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Motor cortical activity in a memorized delay task

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Summary. Two rhesus monkeys were trained to move a handle on a two-dimensional (2D) working surface in directions specified by a light at the plane. They first captured with the handle a light on the center of the plane and then moved the handle in the direction indicated by a peripheral light (*cue* signal). The signal to move (*go* signal) was given by turning off the center light. The following tasks were used: (a) In the *non-delay* task the peripheral light was turned on at the same time as the center light went off. (b) In the *memorized delay* task the peripheral light stayed on for 300 ms and the center light was turned off 450–750 ms later. Finally, (c) in the *non-memorized delay* task the peripheral light stayed on continuously whereas the center light went off 750–1050 ms after the peripheral light came on. Recordings in the arm area of the motor cortex ($N=171$ cells) showed changes in single cell activity in all tasks. In both delay tasks, the neuronal population vector calculated every 20 ms after the onset of the peripheral light pointed in the direction of the upcoming movement, which was instructed by the cue light. Moreover, the strength of the population signal showed an initial peak shortly after the cue onset in both the memorized and non-memorized delay tasks but it maintained a higher level during the memorized delay period, as compared to the non-memorized task. These results indicate that the motor cortex is involved in encoding and holding in memory directional information concerning a visually cued arm movement and that these processes can be visualized using neuronal population vector analysis.

Key words: Motor cortex – Visuomotor memory – Arm movement – Movement direction – Monkey

Introduction

The role of motor cortex in motor control is well documented [see Evarts (1981) for a review]. Moreover, cell activity in the motor cortex has also been shown to reflect more complex processes [see Georgopoulos (1991) for a review] involving, for example, spatial transformations (Georgopoulos et al. 1989; Lurito et al. 1991), spatial trajectory operations (Hocherman and Wise 1991), precuing information concerning movement direction (Riehle and Requin 1990), preparation for memorized movements (Alexander and Crutcher 1990), and preparation for movement sequences (Clark et al. 1991; Crammond and Kalaska 1991; Kettner et al. 1991; Marcario et al. 1991). Indeed, several studies have indicated that the difference between the involvement of motor cortex and premotor areas in complex visuospatial processes may be one of degree rather than of kind (Lecas et al. 1986; Alexander and Crutcher 1990; Riehle and Requin 1990; Chen et al. 1991; Clark et al. 1991; Hocherman and Wise 1991; Tanji et al. 1991), in the sense that qualitatively similar patterns of activity may be observed in these areas, although at higher proportions in premotor areas than in the motor cortex. In previous studies (Georgopoulos et al. 1989a, b) we documented changes in activity of motor cortical cells during an instructed delay period and showed that the neuronal population vector (Georgopoulos et al. 1983) calculated in time (Georgopoulos et al. 1984) predicted, during the delay period, the direction of the upcoming of movement in space. In the present paper, we extend these studies to the case of arm movements towards memorized targets. Changes in cell activity during delay periods preceding eye movements to memorized targets have been documented in the substantia nigra (Hikosaka and Wurtz 1983), the frontal eye field (Bruce and Goldberg 1985), the parietal cortex (Gnadt and Andersen 1988) and the prefrontal cortex (Funahashi et al. 1989). We sought to determine whether such changes in cell activity are also observed in the motor cortex, with regard to arm movements, and whether the neuronal population vector would predict the memorized direction during the delay period. Preliminary results have been reported (Smyrnis et al. 1991).

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Materials and methods

Animals

Two rhesus monkeys (one male and one female, 3.5–4.5 kg body weight) were used.

Apparatus

A 2D planar working surface and an articulated manipulandum were used. The working surface was a 25 × 25 cm Plexiglas square, tilted 15° from the horizontal towards the animal. The apparatus has been described previously (Georgopoulos et al. 1981). The monkeys grasped the distal end of the manipulandum with their left hand pronated, next to a 5 mm radius Plexiglas circle. Motion of the manipulandum over the working surface was free and almost frictionless. A circular pattern of light emitting diodes (LED) was used, with one LED at the center and eight at the circumference of a circle of 6 cm radius. (For the second monkey this radius was 3 cm.) The peripheral LEDs were arranged equidistantly on the circle so that the direction of movement from the center to peripheral LEDs ranged over the whole directional continuum of 360° every 45°.

