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## Dorsal premotor areas of nonhuman primate: functional flexibility in time domain

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**Abstract** A voluntary motor act requires recognition of the informational content of an instruction. An instruction may contain spatial and temporal information. The recently proved role of the monkey frontal cortex in time computation, as well as in motor preparation and motor learning, suggested that we investigate the relationship between premotor neuron discharges and the temporal feature of the visual instructions. To this purpose, we manipulated the duration of an instructional cue in a visuomotor task while recording unit activity. We found two types of premotor neurons characterised by a discharge varying in relation to the duration of the cue: (1) “motor-linked” neurons, with a specific premotor activity constantly bounded to the motor act; (2) “short-term encoders” neurons, with a premotor activity depending on the cue duration. The cue duration was the critical factor in determining the behaviour of the short-term encoders cells: when the cue ranged from 0.5 s to 1 s, they presented a preparatory activity; when the cue was longer, up to 2 s, they lost their preparatory activity; when the cue was blinked the

cells anticipated their discharge. The activity changed in few trials. These data confirm and highlight the role of frontal cortex in encoding specific cues with a temporal flexibility, which may be the expression of temporal learning and represent an extended aspect of cortical plasticity in time domain.

**Keywords** Frontal cortex · Nonhuman primate · Unit activity · Instructional cue · Temporal neuronal flexibility

### Introduction

To achieve their goal, motor actions must be spatially and temporally ordered. The consequence is that movements are precisely planned before being executed. The motor program is conceived as made up of a set of instructions specifying the spatial and temporal parameters of movements and the time computation is particularly relevant when a motor act has to be executed in relation to an instruction.

The premotor area (PM), which is a part of frontal cortex, is known to be involved in movement programming and execution (Weinrich and Wise 1982; Goldberg 1985; Schlag and Schlag-Rey 1987; Caminiti et al. 1990; Mushiaki et al. 1991; Schall 1991; Kurata 1993; Rizzolatti et al. 1998; Tehovnik et al. 2000), in cognitive and attentional phenomena (Rizzolatti et al. 1983; Bon and Lucchetti 1997; Lucchetti et al. 1998; Olson et al. 2000), and in motor and temporal learning (Mitz et al. 1991; Chen and Wise 1995; Deiber et al. 1997, Wise et al. 1998; Lucchetti and Bon 2001). In addition, the role that the premotor area plays in timing ability has been confirmed by lesion studies in humans (Halsband et al. 1993).

In particular, “set related” activity is a pattern of activity observed in the dorsal part of monkey premotor area (PMd); it is considered to reflect both sensory information processing and motor aspects of preparatory set. To better study the characteristics of this pattern of activity, in many experiments the behavioural

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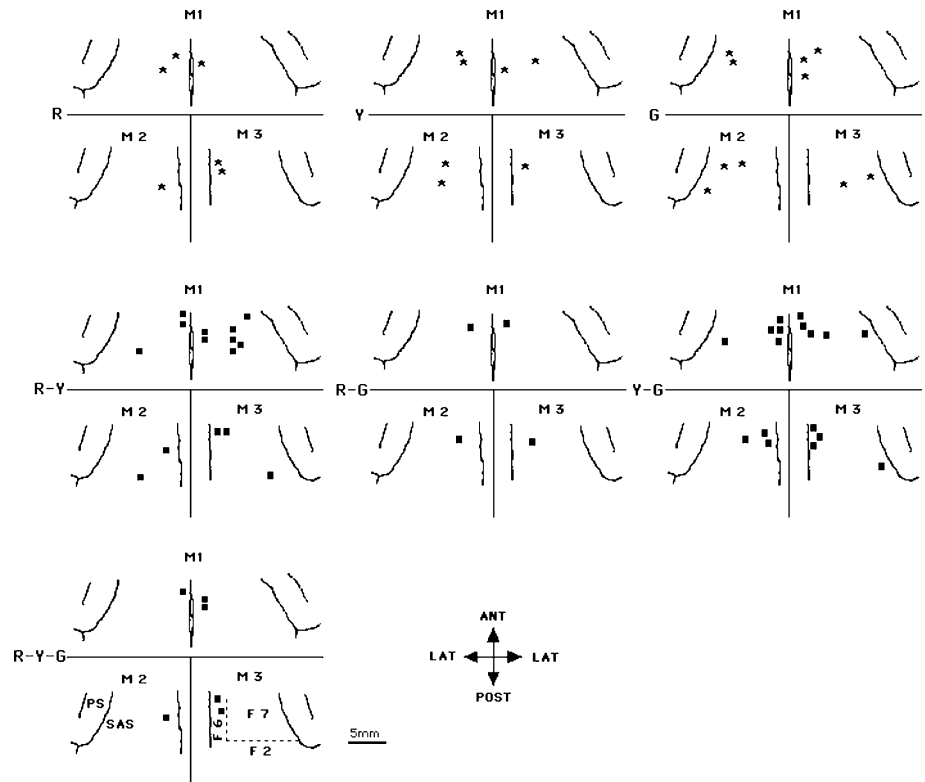
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**Fig. 1** The reconstructed maps of tracks in four hemispheres of three monkeys (M1, M2 and M3). Each penetration is represented with a symbol depending on the type of cells recorded. *R* red cells; *Y* yellow cells; *G* green cells; *R-Y* red-yellow cells; *R-G* red-green cells; *Y-G* yellow-green cells; *R-Y-G* red-yellow-green cells. *PS* principal sulcus, *SAS* superior arcuate sulcus. F7-F2 PMd, F6 pre-SMA



paradigm comprises an instructed delay period in which an instructional cue provides prior information about the characteristics of a movement to be executed after the delay. In this experimental paradigm, neuronal activity during the instructed-delay period running between the cue and subsequent go-signal is presumed to reflect early planning stages initiated by the prior information (Riehle and Requin 1989; Crammond and Kalaska 2000).

