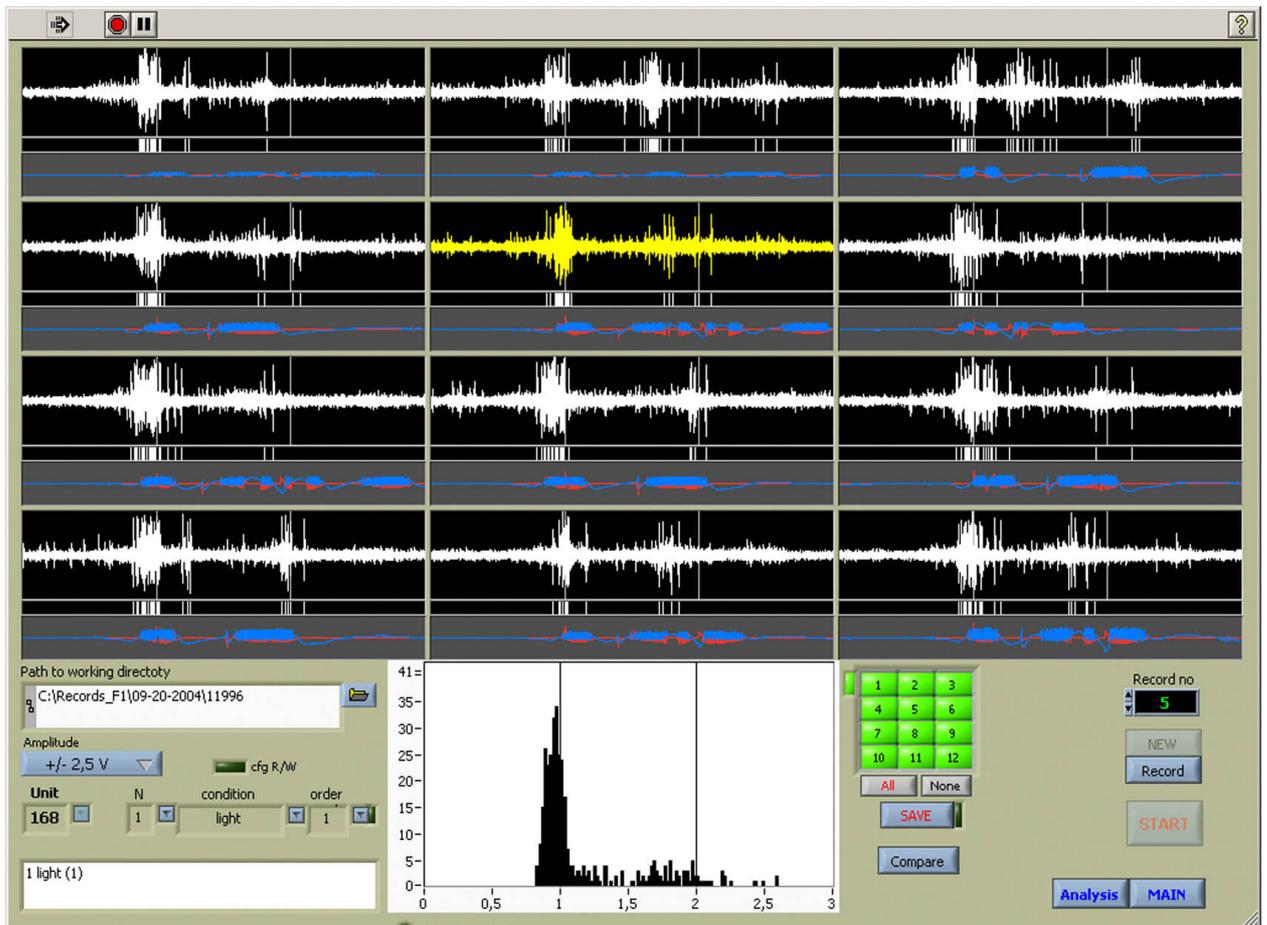


Figure 12. The acquisition software screenshot. Each individual panel (12 total) shows, from above, raw neuronal data, hardware triggered spikes, trigger and infrared sensor data. The sum histogram of the triggered activity was shown online in the bottom white panel.

2.5 Spike sorting and data analysis

All signal processing and visualization procedures, Principal Component Analysis, Fuzzy C-mean clustering and other math functions were implemented with LabView 7 Express software (National Instruments, USA).

Spike extraction

At the first stage individual action potentials were extracted from the sampled neural signal. For each peak, the quadratic fit was tested against a threshold level, interactively adjusted for each recording site. Peaks with amplitudes lower than the threshold level were ignored. Six samples before the peak and 12 samples after it (1.8 ms in total) were collected for each spike for further analysis.

Spike shapes were then interpolated twice (over-sampled) by using a SPLINE interpolation method to obtain 36 samples per peak, and stored in a quadratic matrix $N \times 36$. Six samples at the beginning and at the end of each interpolated shape were then removed, thus leading to 24 sample waveforms, each with 1.2 ms of signal duration. A $N \times 24$ indexed array was then filled with these peak data (figure 13).

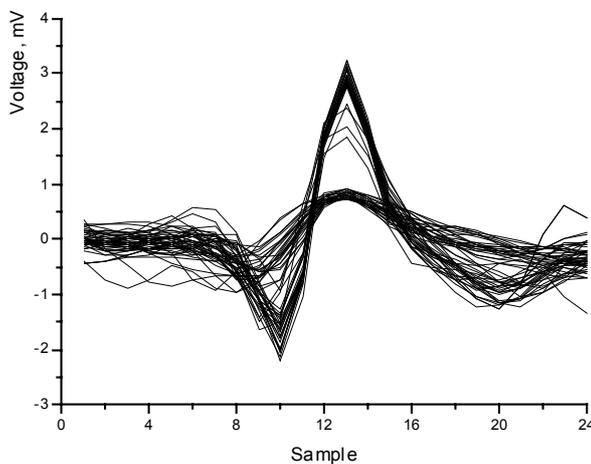


Figure 13. The interpolated, re-sampled signals (two overlapping spikes are clearly distinguishable).

This first processing gave origin to a data matrix $\mathbf{X}^{(N \times M)}$ containing the to-be-analyzed spike shapes (N indicates the total number of spikes contained in \mathbf{X} , M are the samples describing each spike). The matrix \mathbf{X} is a *two-mode* data set where the N spikes represent the 1st mode, and the M samples the second mode.

$$\mathbf{X} = \begin{bmatrix} x_{11} & x_{12} & \cdots & x_{1N} \\ x_{21} & x_{22} & \cdots & x_{2N} \\ \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots \\ x_{M1} & x_{M2} & \cdots & x_{MN} \end{bmatrix}$$

This representation gave us the possibility to apply vector-based procedures with algebraic matrices, instead of scalar ones, for centering, scaling and data normalization. These procedures were done in series for correct calculation of Principal Components (PC) and better separation of low-amplitude spikes.

a. Centering across the 1st mode. The resulting matrix \mathbf{Y} follows from the offset subtraction:

$$\mathbf{Y} = \mathbf{X} - \mathbf{1}\mathbf{m}^T \quad \text{Where} \quad \mathbf{m} = \begin{bmatrix} \bar{x}_1 \\ \vdots \\ \bar{x}_M \end{bmatrix} \text{ and } \mathbf{1} = \begin{bmatrix} 1 \\ \vdots \\ 1 \end{bmatrix} \text{ is a } N\text{-value column vector of 1}$$

b. Scaling within the 1st mode. The resulting matrix \mathbf{Y} follows from the vector multiplication:

$$\mathbf{Y} = \mathbf{X}\mathbf{W} \quad \text{Where} \quad \mathbf{W} = \text{diag}(w_1, w_2, w_3, \dots, w_N)$$

c. Normalization of rows follows from the multiplication of each row element with the corresponding row norm:

$$\mathbf{Y} = \mathbf{X} \cdot \frac{1}{\|x_i\|} \quad \text{where} \quad \|x_i\| - \text{corresponding row norm, } i = 1, \dots, N$$

The most important advantage of using these vector-based operations in LabView was a dramatic increase of speed of data operations, allowing on-line processing of the data set.

Principal Components Analysis

The goal of Principal Component Analysis (PCA) is to extract significant information from a data set while reducing the dimensionality of the data.