Behavioral tasks

A trial started after a variable intertrial interval (1–3 s) during which the all lights were turned off. The center light was then turned on and the monkey was required to capture it within a 12-mm-radius circular positional window ("center window"). After a variable period of time (500–1500 ms) one of the peripheral lights (*cue* signal) was turned on to provide the cue for the upcoming movement direction; the signal to move (*go* signal) was given by turning off the center light. The following kinds of tasks were used, depending on when the *cue* and *go* signals were given (Fig. 1): (a) The *cue* and *go* signals were given simultaneously (non-delay task). (b) The *cue* signal stayed on for 300 ms and the *go* signal was given 450–750 ms later (memorized delay task). Finally, (c) the *cue* signal stayed on continuously whereas the *go* signal was given 750–1050 ms after the *cue* signal (non-memorized delay task). In all trials the animals were rewarded when the handle entered a 12.5 mm radius circular window centered on the peripheral light ("target window"). For the first animal, each of the three tasks was performed in a separate block of trials; for each task, five trials of each position of the peripheral light were presented in a randomized block design. With the second animal, tasks and target positions were mixed and five trials of each combination were presented in a randomized block design.

Neural recordings

After an animal was trained in the task, neural recordings of the extracellular signs of cell activity in the motor cortex were initiated. The electrophysiological techniques used to record the electrical signs of activity of single cells have been described in detail previously (Georgopoulos et al. 1982). A multielectrode recording system (Mountcastle et al. 1991) was used.

Electromyographic (EMG) recordings

The EMG activity of anterior deltoid, posterior deltoid, pectoralis major, triceps and biceps was sampled in the task using intramuscular, Teflon coated, multistranded, stainless steel wires. EMG recordings were made separately from neural recording sessions. The EMG signals were recorded differentially with an approximate gain of 3000 and a bandpass of 100–500 Hz. They were then rectified and sampled every 10 ms.

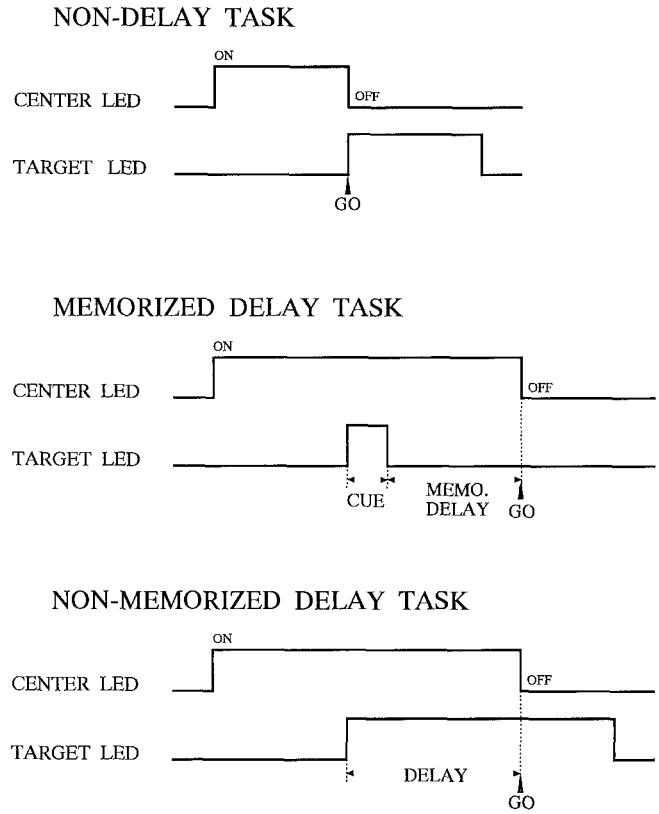

Fig. 1. Schematic diagram of the tasks used

Data collection

A PDP11/34 laboratory minicomputer was used to control the lights on the plane, to monitor and record behavior, and to collect data. Neural data were collected as interspike intervals with a resolution of 0.1 ms. The position (x, y) of the manipulandum was sampled every 10 ms with a resolution of 0.125 mm.

Data analysis

Standard analysis (Sokal and Rohlf 1969; Winer 1971; Mardia 1972; Snedecor and Cochran 1980) and display (rasters, histograms) techniques were used to inspect, evaluate and analyze the data. Of the eight positions of the *cue* light used, three (comprising the lower left quadrant) were partially obstructed by the animal's hand, and, although the animals moved to them in the non-delay task, they had great difficulty in the memorized delay task in which the light stayed on for only 300 ms. Therefore, analyses regarding the delay tasks were confined to the remaining five positions. The BMDP/386 statistical package (Los Angeles, Calif.) was used in some of the analyses.

Behavioral epochs. The following behavioral epochs were distinguished and are indicated in Fig. 1.