On the other hand, estimation of time intervals is also an essential aspect of motor preparation. Although a correct estimation of exactly when to move is necessary to initiate voluntary movement, time representation in cerebral cortex is still under investigation. Electrophysiological and positron emission tomography (PET) studies on humans suggest that supplementary motor area (SMA), medial prefrontal cortex and primary motor cortex play a role in temporal mechanisms (Sasaki and Gemba 1982; Vidal et al. 1995; Maquet et al. 1996). Lesion studies in humans (Halsband et al. 1993) and functional magnetic resonance imaging (fMRI) investigations (Rao et al. 1997; Gruber et al. 2000; Bengtsson et al. 2004) confirmed the role of the SMA and premotor cortex in timing ability. Leon and Shadlen (2003) found that neurons in the posterior parietal cortex of the monkey encode signals related to the perception of time. Roux et al. (2003) showed that timing is represented in neuronal activity of the monkey motor cortex in a strictly context-dependent manner, since it only takes place when information for a task aspect is provided. Lucchetti and Bon (2001) suggested that PMd is also

involved in temporal learning. Infact, the discharge of arm-related build-up neurons was modified in relation to the predictability or unpredictability of the duration of an instructional pre-cue stimulus in a visuomotor task and the authors suggested that these neurons are involved in a learning process in the time domain.

The flexibility of premotor cell discharge in relation to the informational content of the cue (Tanji et al. 1980; Riehle and Requin 1989; Crammond and Kalaska 2000; Lucchetti and Bon 2001) suggested that we investigate whether the premotor neuron discharge also presents some degree of functional flexibility in relation to the temporal characteristics of an instructional cue. For this purpose, we aimed at generating a succession of different temporal cue durations to ascertain whether premotor cortex neurons changed their activity after the duration of an instructional cue changed.