To obtain Principal Components of given spike shapes, the singular value decomposition (SVD) of a given $N \times 24$ real matrix A was performed. Such SVD factorization produces three matrixes U , D , and V so that the following equation is true.

$$A = UDV^T$$

Here, U is an $N \times 24$ matrix, containing the singular values (the Principal Components) of the original matrix, and V^T is an 24×24 square matrix. D is a diagonal matrix formed by 24 singular values in decreasing order (Press et al., 1992). The first principal component accounts for as much variability as possible (figure 14), and each next component individually accounts for as much of the remaining variability as possible. After calculation of PCs for the first experimental condition all other spike shapes recorded from the same site were projected onto the first three PCs, and clustered according to the previously determined 3D PCs space depending on their shape.

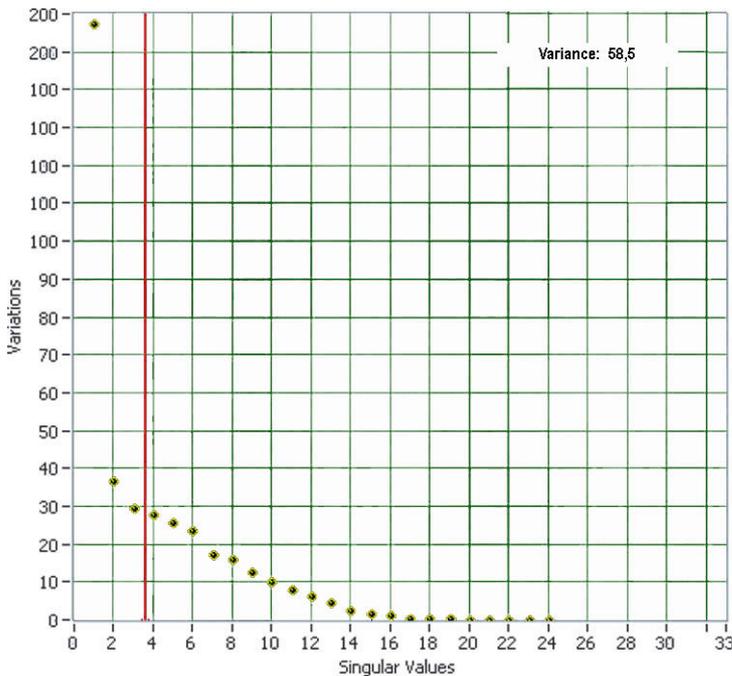


Figure 14

Fuzzy C-mean clustering

We used the iterative Fuzzy C-means (FCM algorithm) for classification of spikes in the principal components (PC) space. This algorithm is based on the classical isodata method of Ball and Hall (1967). The number of clusters c to be found needs to be given beforehand, where c is greater than or equal to two and less than or equal to the number of objects K . In addition, exponent m ($m > 1.0$) determines the degree of fuzziness of the resulting clustering process has to be given. As $m \rightarrow 1$ the fuzziness of the clustering result tends to the results derived with the

classical isodata method. As $m \rightarrow \infty$ the membership values of all the objects to each

cluster tend to the reciprocal of the number of classes $\frac{1}{c}$. The clustering (or training) algorithm of the fuzzy c-means algorithm reads as follows:

A. Initialize the membership values μ_{ik} of the k objects x_k to each of the i clusters for $k = 1, \dots, K$ randomly such that:

$$\sum_{i=1}^c \mu_{ik} = 1 \quad \forall k = 1, \dots, K \quad \text{and} \quad \mu_{ik} \in [0,1] \quad \begin{array}{l} \forall i = 1, \dots, c \\ \forall k = 1, \dots, K \end{array}$$

B. Calculate the cluster centers v_i using these membership values μ_{ik} :

$$v_i = \frac{\sum_{k=1}^K (\mu_{ik})^m \cdot x_k}{\sum_{k=1}^K (\mu_{ik})^m}, \quad \forall i = 1, \dots, c$$

C. Calculate the new membership values μ_{ik}^{new} using these cluster centers v_i :

$$\mu_{ik}^{new} = \frac{1}{\sum_{j=1}^c \left(\frac{\|v_i - x_k\|}{\|v_j - x_k\|} \right)^{\frac{2}{m-1}}}, \quad \begin{array}{l} \forall i = 1, \dots, c \\ \forall k = 1, \dots, K \end{array}$$

D. If $\|\mu^{new} - \mu\| > \varepsilon$, let $\mu = \mu^{new}$ and go to step 2.

To calculate the vector distances in step C a Euclidean distance was chosen. The process ends when the distance between two successive membership matrices μ falls below a stipulated convergence threshold ε . To calculate the distance, a suitable matrix norm needs to be chosen. The process ends by comparing two successive cluster center matrices, the matrix norm being the sum of the vector components. In addition to providing the position of the cluster centers, with the aid of step C, the fuzzy c-means algorithm also provides the membership values of the individual objects to the different clusters. This permits the classification of new objects and their membership values to the different classes for the given cluster centers.

The cluster centers than automatically labeled with the following algorithm:

E. Calculating the membership function μ_{ik} of the object x_k of the class(k) to all class centers v_i :

$$\mu_{ik} = \frac{1}{\sum_{j=1}^c \left(\frac{\|v_i - x_k\|}{\|v_j - x_k\|} \right)^{\frac{2}{m-1}}}, \quad \forall i = 1, \dots, c$$

Rasters, histograms, and statistic files were then automatically created by the program for each class (figure16).

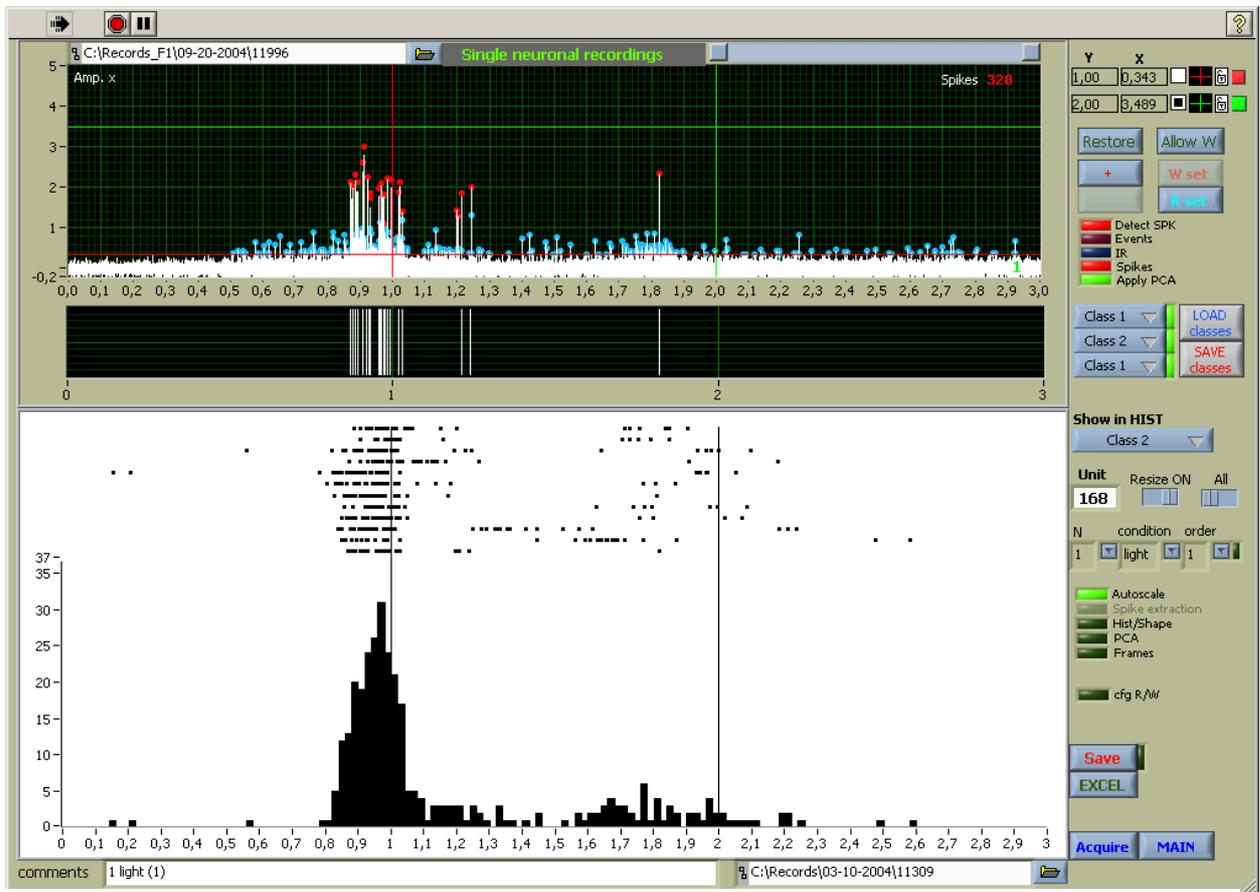


Figure 16. Example of rasters and histogram of a neuron (marked here by red dots on the original signal) separated by the fuzzy clustering procedure.