Non-delay task. (a) The time during which the animal held the manipulandum within the center window, from the capture of the center light until the appearance of the peripheral light, was the *center hold time.* (b) The time from the moment that the center light was turned off until the manipulandum exited the center window was the *reaction time* (RT). This estimate of the RT from position was conservative; we chose to measure the RT with high certainty from the crossing of the center window, for we wanted to eliminate completely false-positive cases. However, we also calculated a "liberal" estimate of the onset of movement, based on the change of

speed. For this purpose, (1) the tangential speed was calculated for every trial; (2) the maximum speed and its time of occurrence were noted; (3) a backwards search was performed, starting from the time of maximum speed and moving towards the beginning of the trial (the search was made backwards to avoid wrong positive errors); (4) the search was stopped the first time that the speed became less than 10% of the maximum speed, and the time of that point noted. In the remainder of the paper we use *reaction time* to refer to the crossing the center positional window, and *movement onset* to refer to movement onset determined from the change in speed. Finally, (c) the time elapsed from crossing the center window until the target window was entered was the *movement time*.

Memorized delay task. The center hold, reaction and movement times were as defined above. However, two additional epochs were defined in these tasks. One comprised the first 300 ms after the appearance of the peripheral light, that is, the time for which the cue stayed on *(instructed delay period)*. The second epoch was from the time that the cue light was turned off until the center light went off; during this time there was no peripheral light and therefore its direction had to be kept in memory *(memorized delay period)*. The trial was a *success* if the animal waited during the whole delay period holding the handle within the center window, and then moved following the *go* signal and entered the target window. Sometimes the animal moved prematurely out of the center window during the delay period; this was called a *delay error*.

Non-memorized delay task. In this task, the peripheral light stayed on throughout the trial; therefore, the instructed delay period was from the time that the peripheral light came on until the center light was turned off. However, in order to compare the cell activity at similar periods in the memorized and non-memorized delay tasks, the instructed delay period in this task was arbitrarily divided into two parts, the first comprising the initial 300 ms and the second the remaining period until the center light went off. The center hold, reaction and movement times, and successes and delay errors were as defined above.

Directional performance. The direction of movement was determined when the center window was crossed. We wanted to know whether and how much the direction of the movement differed among the tasks used. For this purpose, the mean direction and circular standard deviation (Mardia 1972) were calculated from all successful trials in which neural recordings were made in each task.

Assessment of change in cell activity. A paired *t*-test (Snedecor and Cochran 1980) was used to assess the statistical significance of the change in mean cell activity during (a) the first 300 ms and (b) the remaining of the delay period for each direction, relative to the activity observed during the center hold time.

Analysis of variance (ANOVA). The frequency of cell discharge during the (a) the instructed delay period (first 300 ms following the presentation of the cue), and (b) the remaining delay period (memorized and non-memorized) in the memorized and non-memorized delay tasks was analyzed using a repeated measures ANOVA (Winer 1971; Snedecor and Cochran 1980) to test differences in mean cell activity between these two tasks.

Directional tuning. The directional tuning was analyzed from the frequency of discharge during the RT in the non-delay task using all eight directions of movement. The *preferred direction* was calculated using standard directional statistics (Mardia 1972). The value of the preferred direction thus calculated is identical to that calculated using multiple regression statistics (Georgopoulos et al. 1982). For the *statistical significance of the directional tuning* we used a non-parametric, statistical bootstrapping technique to determine whether the frequency of cell discharge during the RT was directionally tuned. This technique has an advantage over the multiple regression and other techniques used before (Georgopoulos et al. 1982) in that it does not depend on distributional assumptions for a test of statistical significance which, in this case, is generated from the data themselves. The technique has been described in detail (Lurito et al 1991).

Calculation of the neuronal population vector. The neuronal population vector is the weighted sum of vectorial contributions of individual cells (Georgopoulos et al. 1983, 1984, 1986, 1988). It provides information concerning the directional tendency of the neuronal ensemble and can be calculated from the average cell discharge in a particular epoch (Georgopoulos et al. 1983, 1986, 1988) or in an ongoing, time-varying fashion (Georgopoulos et al. 1984, 1988). For the calculation of the population vector, peristimulus time histograms (20 ms bin width) were computed using counts of fractional intervals as a measure of the intensity of cell discharge. For a given time bin, each cell made a vectorial contribution in the direction of its preferred direction and of magnitude equal to the change in cell activity from that observed during 0.5 s preceding the onset of the peripheral stimulus ("control rate", that is, while the monkey was holding the handle at the center of the plane). The population vector **P** for the j^{th} class and k^{th} time bin is

$$\mathbf{P}_{j,k} = \sum_{i}^{N} w_{i,j,k} C_i \qquad (1)$$

where C_i is the preferred direction of the i^{th} cell and $w_{i,j,k}$ is a weighting function

$$w_{i,j,k} = (d_{i,j,k}) - a_{i,j} \qquad (2)$$

where $d_{i,j,k}$ is the square-root transformed discharge rate of the i^{th} cell for the j^{th} class and k^{th} time bin, and $a_{i,j}$ is the similarly transformed control rate of the i^{th} cell for the j^{th} class. The square root transformation was used as a variance stabilizing transformation for counts (Snedecor and Cochran 1980). The statistical significance of the directionality of the population vector at a given time bin was assessed using a modified Rayleigh test for vectors (Moore 1980).