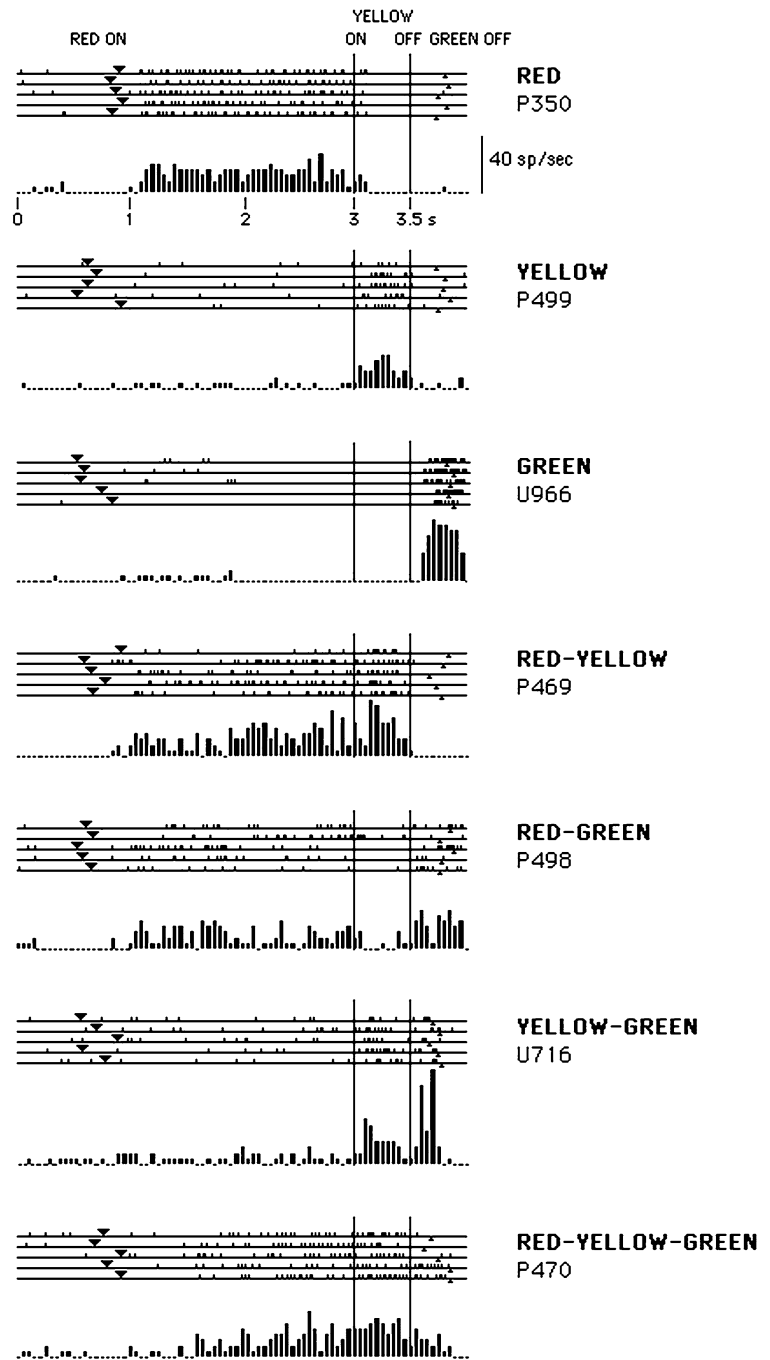
## Methods

### Behavioural methods

The experiments were carried out on three monkeys (*Macaca fascicularis*) in four hemispheres. The animals were trained for three visuomotor tasks: (1) fixation task; (2) fixation-blink task; (3) saccade task.

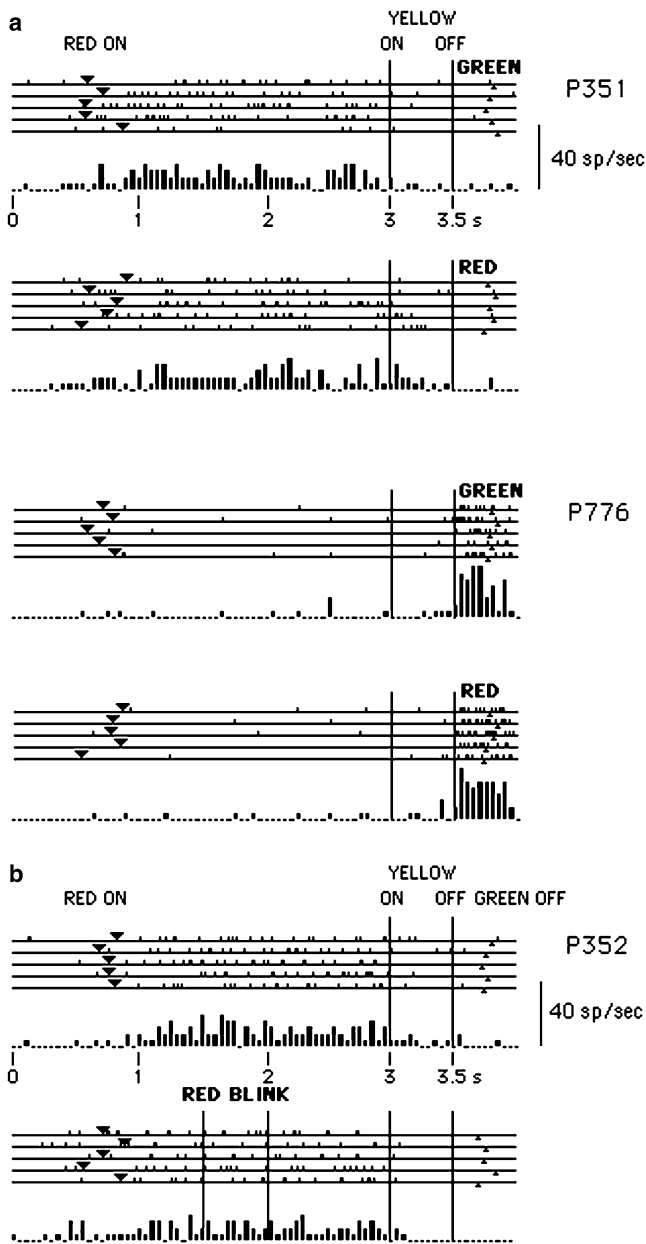
The fixation task was utilised, as probe task, in different spatial positions: the targets (nine in all) were displayed in the form of a cross on a tangent screen placed at a distance of 114 cm from the animal's face.

**Fig. 2** Task-related arm movement cells. For each example, the raster of five trials and the corresponding histogram, aligned with the onset of the yellow period, are reported. In each raster, the *arrows* on the left side show the red onset, while the *arrows* on the right side show the green offset, i.e., the bar release. Bin width of 50 ms



One target was located at the intersection of the cross; the other eight were distributed two to each arm, 10 and 20 degrees, respectively, from the intersection in an up-down, left-right configuration. The animal had to press a bar, positioned before it on the same side as the hand contralateral to the hemisphere being recorded; a target (a tricoloured light-emitting diode (LED)) would then go on red (red period, Rp), after which the LED turned yellow (yellow period, Yp) and then green (green period, Gp). In the first 500 ms of red period the animal had to direct its gaze towards the target and maintain fixation until the green light went on. During green period the

animal had to release the bar to receive some drops of sweetened water as a reward. During training, Rp lasted a random time ranging from 2 s to 2.5 s, Yp was fixed at 0.5 s and Gp was chosen between 0.5 s and 0.8 s in relation to the monkey's characteristic reaction time. The red stimulus, representing an instructional pre-cue, required the monkey to maintain fixation and keep the bar pressed; the yellow stimulus, representing an instructional cue, required it to maintain fixation and prepare to release the bar, while the green stimulus was the go-signal to release the bar. During the recording sessions, the arm-related cells that presented premotor



**Fig. 3** The cellular activity is neither colour related nor visual **a** *Upper part*: the substitution of the green light with the red one does not modify the activity of a red cell. *Lower part*: the substitution of the green light with the red one does not modify the activity of a green cell. **b** The fixation-blink task does not modify the activity of a red cell. In each raster, the *arrows* on the left side show the red onset, while the *arrows* on the right side show the green offset, i.e., the bar release. Bin width of 50 ms

characteristics, that discharge during Yp, were tested changing the Yp duration. Four values were chosen for Yp duration: 0 s, 0.5, 1 and 2 s, each presented in blocks of ten trials. Then, within a block of trials, Yp had a fixed duration and a temporal uncertainty was not generated. The Yp duration was, however, changed from block to block.