2.6 Kinematic study

During the experimental paradigm the kinematics of the reaching and grasping movements (namely, grasping and holding phase) of the monkey's hand were analyzed. An infrared-sensitive, digital camera (Philips ToUCam Pro) with high frequency acquisition (60 frames/s) was placed in front of the experimental scene and directed perpendicularly to the main direction of monkey's reaching movement. Twelve trials were recorded for each experimental condition (see Methods). The time of maximal hand opening during reaching, the time of first contact with the door, the time of trigger event and the time of door opening were determined by analyzing the videos frame by frame. The differences in time intervals between the various experimental conditions were analyzed by using a two-tailed t-test. More recently, the 1 kHz ProReflex tracking system (Qualisys AB, Sweden), has been used to record and reconstruct the three-dimensional trajectories of three reflecting markers positioned on the wrist, the index

finger and the thumb, respectively. In a third study, indirect kinematic data (recorded by the pyroelectric sensor during the neural recordings) have been analyzed too.

2.7 Statistical analysis

Statistical analysis (ANOVA) was carried out using commercial statistical software (Statistica, StatSoft, Inc., USA). Response histograms for each experimental condition were built on rasters aligned with the instant at which the monkey touched the target handle. Histogram values were obtained by summing up all spikes occurring in each bin (20 ms) across the twelve rasters recorded during each condition. Figure 17 shows a typical histogram composed in such a way. The same figure shows also the temporal segmentation of the data into epochs (from 1st to 5th) to analyze the task-related response of the recorded neurons.

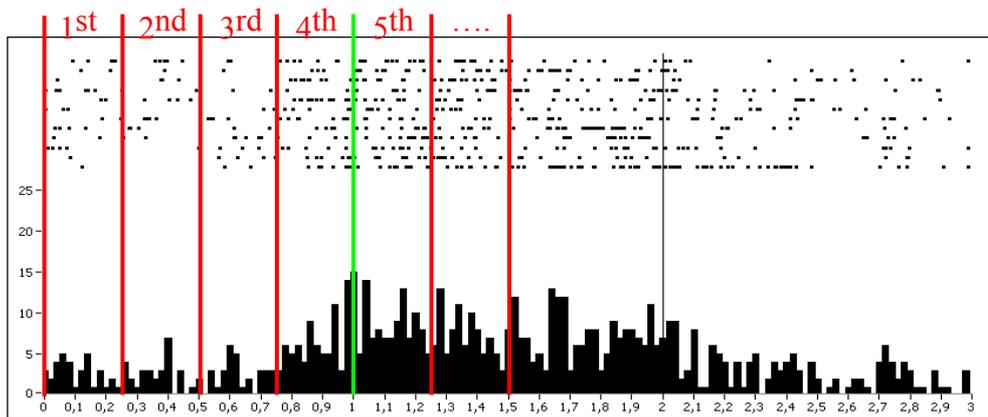


Figure 17. A typical response histogram. Each black column describes the number of spikes occurring during the corresponding 20 ms bin in all the 12 recorded rasters (shown in the uppermost part of the figure). Red lines across rasters and histogram delimitate the epochs considered for the statistical analysis. The green line indicates the instant at which the monkey touches the target handle. Rasters and histogram are aligned with this instant. Abscissae: seconds, ordinates: spikes per bin.

The following epochs have been considered for statistical analysis: (1st) Background activity, represented by the first 250 ms of each trial: Monkey's hand is still at the starting position. (2nd) Hand shaping epoch, from 250 ms before to the touch of the target handle with both thumb and index finger (precision grip). (3rd) Touch/manipulation epoch, from handle grasping to 250 ms after (door opening). For each raster, the mean spikes quantity was calculated for each epoch and compared with the spike counts in other conditions and epochs using multilevel ANOVA for repeated measures followed by Newman-Keuls post-hoc analysis, with a threshold of $P < 0.05$. Spikes counts in different epochs (spontaneous activity, hand shaping, touch/manipulation) were the dependent variable; conditions (light, dark, flash during

reaching, flash with touch, prism conditions) were the factors. Neurons in which the spikes count was not statistically different between the first epoch (background activity) and at least one of action related epochs (shaping, touch/manipulation) were rejected as not specifically responding to the experimental paradigm. All statistical results were then pasted into a Microsoft Access database to summarize the various categories of neurons.

3. Results

3.1. General characteristics of recorded neurons

After clinical testing and selection, grasping neurons recorded from area F5 (two hemispheres) and area F1 (one hemisphere) were submitted to formal testing according to the behavioral task described in the Methods section. It should be stressed here that particular care was taken to select neurons **with motor properties only**. The aim of the present work was indeed to investigate if F5 purely-motor neurons were modulated by the vision of the monkey's own acting hand. As a control, a series of recordings in area F1 (primary motor cortex) has been performed, too. A total of 112 recording sites in area F5 and 71 in area F1 have been investigated during the project. From these sites (more than 500 neurons clinically studied), 112 neurons from area F5 (out of 187 recorded) and 71 from area F1 (out of 109 recorded) were acquired during the whole formal testing. Among these, more than two thirds were completely stable during the whole testing procedure, as assessed by a statistical analysis comparing the response intensity of the first with that of the last condition (valid neurons).

3.2 General comparison between light and dark conditions

This section illustrates the results of the statistical comparison between grasping with the hand fully visible (light condition) and grasping without hand vision (dark condition). Epochs 4th (hand shaping) and 5th (touch/manipulation) have been considered for this comparison. Figures 18 and 19 illustrate one modulated and one not-modulated neuron, respectively.

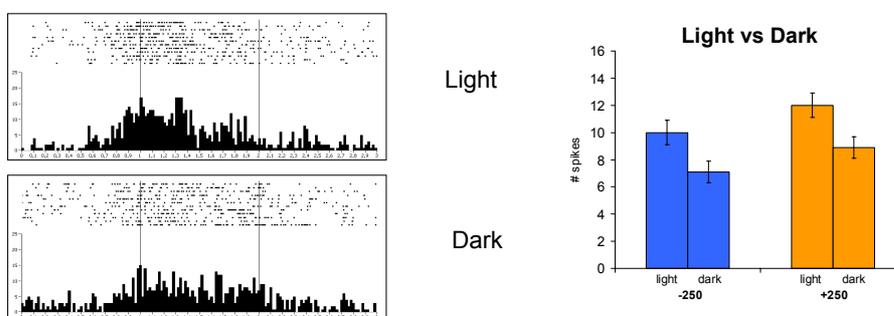


Figure 18.

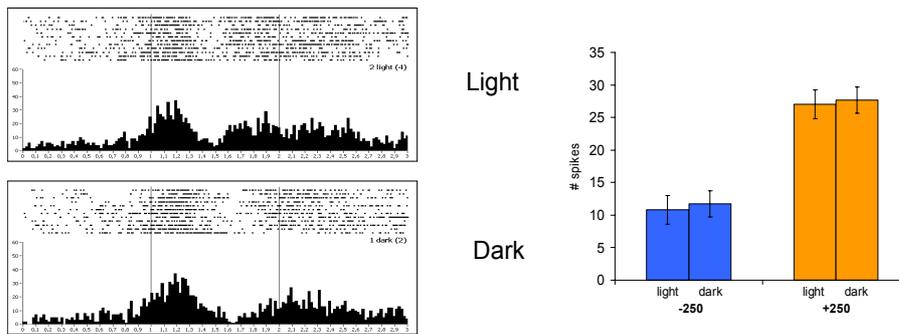


Figure 19.

In area F5, 44 out of 97 valid neurons (45,4%) showed different activity in at least one of the two action-related epochs (4th and 5th, 250 ms before touch and 250 ms after touch, respectively, see figures 18 and 19). In area F1, 34 out of 56 valid neurons (60,7%) showed different activity in at least one of the two action-related epochs. At first glance this result may appear somehow paradoxical: the percentage of modulated neurons in area F1 largely exceeded that of area F5. Although modulation could occur in both directions (i.e. response in dark larger than in light or vice versa), we were particularly interested in neurons showing a reduction of their activity in the dark condition with respect to the light one. If one takes into account the modulation critical for our hypothesis, i.e. the negative one (less activity in the dark condition with respect to the light), the prevalence of modulation in area F1 dramatically reverses. Only 5 neurons out of 34 (14,7%) satisfy this criterion. On the contrary, 25 F5 neurons out of 44 (56,8%) reduced their activity when the grasping hand was not visible. Figure 20 shows the overall modulation in F5 and F1, figure 21 depicts the positive/negative modulation during the dark condition with respect to the light one.

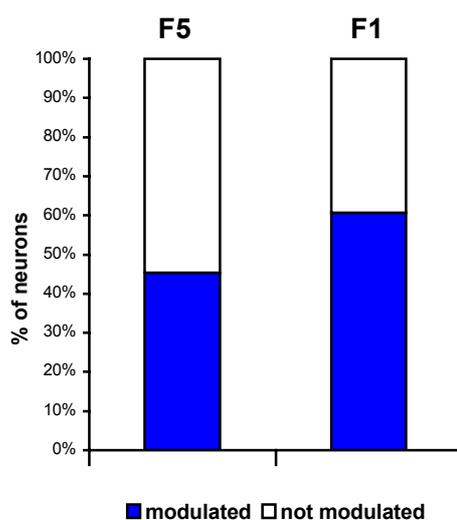


Figure 20. The relationships between modulated and not-modulated neurons in the dark condition (with respect to the light condition).

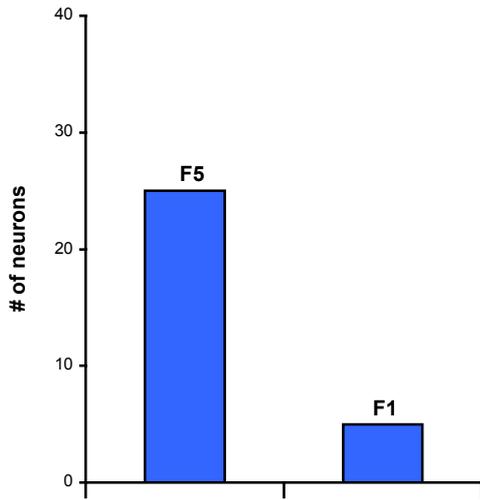


Figure 21. Negatively modulated neurons (decreasing their activity in the dark condition with respect to the light condition) in area F5 (56.8%) and in area F1 (14.7%).

In order to better clarify light/dark differences, we analyzed neurons' behavior according to the epoch-dependent modulation. Figure 22 shows the results of this analysis.

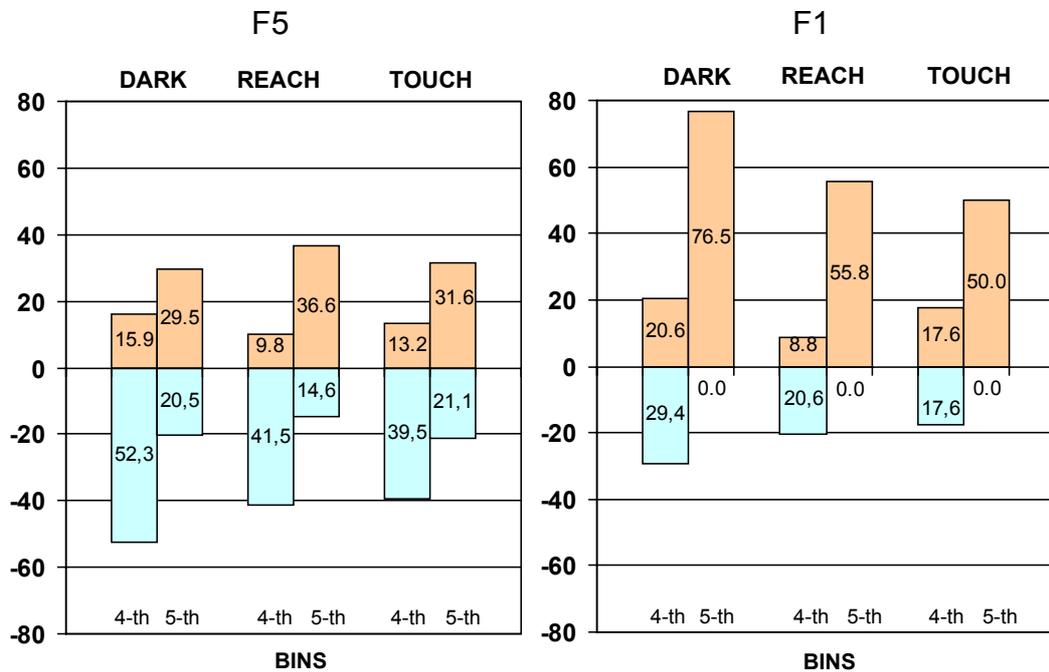


Figure 22. Percentage of statistically significant differences between light and (i) dark, (ii) hand shaping feedback (REACH), and (iii) touch feedback (TOUCH) conditions in areas F5 and F1 in the 4th and 5th epochs. Sign of ordinates refers to the direction of the modulation (see text above).

As it emerges from figure 22, apart from the already described prevalence of negative modulation in area F5 and of positive modulations in area F1, when the modulation is negative it mainly concerns the 4th epoch (instantaneous feedback during hand shaping), while when the modulation is positive, it affects mainly the 5th epoch (instantaneous feedback during handle touching). This result is particularly interesting because, in addition to the differential modulation in the two areas in terms of ‘sign’, it demonstrate a prevalence of ‘predictive’ responses (when the 4th bin is influenced by the negative effect) in area F5.

3.3 Neuronal responses to sudden hand appearance during hand shaping and during handle touch

A further aspect of our analysis was concerned with the effect on neuronal discharge of a brief flash of light, which caused a sudden appearance of the acting hand. There were two different flash conditions, during reaching and hand shaping (flash at hand shaping) and at the moment in which thumb and index finger touched the to-be-grasped handle (flash at touch). For this analysis, only light/dark negatively modulated cells were selected (see figure 21). Figure 23 shows one F5 neuron whose activity was modulated in both the aforementioned flash conditions.

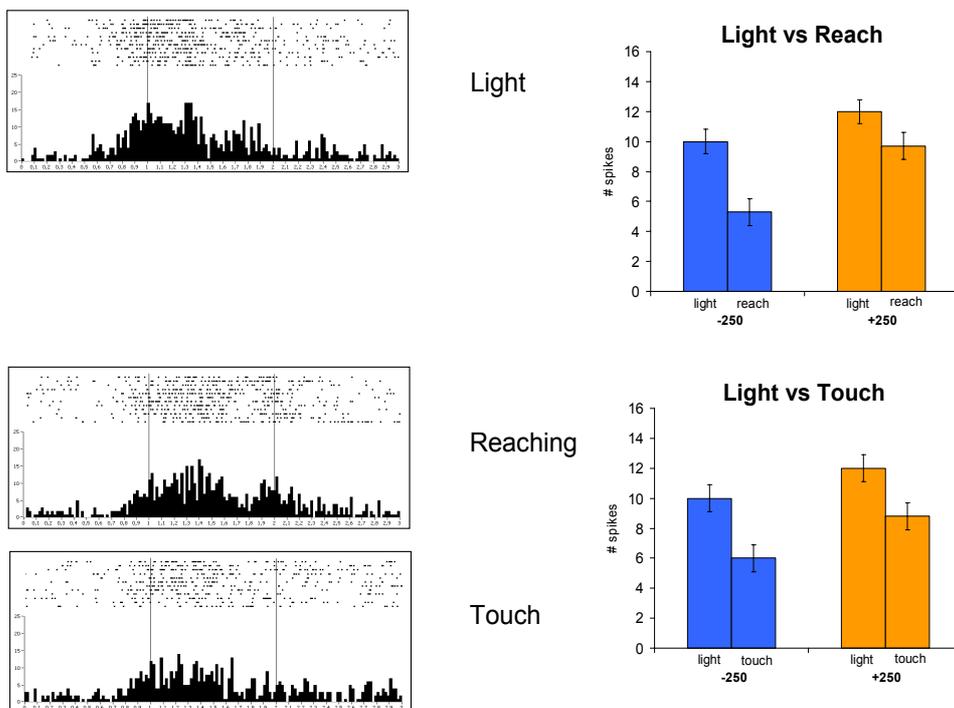


Figure 23. F5 neuron modulated by both flash conditions.