The fixation-blink task was used to test the presence of visual response: this task was similar to the fixation

task but during the Rp the LED was switched off for 0.5 s, and the animal had to maintain the fixation towards the target. The end of the blink preceded the yellow onset by 1 s.

The saccade task, in which the red light jumped between two or more targets before becoming green, was used to interrupt the repetition of fixation task in order to avoid learning phenomenon.

Before the recording sessions, we trained the animals to use only the arm contralateral to the recording hemisphere, thereby eliminating the simultaneous contraction of muscles in the ipsilateral arm. We also monitored the monkey's behaviour during the experimental sessions by means of an infrared TV system. All tasks were executed in total darkness. An acoustic cue was switched on at the beginning of each session and switched off at the end, thus signalling to the monkey the beginning and the end of the working period.

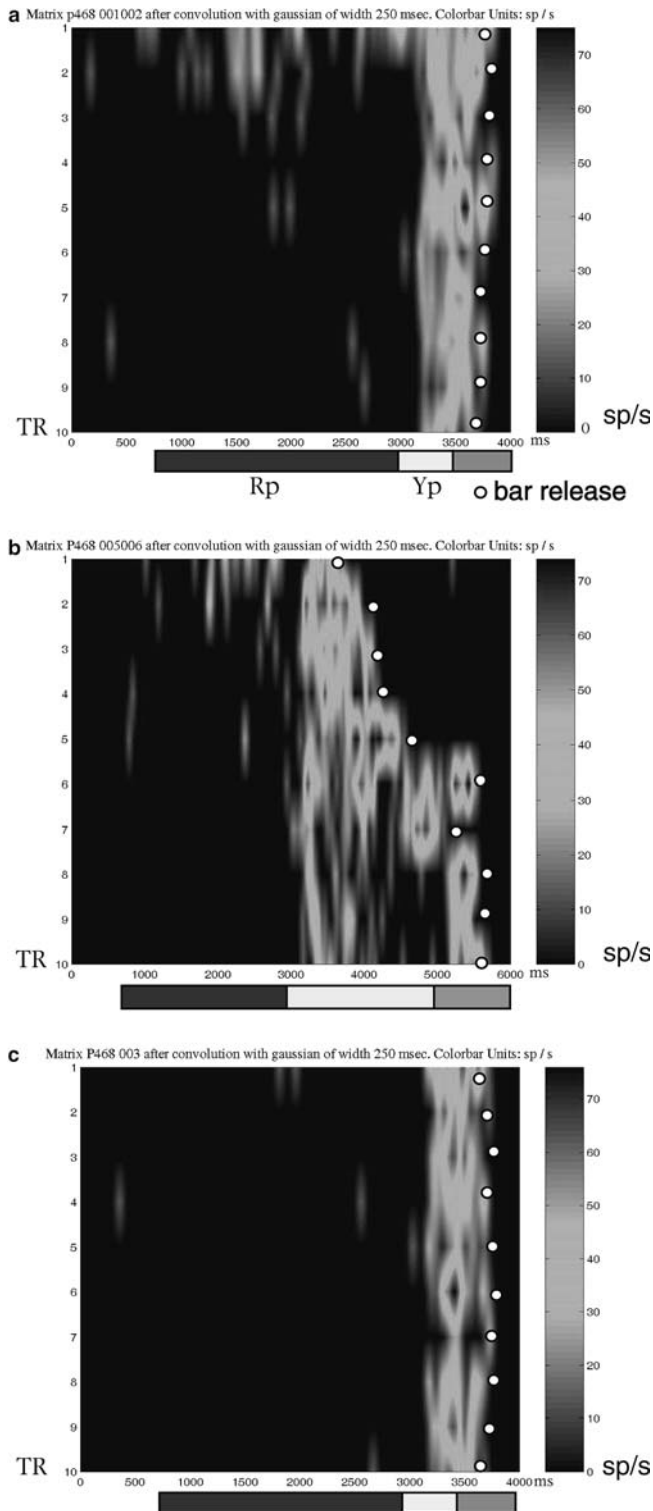
### Surgical methods

Using aseptic techniques and under general anaesthesia (10 mg/kg ketamine and 0.1 mg/kg xylazine, i.m. or 10 mg/Kg Zoletil "Tiletilamina + Zolepam"), a stainless steel cylinder was attached to the skull with four screws and cemented (Palacos R) in place to allow a painless fixation of the head. A search coil was implanted subconjunctivally (Judge et al. 1980). Outside the experimental sessions, when task-related cells were recorded, EMGs of contralateral masseter, supraspinatus, sternohydeus, sternomastoideus, rombocervicalis, spinodeltoideus, trapezius, palmaris, ulnaris, radialis, triceps and biceps were recorded by needle electrodes. A stainless steel chamber for the electrophysiological investigation was then implanted vertically above each hemisphere. After each surgery session, treatment with antibiotics, cortisone and analgesics was administered for up to one week.