Although the dimension of our sample does not allow drawing a conclusive picture on neurons' behavior during flash conditions, it is important to stress that these two conditions were included to control for the presence of phasic modulation of activity due to own hand vision. They were therefore not necessary to validate results. Furthermore, we decided to investigate two flash conditions (one would have been enough to assess the presence of phasic activity) in order to be sure that the phasic modulation was not dependent on unspecific factors, i.e. the flash itself. We considered as particularly interesting only those neurons showing a flash-dependent modulation (in either one of the two conditions) that produced a temporal shift of the peak response. Few cells (about 10% of the modulated ones), showed this very specific phase-dependent modulation. Figure 24 shows one of these neurons, which clearly anticipated its peak during the flash at hand shaping (white trace) with respect to the dark condition (green trace).

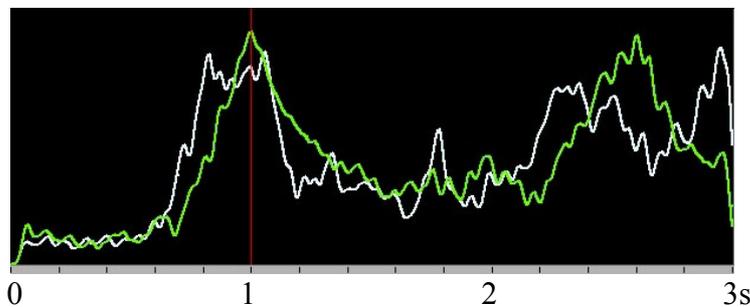


Figure 24.

3.4 The effect of prisms

This test has been performed on F5 neurons only. Although only some neurons have been tested with the prisms paradigm (about 30) because of the difficulty to keep stable the recording during the whole procedure, the effect induced by this condition was quite homogeneous in all units submitted to the full test. As shown by figure 25, the presence of visual perturbation mainly affected the touch-related epoch with a significant prevalence of positive modulation.

Although preliminary, this is an important result because it demonstrates that the prevalence of negative modulation, does not relate to some peculiar property of area F5 concerning its response to an increased feedback involvement, but is really dependent on the presence of the visual hand.

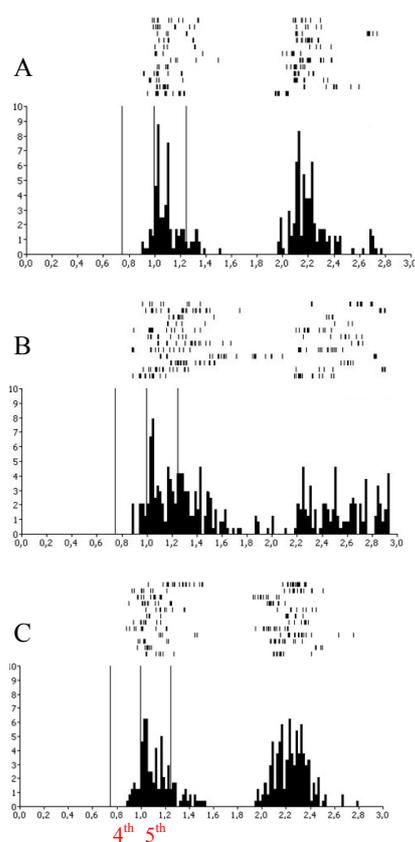


Figure 25. Example of F5 neuron recorded during grasping in light (A), grasping in light with the visual field laterally displaced of 15° by a prism (B), control condition (the same as in B but with fake prism, C). Note in B the increase of activity during the 5th epoch.

3.5 Kinematic study

The recordings of the kinematics of the hand movements during execution of experimental paradigm obtained as described in the Methods section were analyzed by taking the maximal hand aperture as the initial temporal landmark. The time intervals between this instant and the first contact with the door, the trigger event, and the door opening, were determined in this series of observations. The statistical analysis (One-Way ANOVA, $P < 0,05$) performed on time intervals in the different experimental conditions did not reveal significant differences.

4. Discussion

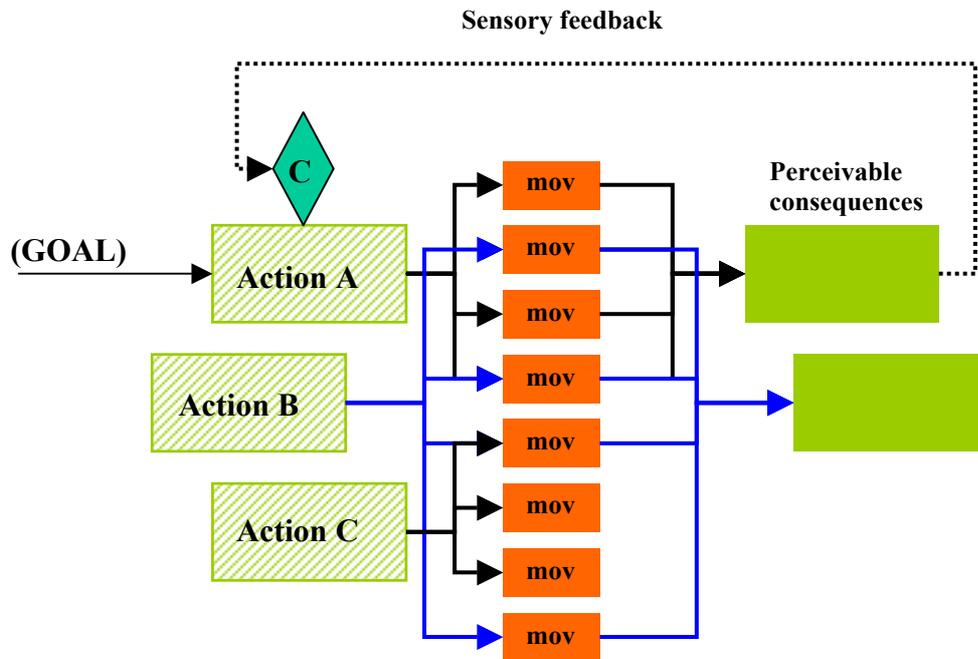
The results of monkey experiments presented in this deliverable are, in our view, of great interest. They firstly demonstrate that within a premotor area, involved in hand action programming and execution, there are motor neurons specifically modulated by the vision of monkey's own acting hand. To reach this conclusion we manipulated the visual feedback in several ways: from the simplest situation in which the monkey was requested to grasp an object in the dark, to more elaborated visual manipulations, such as the flash and prism conditions. Care was taken to preserve, as much as possible, the constancy of the actual movements during the different experimental conditions. The presence of an illuminated to-be-grasped handle (the level of light was, however, so low that the approaching fingers never became visible to the animal) was probably the most effective solution adopted to allow the animal to reach the target in the dark with sufficient movement smoothness. The analysis of kinematic data, recorded during light and dark trials, confirmed this substantial constancy of the grasping movement across all conditions. In addition, we performed some neuron recordings in area F1 (primary motor cortex), whose elements are considered more movement-related than those of area F5. A difference in neuronal modulation between these two areas could therefore reinforce our starting hypothesis.

The first important result achieved by these experiments is related to the direction of the modulation. In contrast with area F1, F5 motor neurons are negatively modulated by the absence of the visual hand. This reduction of the response could be, very likely, attributed to the lack of the hand-related visual input reaching F5 neurons during grasping in light. The second result is that, when a negative modulation occurs, in general involves the epoch preceding handle touching. If one considers that prediction is strongly embedded in feed-forward control systems, this anticipatory effect, specific for area F5, speaks in favor of a control role played by this area. Let us now discuss more in detail this possibility and, then, try to link this control function to the more general problem of action recognition.

A series of neurophysiological evidence is in favor of the idea that motor programs are characterized by a hierarchical structure, in which lower level procedures are embedded into more and more general action representations. For example, the representation of the action "to-grasp-an-apple" contains, embedded, the reaching and grasping programs, which in turn, are composed of low-level routines for the control of muscular synergies and, finally, even for single joints mobilization.