### Physiological methods

Single neurons were isolated with epoxy-coated tungsten electrodes passed through the dura with a hydraulic microdrive (Narishige MO-95B). The micro-electrode signal was amplified (Bak MDA-4) and passed through a custom-built band-pass filter (500–7500 Hz) to eliminate artefacts from the 50 KHz and 75 KHz coil drivers. The unit activity was selected by a SPS 8701E Waveform Discriminator System.

Eye movements were recorded using a magnetic field technique (Rommel 1984). The horizontal and vertical components of eye position, the unit activity, the bar and LED status and the EMG activities were sampled (1 kHz) and stored by a computer (Macintosh) for off-line analysis. SuperScope II (GWI) software was used for data acquisition and analysis.



**Fig. 4** Example of motor-linked cell. Each block is made up of ten trials. **a** Yp duration is 0.5 s; **b** Yp duration is 2 s; **c** Yp duration is 0.5 s. Each frame is a two-dimensional representation on a grey-scale of the “discharge density” function after the convolution of each spike with a gaussian of 250 ms. Trial periods and durations are reported on X-axis, trials succession on Y-axis. *Open circles*: bar release

Coagulation marks were made using direct current (10  $\mu$ A for 15 s) at some recording sites for histological reconstruction. At the end of the experiments, under deep anaesthesia, the animal was perfused with a 0.9% NaCl solution followed by 5% formalin. The brain was then sectioned in 60  $\mu$ m slices, stained with thionine and the map of penetrations was reconstructed.

All phases of the experimental procedure followed the standards established by the European Community and Italian law (D.L. 116/92). The project was approved by the “Istituto Superiore di Sanità” and authorised by the “Ministero Nazionale di Sanità”. A veterinary surgeon checked the health of animals in all experimental phases.

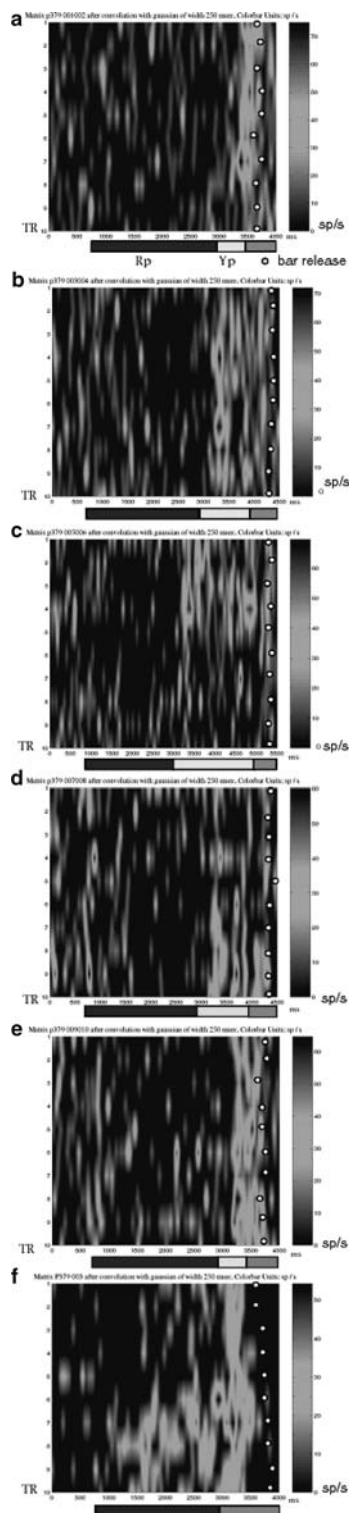
#### Data analysis

We used Wilcoxon-signed-rank test to define the behaviour of each cell, evaluating the discharge frequency difference between one or more task periods and a rest period of the same duration.

Moreover, for a better comprehension of the neuronal discharge behaviour in the time domain, for each trial we constructed an activity density function, obtained by convolving the corresponding original neuronal discharge values with symmetric gaussian functions of a given width. This provided a function, the value of which depends on the number of spikes per time unit. The values and the shape of this activity density function depend both on shape and width of the convolving gaussian function that is used. In this work, we initially evaluated three different widths for the gaussian functions, i.e., 100, 250 and 500 ms; in all those cases the standard deviations were kept as one eighth of the width. On the basis of the preliminary results obtained, we decided to fix the width at 250 ms for all the subsequent graphical and statistical elaborations, since this value gave the clearest representation. Then the discharge density values (expressed in spikes/s) of the ten trials of each block, have been graphically represented by means of appropriate two-dimensional grey-scale maps.

Moreover, for a deeper evaluation of the discharge density function, principal component analysis (PCA) has also been performed on the same data for each block of trials.

Principal component analysis is a technique for concentrating the information in a data set into fewer dimensions (Massart 1997). It does this by creating new variables, the so-called principal components, PCs, linear combinations of the original variables (i.e., in our case, the activity density values at the different times) which account for maximum possible variance in the data set. Each PC, thus, represents a different fundamental property of a system, where all the original variables that are partially or largely redundant in information content influence the same PC in the same



**Fig. 5** Example of short-term encoders cell. Each block is made up of ten trials. **a** Yp duration is 0.5 s; **b** Yp duration is 1 s; **c** Yp duration is 2 s; **d** Yp duration is 1 s; **e** Yp duration is 0.5 s; **f** Yp duration is 0. Each frame is a two-dimensional representation on a grey-scale of the “discharge density” function after the convolution of each spike with a gaussian of 250 ms. Trial periods and durations are reported on X-axis, trials succession on Y-axis. *Open circles*: bar release

direction. This is evident in the loadings plot, which shows correlations between the PCs and the original variables, i.e., in our case, the different times. Moreover, if necessary, it is possible to view how the different objects of the data set, i.e., in our case, the different trials, are distributed in the space of the PCs by means of the scores plot. The number of significant PCs indicate the number of fundamentally different properties exhibited by the data set, and it is chosen based on the variance captured by each of the PCs. In this work, given the amount of random variability of the analysed data, we considered only those PCs accounting for a percentage of 15 % of the total amount of variance.

## Results

We recorded 232 arm-related cells in four hemispheres of three monkeys in the dorsal premotor areas.

The cells discharged during task execution (bar pressing and releasing) as well as outside the task (reaching and/or grasping of natural stimuli, as pieces of fruit or raisins, presented by the experimenter); 148 (63%) of these were task-related and were active only during one or more periods of the task. Forty-nine out of 148 presented a build-up activity during the red-yellow period and were described in a previous paper by Lucchetti and Bon (2001). In all task-related cells, there was no significant difference in discharge in the different spatial positions of the eye, then they were tested prevalently with fixation task in the central position.

Histological reconstruction, performed at the end of the experiments, showed that the recorded neurons were not clustered in one field but distributed through a large portion of frontal cortex, comprehending the PMd, from its rostral part (F7) to its caudal part (F2) and the pre-SMA (F6) (Matelli et al. 1991) (Fig. 1).

We found different cellular behaviours in relation to the visual instructions, statistically testing the discharge frequency difference between one or more task periods and a rest period of the same duration (Wilcoxon test,  $P < 0.001$ ): (a) 14 red cells, (b) 12 yellow cells, (c) 16 green cells, (d) 49 red-yellow cells, (e) 6 red-green cells, (f) 43 yellow-green cells, (g) 8 red-yellow-green cells. (Fig. 2). Moreover, to ascertain whether the discharge was related to the colour, we changed the colour of one of the visual instruction. Specifically, in the cells active only during Rp, we substituted the green light with the red one and the pattern of activity did not change; similarly, in the cells active only during Gp, we substituted the green light with the red one and also in this case the pattern of activity did not change even if activity was reduced in the first trials (Fig. 3a). This test showed at the same time that cells were not related to the colour but to the succession of events. However, in the standard experimental sessions, we maintained the same colour succession as in the training sessions.

In addition, to rule out the existence of any foveal visual receptive field, the fixation-blink task was used, blinking the red light while monkey fixated did not change cell discharge (Fig. 3b).

We decided to test the effects of the instructional cue duration on the yellow–green cells, since these neurons may be considered more strictly linked to the preparation and execution of motor act. Then, while recording these cells, we manipulated the cue temporally, lengthening its duration from 0.5 s to 1 s and/or 2 s. In this way we could divide this cellular category into two groups: (1) “motor-linked” cells (13 cells) (2) “short-term encoders” cells (30 cells).

The activity of the motor-linked cells, invariably precedes the go-cue of 300–500 ms: this implies that they are able to delay their discharge in relation to the visual instruction duration. An example of this behaviour is reported under the form of two-dimensional grey-scale maps in Fig. 4: the cell active for the Yp of 0.5 s (Fig. 4a) presents the same type of discharge when the cue is lengthened to 2 s (Fig. 4b), and when Yp is shortened again to 0.5 s (Fig. 4c). We have chosen this cell as an example of the motor-linked cells, since it highlights the link between the neuronal discharge and the motor act even when the animal erroneously releases the bar during Yp (Fig. 4b, trials from first to fifth). This type of cells shows a unique behaviour, presenting a peak discharge that is temporally constant with respect to the offset of the yellow cue. This temporal constancy is also maintained when the cue is lengthened, even if the animal reaches a steady performance after a series of error trials.