Ventral premotor cortex forms a reservoir (vocabulary) of action representations such as

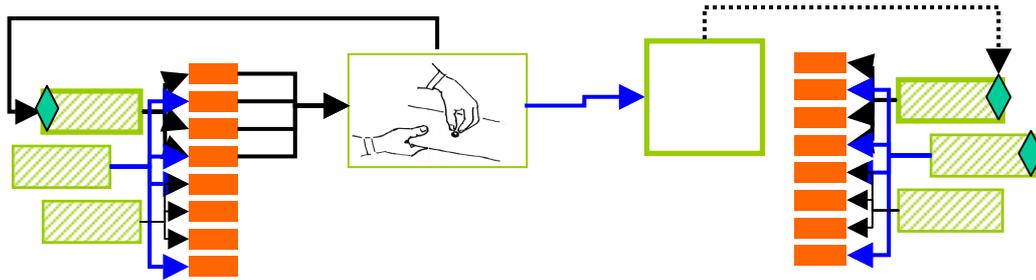
‘grasping’, ‘taking possession of’, ‘manipulating’, etc. The degree of specification of each of these ‘motor words’ may vary among different neurons (from a very general representation of ‘grasping’ to a very specified one, such as ‘grasping a small, soft, object with the thumb and the index finger’) (see Rizzolatti and Fadiga, 1998). A schematic description of this hierarchical structure is given by the next figure.



Here one can see, depicted in a very simplified way, a model of action representation. Actions (F5 level) are represented in the leftmost part of the figure, they are driven by the ‘desire’ to achieve a certain goal and, if activated, activate in turn a set of motor synergies, here depicted in orange (mov, F1 level). Action generation, however, does not produce consequences only on the external environment. On the contrary, a series of afferent signals come back, from the periphery to the brain. These proprioceptive, visual, auditory signals (perceivable consequences, in the figure), are constantly monitored by the brain and used to control the development of the ongoing action, signaling also the goal achievement. The hypothesis we suggest (**and that has been also tested by the general model of action recognition coming out from the MIRROR project**) is that proprioceptive and motor information, biologically invariant by definition during the actuation of a same motor command, are used by the brain to generalize (and to validate) the visual inputs related to the ongoing action. These visual inputs, that continuously vary depending on the position of the head with respect to the acting hand, are forcedly considered as homogeneous because are generated by the same (or very similar) motor program. This visuomotor coupling mechanism should play a very relevant role during

development, when our motor competencies are growing up. The data we present here demonstrate that this visuomotor coupling is at work also in adult individuals, and that premotor neurons, apparently devoid of any visual property, indeed receive facilitatory inputs activated by the vision of one's own acting hand.

Which is the relationship between the hypothesis described above and the mirror neurons, originally found in area F5? A possible answer to this question can be given by the next figure.



This figure depicts two individual 'brains', each one organized according to the scheme of the previous figure. When the individual on the left grasps a small object (s/he is left handed, but this is irrelevant for our purposes) her motor system receives a visual description of the ongoing movement that could be used to control its correct execution. At the same time, however, the right 'brain' sees the same scene (with some changes of perspective). Due to the visuomotor coupling s/he created for her own movements through the process previously described, this visual representation of the seen action could gain the access to the correspondent motor representation (following the dotted line). This is, in our view, the 'recognition' operation played by mirror neurons. The finding of the present experiment that in area F5 there are neurons satisfying the condition we postulated in our hypothesis, is a strong argument in favor of this interpretation. More additional experiments are required to definitely demonstrate it, such as extend our testing to F5 mirror neurons, as well as the extension of the investigation to areas possessing mirror properties different from area F5. This possibility was indeed programmed in our original proposal but, in consideration of the results coming out from the experiment described in this deliverable, we preferred to more deeply investigate the found effect in order to demonstrate its validity, before moving outside area F5 to explore different cortical areas.

PART B: TMS EXPERIMENTS

A series of studies on the brain correlates of the verbal function demonstrate the involvement of Broca's region (BA44) during both speech generation (see Liotti et al. 1994 for review) and speech perception (see Papathanassiou et al. 2000 for a review of recent papers). Recently, however, several experiments have shown that Broca's area is involved also in very different cognitive and perceptual tasks, not necessarily related to speech. Brain imaging experiments have highlighted the possible contribution of BA44 in "pure" memory processes (Mecklinger et al, 2002; Ranganath et al. 2003), in calculation tasks (Gruber et al 2001), in harmonic incongruity perception (Maess et al. 2001), in tonal frequency discrimination (Muller et al, 2001) and in binocular disparity (Negawa et al, 2002). Another important contribution of BA44 is certainly found in the motor domain and motor-related processes. Gerlach and colleagues (2002) found an activation of BA44 during a categorization task only if performed on artifacts. Kellenbach and colleagues (2003) found a similar activation when subjects were required to answer a question concerning the action evoked by manipulable objects. Several studies reported a significant activation of BA44 during execution of grasping and manipulation (Binkofski et al, 1999ab; Gerardin et al, 2000; Grezes et al, 2003; Hamzei et al, 2003; Lacquaniti et al, 1997; Matsumura et al, 1996; Nishitani et Hari, 2000). Moreover, the activation of BA44 is not restricted to motor execution but spreads over to motor imagery (Binkofski et al, 2000; Gerardin et al, 2000; Grezes et Decety, 2002).

From a cytoarchitectonical point of view (Petrides and Pandya, 1997), the monkey's frontal area which closely resembles human Broca's region is a premotor area (area F5 as defined by Matelli et al. 1985). Single neuron studies (see Rizzolatti et al. 1988) showed that in area F5 are represented hand and mouth movements. The specificity of the goal seems to be an essential prerequisite in activating these neurons. The same neurons that discharge during grasping, holding, tearing, manipulating, are silent when the monkey performs actions that involve a similar muscular pattern but with a different goal (i.e. grasping to put away, scratching, grooming, etc.). All F5 neurons share similar motor properties. In addition to their motor discharge, however, a particular class of F5 neurons discharge also when the monkey observes another individual making an action in front of it ("mirror neurons"; di Pellegrino et al., 1992, Gallese et al., 1996; Rizzolatti et al., 1996a). There is a strict congruence between visual and motor properties of F5 mirror neurons: e.g., mirror neurons motorically coding whole hand prehension discharge during

observation of whole hand prehension performed by the experimenter but not during observation of precision grasp. The most likely interpretation for the visual response of these visuomotor neurons is that, at least in adult individuals, there is a close link between action-related visual stimuli and the corresponding actions that pertain to monkey's motor repertoire. Thus, every time the monkey observes the execution of an action, the related F5 neurons are addressed and the specific action representation is "automatically" evoked. Under certain circumstances it guides the execution of the movement, under others, it remains an unexecuted representation of it, that might be used to understand what others are doing.

Transcranial magnetic stimulation (TMS) (Fadiga et al. 1995; Strafella and Paus 2000) and brain imaging experiments demonstrated that a mirror-neuron system is present also in humans: when the participants observe actions made by human arms or hands, motor cortex becomes facilitated (this is shown by TMS studies) and cortical activations are present in the ventral premotor/inferior frontal cortex (Rizzolatti et al. 1996b; Grafton et al. 1996; Decety et al. 1997; Grèzes et al. 1998; Iacoboni et al. 1999, Decety and Chaminade; 2003; Grèzes et al. 2003). Grèzes et al. (1998) showed that the observation of meaningful but not that of meaningless hand actions activates the left inferior frontal gyrus (Broca's region). Two further studies have shown that observation of meaningful hand-object interaction is more effective in activating Broca's area than observation of non goal-directed movements (Hamzei et al, 2003; Johnson-Frey et al, 2003). Similar conclusions have been reached also for mouth movement observation (Campbell et al, 2001). In addition, direct evidence for an observation/execution matching system has been recently provided by two experiments, one employing fMRI technique (Iacoboni et al 1999), the other using event-related MEG (Nishitani and Hari, 2000), that directly compared in the same subjects action observation and action execution.

The evidence that Broca's area is activated during time perception (Schubotz et al 2000), calculation tasks (Gruber et al 2001), harmonic incongruity perception (Maess et al. 2001), tonal frequency discrimination (Muller et al, 2001), prediction of sequential patterns (Schubotz and von Cramon 2002a) as well as during prediction of increasingly complex target motion (Schubotz and von Cramon 2002b), suggests that this area could play a central role in the representation of sequential information in several different domains. This could be crucial for action understanding, allowing the parsing of observed actions on the basis of the predictions of their outcomes. Others' actions do not generate only visually perceivable signals. Action-generated sounds and noises are also very common in nature. In a very recent experiment Kohler and colleagues (2002) have found

that 13% of the investigated F5 neurons discharge both when the monkey performed a hand action and when it heard the action-related sound. Moreover, most of these neurons discharge also when the monkey observed the same action demonstrating that these ‘audio-visual mirror neurons’ represent actions, independently of whether they are *performed, heard or seen*. The presence of an audio-motor resonance in a region that, in humans, is classically considered a speech-related area, prompts the Liberman’s hypothesis on the mechanism at the basis of speech perception (motor theory of speech perception, Liberman et al., 1967; Liberman and Mattingly, 1985; Liberman and Wahlen, 2000). This theory maintains that the ultimate constituents of speech are not sounds but articulatory gestures that have evolved exclusively at the service of language. Speech perception and speech production processes could thus use a common repertoire of motor primitives that, during speech production, are at the basis of articulatory gesture generation, and during speech perception are activated in the listener as the result of an acoustically evoked motor “resonance”. According to Liberman’s theory, the listener understands the speaker when her articulatory gestures representations are activated by the listening to verbal sounds. Although this theory is not unanimously accepted, it propose a plausible model of an action/perception cycle in the frame of speech processing.

To investigate if speech listening activates listener’s motor representations, we administered TMS on cortical tongue motor representation (Fadiga et al., 2002), while subjects were listening to various verbal and non-verbal stimuli. Motor evoked potentials (MEPs) were recorded from subjects’ tongue muscles. Results showed that during listening of words formed by consonants implying tongue mobilization (i.e. Italian ‘R’ vs. ‘F’) MEPs significantly increased. This indicates that when an individual listens to verbal stimuli, his/her speech related motor centers are specifically activated. Moreover, words-related facilitation was significantly larger than pseudo-words related one.

The presence of “audio-visual” mirror neurons in the monkey and the presence of “speech-related acoustic motor resonance” in humans, suggests that, independently from the sensory nature of the perceived stimulus, the mirror-neuron resonant system retrieves from the action vocabulary (stored in the frontal cortex) the stimulus-related motor representations. It is however unclear if the activation of the motor system during speech listening is causally related to speech perception, or if it is a mere epiphenomenon due, for example, to an automatic compulsion to repeat without any role in speech processing. One experimental approach to answer this question could be to interfere with speech

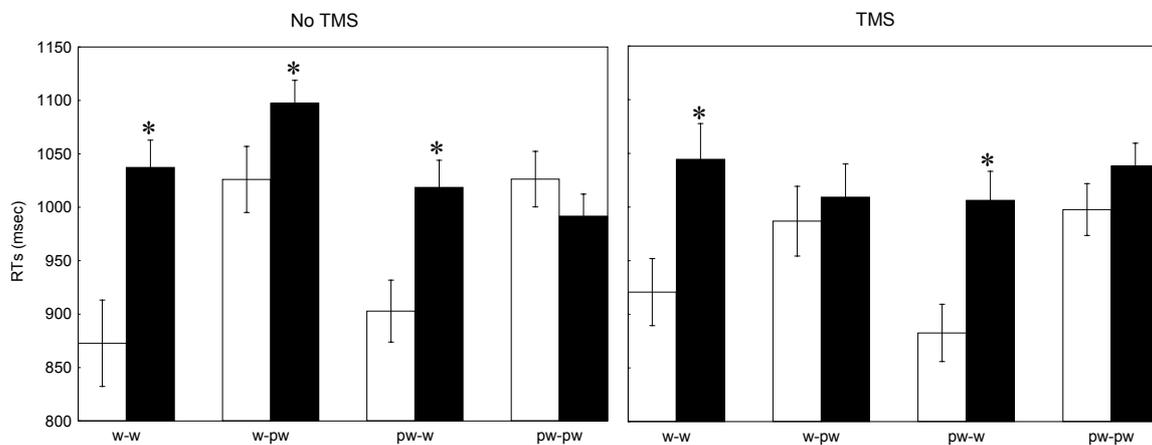
perception by applying TMS on speech-related areas. Although classical theories consider the inferior frontal gyrus as the “motor center” for speech production, cytoarchitectonical homologies with monkey area F5, and brain imaging and patients studies (among more recent publications see Watkins and Paus, 2004; Dronkers et al. 2004, Wilson et al 2004) suggest that this region may play a fundamental role in perceived speech processing. Broca’s area was therefore selected as the better candidate for our study.

In order to investigate a possible role of Broca’s area in speech perception, both at the lexical and at the phonological level (Fadiga et al. 2002 showed that both these speech-related properties influence motor resonance) we selected a priming paradigm. Priming experiments, in general, demonstrate that whenever a word (target) is preceded by a somehow related word (prime) it is processed faster than when it is preceded by an unrelated word. The prime can therefore have either a semantic or phonologic relation with the target. Our starting aim was to test the possibility to modulate this facilitation by interfering on Broca’s activity with TMS. A magnetic stimulus delivered immediately after the listening of the prime, on a functionally-related brain region, should impair prime processing, resulting in a modification in the priming effect. In our experiment we used the paradigm by Emmorey et al. (1989) in which subjects are requested to perform a lexical decision on a target preceded by a rhyming or not rhyming prime. By manipulating the lexical content of both the prime and the target stimuli (Emmorey et al. used only word prime), in addition to the rhyming effect, we tested also the role played by Broca’s area at the lexical level. Single pulse TMS was administered on Broca’s region in 50% of the trials, while subjects were submitted to a lexical decision task on the target. Subjects had to respond by pressing one of two switches with their left index finger. TMS was administered during the 20 msec pause between prime and target acoustic presentation (ISI). The click of the stimulator never overlapped with the acoustic stimuli. The pairs of verbal stimuli could pertain to four categories which differed for presence of lexical content (words vs pseudo-words) in the prime and in the target (Table 1).

Table1. Example of the stimuli used in the experiment.

	Rhyming	Not-rhyming
Word/word	zucca (pumpkin)- mucca (cow)	fiume (river)- scuola (school)
Word/pseudo-word	freno (brake)- preno	strada (street)- terto
Pseudo-word/word	losse-tosse (cough)	stali-letto (bed)
Pseudo-word/pseudo-word	polta-solta	brona-dasta

From data analysis on trials without TMS (see the figure) an interesting (and unexpected) finding emerged: lexical content of the stimuli modulates the phonological priming effect. No rhyming effect was found in the pseudo-word/pseudo-word condition in which neither the target nor the prime has the access to the lexicon. In other words, in order to have a phonological effect it is necessary to have the access to the lexicon. In trials during which TMS was delivered, a TMS-dependent effect was found only in pairs where the prime was a word and the target was a pseudo-word, and consisted in the abolition of the phonological priming effect. Thus, TMS on Broca's area made the pairs word/pseudo-word similar to the pseudo-word/pseudo-word ones.



Legend: Reaction times (RTs \pm SEM in msec) for the lexical decision during the phonological priming task, without (left panel) and with (right panel) TMS administration. White bars: conditions in which prime and target share a rhyme. Black bars: no rhyme. Asterisk on the black bar means the presence ($p > 0.05$, Newman-Keuls test) of a phonological priming effect (response to rhyming target faster than response to not-rhyming target) in the relative condition. TMS administration did not influence the accuracy of the participants that was almost always close to 100%. W-W, prime-word/target-word; W-PW, prime-word/target-pseudo-word; PW-W, prime-pseudo-word/target-word; PW-PW, prime-pseudo-word/target-pseudo-word.