PCA analysis has been performed on the three blocks of ten trials each. In particular, for the trials of the first block, the first principal component (PC1) accounts for 77.94% of the total amount of variance, the other components being negligible. In our analysis, in general, PC1 reflects the time regions containing the greatest deviations from the zero value of the activity density function, averaged over all ten trials. In this case, the PC1 loadings plot shows that the greatest contribution (in absolute value) is given by the time region ranging from about 3100 ms to about 3800 ms, corresponding to Yp–Gp. A different situation is observed for the trials of the second block: the variance is spread over quite a high number of PCs, this fact indicating that a more heterogeneous behaviour is observed for this block of trials than seen in the former block. The PC1 loadings plot (52.68% captured variance), indicates that there are two portions in the time domain where the discharge density values are particularly high: a first one from about 3000 ms to 4400 ms, and a second, centred at about 5500 ms. On the other hand, in PC2 loadings plot (15.38% captured variance), trials 1–5 stand out with high values in the region from 3300 ms to 4600 ms (with high positive loadings values) while trials 6–10 have high values in the region from 4600 ms to 6000 ms (with high negative loadings values). Finally, the trials of the third block show behaviour very similar to that of the first

block: again in this case, most of the variance is accounted for by PC1 (88.45% captured variance), the other components being negligible. The PC1 loadings plot indicates that the discharge density values are almost entirely included in the time region from 3100 ms to 3800 ms.

Hence the PCA analysis confirms that this type of cells shows a unique behaviour, presenting a peak discharge that is temporally constant with respect to the motor act, i.e., to the bar release, on the one hand, and is variable in relation to the cue duration on the other hand. This behaviour is also maintained when the cue is lengthened, even if the animal reaches a steady performance after a series of errors. In other words, the cell discharge is variable depending on the cue duration.

The activity of the short-term encoders cells is also variable depending on the cue duration, but in a different way. An example of this behaviour is reported under the form of two-dimensional grey-scale maps in Fig. 5: the cell active for the Yp of 0.5 s (Fig. 5a), progressively changes its discharge when the cue is lengthened to 1 s (Fig. 5b), and progressively changes again when the cue is lengthened to 2 s (Fig. 5c). Moreover, in the last temporal condition, the cell stops discharging after few trials. When Yp is shortened again to 1 s (Fig. 5d), the cellular discharge reappears after the first trials and when Yp returned to 0.5 s (Fig. 5e), the cellular discharge shortens and presents its original duration.

The PCA analysis has been performed on the five blocks of ten trials each. In particular, for the first block of ten trials (Fig. 5a), with Yp duration of 0.5 s, the PC1 loadings plot (66.42% captured variance) indicates that the unique systematic contribution to the variability of this block is given by the strictly defined time region ranging from about 3200 ms to 3800 ms, which corresponds to Yp–Gp. In the case of the second block, with Yp duration of 1 s (represented in Fig. 5b), PC1 captures 61.72% of the total variance and its loadings plot shows that the greatest contribution (in absolute value) is given by a wider time region, ranging from about 3100 ms to 4400 ms, which corresponds to the lengthened Yp–Gp. Conversely, for the trials of the third block (Fig. 5c) where Yp duration is 2 s, the variation is no longer localised in a strictly defined time region, thus indicating a more heterogeneous behaviour for this block of trials, with respect to the previous ones. In fact, the loadings plot of PC1 (58.39% captured variance) indicates that the portion of the time domain where the discharge density values are higher is rather large (approximately from 3200 ms to 5300 ms, corresponding to the lengthened Yp–Gp), and is not so clearly defined as in the previous cases. Moreover, the scores plot shows a change in the behaviour of the activity density function when passing from the first four trials (in particular trials 1 and 4) to the following trials.

A similar behaviour is observed for the fourth block of trials (represented in Fig. 5d), where Yp duration is

1 s. Again in this case, the PC1 loadings (55.74% captured variance) show a wide portion in the time domain where the discharge density values are particularly high, but not equally so in all the trials. Here, too, the scores plot shows a change in the behaviour of the activity density function when passing from the first four trials to the following trials. Finally, the trials of the fifth block (represented in Fig. 5e) show a behaviour that is very similar to that of the first block (both blocks have Yp duration of 0.5 s). The systematic variance is almost entirely accounted for by PC1 (65.94% captured variance), the loadings of which indicates that the greatest contribution is given by the time region ranging from about 3200 ms to 3800 ms, which corresponds to Yp–Gp. In this case, too, considering the scores, a change may be observed in the behaviour of the activity density function when passing from the first trials to the following ones.

In the group of short-term encoders cells, we blinked the cue instruction to study the effect of a reduction of Yp duration to 0: an example is shown in Fig. 5f, referred to the cell presented in the same figure and described above. In the block of ten trials, the onset of cellular discharge clearly shifts: in the first trials it is confined to 500 ms preceding the bar release, which corresponds to Gp. In the following trials the discharge gradually increases during Rp.

These observations are confirmed by the PCA analysis performed on the block of ten trials. In particular, for PC1 (64.59% captured variance) the greatest contribution is given, to differing extents, by the time region ranging from about 1500 ms to 3500 ms, corresponding to both Rp and Gp. The corresponding PC1 scores for each of the 10 trials, reveal the change in the behaviour when passing from the first five trials to the second five. As was previously evidenced, PC1 accounts for the time regions containing the greatest deviations from zero in the activity density function. In fact, the absolute value of the PC1 scores for the first five trials, where the activity is almost exclusively limited to the time region from about 3000 ms to 3500 ms, is lower than the absolute value of the trials from 6 to 10, where a certain degree of activity is observed even in the time region from about 1500 ms to 3000 ms.