This finding suggests that the stimulation of the Broca's region might have affected the rhyming effect not because it interferes with phonological processing but because it interferes with lexical categorization of the prime. In support to this interpretation are recent results from Blumstein and colleagues (2000) who have found that Broca's aphasics display deficits in the facilitation of lexical decision targets by prime words that rhyme with the target. In contrast, Wernicke's aphasics showed a pattern of results similar to that of normal subjects. Moreover, Milberg et al. (1988), in a phonological distortion study, showed that Broca's aphasics failed to show semantic priming when the

phonological form of the prime stimulus was distorted. The authors interpreted this finding in the framework of the hypothesis that Broca's aphasics have reduced lexical activation levels (Utman et al. 2001). As a result, while in normal subjects an acoustically degraded input is able to activate the lexical representation, in aphasics it fails to reach a sufficient level of activation. However, there is evidence that Broca's aphasics have impaired lexical access even in response to intact acoustic inputs (Milberg et al. 1988). The results of our TMS experiment on phonological priming, together with the data on patients reported above, lead to the conclusion that Broca's region is not the main responsible for the acoustic motor resonance effect shown by Fadiga et al. (2002). This effect was in fact present during listening of both words and pseudo-words and was only partially related to lexical properties of the heard stimuli. The localization of the premotor area involved in such a "low level" motor resonance will be the argument of our future experimental work.

The general interpretation we propose here is that Broca's involvement during speech processing, more than indicating a speech-specific role for this area, may reflect its general involvement in meaningful action recognition. This possibility finds its basis on the observation that, in addition to speech-related activation, this area is activated during observation of meaningful hand or mouth actions. Speech represents a particular case of this general framework: among meaningful actions, phonoarticulatory gestures are meaningful actions conveying words. This hypothesis is moreover supported by the observation that Broca's aphasics, in addition to speech production deficits, show an impaired access to the lexicon (although for some category of verbal stimuli). The consideration that Broca's area is the human homologue of monkey mirror neurons area, opens the possibility that human language may have evolved from an ancient ability to recognize actions performed by others, visually or acoustically perceived. The Liberman's intuition that the ultimate constituents of speech are not sounds but articulatory gestures that have evolved exclusively at the service of language, seems to us a good way to consider speech processing in the more general context of action recognition.

PART C: BEHAVIORAL DEVELOPMENT

During the third year of Mirror we focused our activities on 3 separate problem areas related to the Mirror project: the basic neural processes, the development of predictive action and the development of manipulative capabilities. These different studies are described in the following sections:

1. Basic neural processes

Action control is crucially dependent on the ability to perceive motion. We have earlier found that the ability to smoothly pursue moving objects with the eyes emerges between 6 and 14 weeks of age. The aim of the present research was to identify the cortical changes associated with these emerging abilities. We used high-density EEG (EGI 128 sensor net) in an ERP design to detect neural activity in 2-, 3-, and 5-month-old infants when they watched a static or rotating pattern. It consisted of an inner (smaller) and an outer (larger) set of simple geometric figures, rotating in opposite directions at 60 deg/s. This pattern was chosen because it has a very small tendency to elicit smooth pursuit eye movements in infants. The onset of motion was randomly determined. In addition to the infants, an adult group was examined.

The ERP in the 2-month-olds is a minor response at 290 ms observed in MT region on the left side. The 3-month-olds showed consistent unilateral left side ERP, significant at 260 ms and the ERP of the 5-month-olds was bilateral but had an earlier onset on the left (150 ms) than on the right side (410 ms). Adults showed stable bilateral activation starting at 120 ms on the left, and 150 ms right side respectively. Furthermore, ERP in the parietal region was only observed in the 5-month olds and in the adults, bilaterally in both groups. The results are consistent with other indicators of the development of motion processing competence over this age period and demonstrate the increasing involvement of the MT/V5. Furthermore we found that the latency of the ERP is related to the gain of smooth pursuit calculated from a number of infants (15). The unilateral activation on the left side at 3 months has not been reported before. This result may explain why children with unilateral congenital cataracts, tested at 6 years of age do not show deprived perception of global motion while those with bilateral cataracts do. If the left MT/V5 is connected to both visual fields at this age, it would remain functional even if there is a cataract in one eye. Another suggestion is that the onset of ERP on the right side at 5 month of age is associated with the development of reaching at this age. As found by others, the right hemisphere is involved in visual processing prior to reaching movements. The right temporal area is dominant in processing visual-spatial information for reaching.

2. Predictive action

During this year we have continued our studies on predictive actions in infants. Two kinds of studies have been conducted. Two kinds of actions have been studied: tracking objects over temporary occlusion and catching moving objects.

Tracking objects over temporary occlusion: This is an important cognitive skill because it requires the infant to represent the moving object in its visual absence. Such skills are extremely important in the planning of action in general. We studied the emerging ability to represent an oscillating moving object over occlusions in 7- to 21-week-old infants. The object moved at 0.25 Hz and was either occluded at the center of the trajectory (for 0.3 s) or at one turning point (for 0.7 s). Each trial lasted for 20 s. Both eye and head movements were measured. By using two kinds of motion, sinusoidal (varying velocity) and triangular (constant velocity), infants' ability to take velocity change into account when predicting the reappearance of the moving object was tested. Over the age period studied, performance at the central occluder progressed from almost total ignorance of what happened to consistent predictive behavior. From around 12 weeks of age, infants began to form representations of the moving object that persisted over temporary occlusions. At around 5 months of age these representations began to incorporate the dynamics of the represented motion. Strong learning effects were obtained over single trials, but there was no evidence of retention between trials.

Catching moving objects: We investigated infants' ability to catch moving objects with a new device that presents objects moving on a vertical flat screen. On the back of the screen two orthogonally positioned servomotors are placed that control the motion of a magnet. At the base of the object on the front of the screen there was another magnet. When the magnet on the object's supporting rod was placed on the metal sheet directly over the magnet on the back, the combined attraction held the object in place and caused it to undergo whatever motion was produced by the plotter. We investigated infants' ability to deal with two different kinds of motion. Adults perceive an object that moves on an elliptical path as having constant velocity when it slows down according to a sinusoidal function towards the end points of its longest axis (Viviani and Stucchi, 1992). Infants also perceive an elliptical motion with constant speed to accelerate towards the endpoint of the longest axis. Both kinds of motion are found in nature. The reason why adults perceive object motion in this way could be because they have much experience with biological motion (the sinusoidal case) or it could be an inherent constraint on the perception of motion.

We have found effects of the shape of the trajectory on infants' reaching. The aiming as well as the number of movement units (MU) were affected but the underlying principles remains to be unveiled. This work is in progress and we will continue to analyze MUs and aiming of reaches to get a better understanding of the development of predictive reaching of physical and biological motions.

3. Manipulation.

During the second year of life, infants are fascinated by problems of how to relate objects to each other. For instance, they find it very attractive to pile objects, put lids on pans, and insert objects into holes. The ability to solve such problems reflects infants' developing spatial perception and cognition. To fit an object into an aperture, for instance, the size of the object and aperture must be perceived and the relationship between the two. This information could then be used to plan the fitting action in a prospective and economical way. Thus, the degree of sophistication in the planning of actions on objects is informative about infants' perception of object properties and their ability to use this information in a functional way. This opens a window for studying the development of object perception and spatial cognition. The planning of early reaches shows that infants perceive the orientation and size of the objects reached for. Reaching is also organized differently depending on what the infant intends to do with the object. A ball is picked up in one way if it is going to be fit into a tube and in another way if it is going to be thrown into a tub (Claxton et al, 2003). We studied the understanding of the spatial relationships between objects and apertures in 14- to 26-month-old infants'. The task was to insert objects with various cross-sections (circular, square, rectangular, elliptic, and triangular) into apertures in which they fitted snugly. Task difficulty was increased from a circle to a triangle. The cylinder fitted into the aperture as long as its axis was perpendicular to it, while the right-angled triangular object, in addition, had to be turned in a unique and specific way. Results show that 14-month-olds understand the task and like it but have only vague ideas of how to orient the object to fit the aperture. Younger infants spent more time on transporting the objects to the lid, spent more time trying to fit them into the apertures, and made more explorative adjustments than older ones. 14-month-old infants turned more often to the parent, moved the object from one hand to the other, and conveyed it to the mouth, before transporting it to the lid. Such transactions were less common in the 26-month-olds. The success rate was influenced by the mode of presentation. If the object was lying down when presented, the younger infants often failed to raise it up before trying to insert it into the aperture. At the moment we have expanded these studies to include choices between objects and apertures. I the

infants are shown two objects of which one fits the aperture, they have to figure out which object fits ahead of picking it up. If they are shown one object and two apertures, they have to move the object towards the correct hole optimally from the onset of the movement. Thus both of these tasks reflect the degree of planning in executing them.

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