To verify whether a discharge variation could be correlated with reaction time in bar releasing (RT) variations, RTs were measured during all recording sessions where motor-linked and short-term encoders cells were found. When Yp duration was 500 ms, RT was  $256 \pm 74$  (mean  $\pm$  SD); when Yp duration was 1000 ms, RT was  $401 \pm 79$ ; when Yp duration was 2000 ms, RT was  $420 \pm 53$ ; and when Yp was absent, RT was  $430 \pm 61$ . There was a significant difference between the RTs obtained with a Yp of 500 ms and those obtained with the other experimental conditions (Wilcoxon-signed-ranked test,  $P < 0.005$ ), whereas no significant differences were observed between other conditions ( $P > 0.1$ ).

## Discussion

In previous studies, the relationship between neuronal discharge and the spatial informational content of the instructional cue was investigated. Riehle and Requin (1989), recording from arm-related cells in primary motor and PMd cortex of monkeys performing visually guided wrist flexion and extension, found that neuronal discharge modifies in relation to the informational content of the instructional cue regarding direction and extent of the forthcoming movement. Crammond and Kalaska (2000), recording from arm-related cells in primary motor and PMd cortex of monkeys performing two different types of task, showed that neuronal planning correlates recorded during the instructed-delay period in instructed-delay task trials share common features with post-go activity in reaction time trials; moreover, those response components need not be recapitulated after the go signal in instructed delay trials. Cisek and Kalaska (2002) presented evidence that PMd neurons can simultaneously generate multiple directional signals related to multiple alternative reaching actions before making a decision between them. Moreover, Coe et al. (2002) found that changes in neural activity related to changing target selection for saccadic movements during a free-choice task are very common in supplementary eye field (SEF), which is the part of the PMd principally devoted to eye movement control.

All these data denote the flexibility of PMd neurons which concerns the spatial aspects of motor preparation: in fact, all the tasks were visuo-spatial, the instructional cues informed the animal about the spatial characteristics of the movement to be executed after the delay and the experimenter properly manipulated the spatial informational content of the cue.

On the contrary, in our experiments, the instructional cue did not supply any spatial information about the movement to be executed, which was always a bar release. The only information supplied by the cue was temporal, consisting of the duration of the cue itself, which provided the timing for the final movement. In our task there was no temporal uncertainty, since the cue had a fixed duration within a block of trials. Our experiments aimed at testing whether premotor neurons changed their activity rapidly after the duration of an instructional cue changed.

Two points stand out from our investigation. First of all, we found, in a large portion of frontal cortex, arm-related neurons discharged selectively for one or more visual instructions. Their activity was related to the informational content and not to the colour of the cue. This finding suggest that, in the same cortical area, cells coding single instructions, pre-cue (wait), cue (prepare) and go command, do coexist together with cells that combine two or three different instructions. These cells could form a sort of node in a neural network in which some “simple” nodes are active for only one instruction



and “complex” nodes are active for more than one instruction. Simple nodes may be considered step task analysers; complex nodes may be considered step task combiners. In other words complex nodes should receive convergent inputs from simple nodes.

The second point emerging from our investigation is that lengthening and shortening the duration of the instructional cue in a visuomotor task may affect the premotor cells to different extents.

The motor-linked cells show a discharge delay related to the instructional cue duration such that the discharge always precedes the same amount of time (300–500 ms) the go cue.

The short-term encoders present a different type of discharge switching in relation to the instructional cue duration. In particular, when the cue duration is 0.5 or 1 s, these cells show a premotor discharge lasting for the duration of the instructional cue. The switching requires a few transitional trials before it becomes effective. During the transitional trials, the cells tend to behave as in the last trials of the preceding block. A different phenomenon is present with a cue duration of 2 s: in fact, the cells cease to fire during the cue, as if they would not recognise it as a preparing instruction. The duration of 1 s could be an intrinsic limit of the computational property of these cells, as already observed by Lucchetti and Bon (2001). This result is in accord with the existence, in humans, of slow EEG potentials, recorded in the frontal lobe, called “contingent negative variation” (CNV) (Walter et al. 1964), that precede a motor act when it is performed in response to a sensorial instruction. The early component of CNV starts more than 0.5–0.8 s prior to the onset of the movement.

Moreover, when the cue is blinked, these cells begin to fire during the pre-cue. Then the pre-cue becomes a cue in few trials.

The RTs study in the different experimental conditions shows that, when the cue duration is the usual one, 0.5 s, RTs are significantly shorter than when the cue duration is longer or when it is blinked. This result highlights the relevance that the duration of a preparatory cue, to optimise the animal’s motor performance, has to be inferior to 1 s. These data support the findings in the experiments on build-up neurons in monkeys’ PMd by Bon and Lucchetti (2001).

We thus suggest that the discharge modifications induced by changes in the instructional cue duration are an expression of a rapid functional time-induced flexibility at neuronal level in frontal cortex. This flexibility may be considered functional expression of intelligence (Evarts et al. 1984). In natural behaviour the functional flexibility allows motor adaptation in response to environmental stimuli.

In conclusion, the presence of cells related to the visual instruction, to the premotor built-in activity and to the functional flexibility, and cells that have a predictive temporal behaviour (Lucchetti and Bon 2001), might constitute a neuronal network inside frontal cortex, able to control appropriate motor acts in the time domain.

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