Results

Neuronal and EMG results: General

The activity of 171 arm-related cells was recorded in the motor cortex (2 hemispheres, 2 animals); of those, 117 and 54 cells were recorded in the first and the second monkey, respectively. Each cell changed activity in relation to proximal movements of the contralateral arm as judged by examination of the animal outside the behavioral task. Cells that changed activity in relation to distal movements (e.g., of the hand and/or fingers) were not studied because the movements of the manipulandum in the task were produced by motion about the proximal joints (shoulder and elbow). No obvious responses to visual stimuli (e.g., flashes of light) were observed. Finally, all muscles studied (see Materials and methods) were active in all tasks following the *go* signal; they were inactive during the delay period. Examples from two muscles are illustrated in Fig. 2.

Behavioral performance

By design, the number of successes in both delay tasks were the same ($n = 2{,}675$ trials. This number is less than 171 cells × 5 directions × 5 trials per direction = 4,275 because many cells were recorded simultaneously). The numbers of delay errors (see Materials and methods)

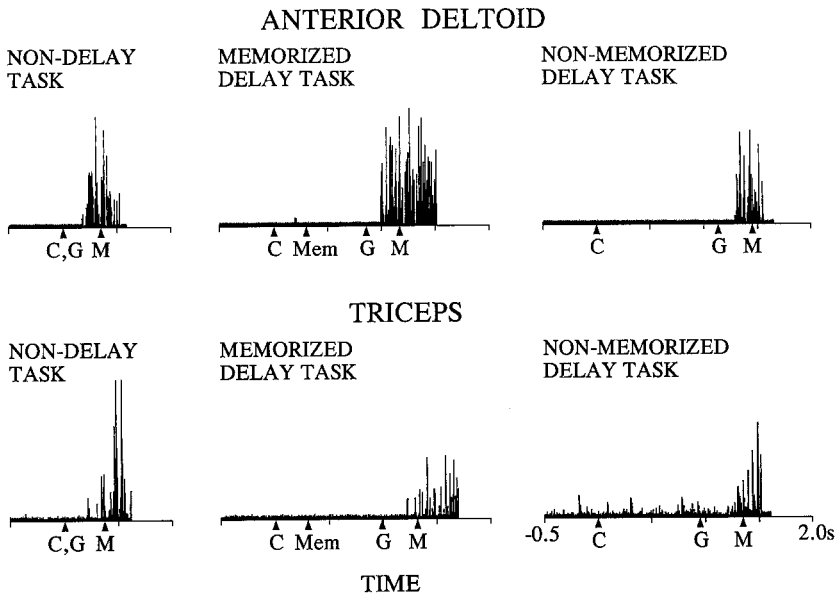

Fig. 2. Example of EMG activity (arbitrary units) of acromiodeltoid and triceps muscles in the three tasks used. Traces are from single trials. *C, cue* onset; *G,* onset of the *go* signal; *M,* exit from center window; *Mem,* onset of memorized delay period (time of *cue* off)

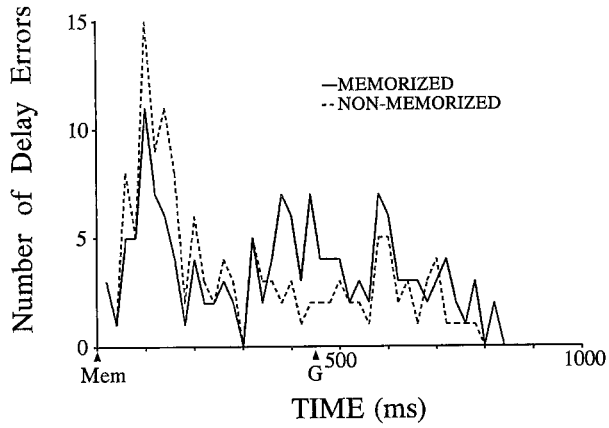

Fig. 3. Time course of delay errors. Abbreviations as in Fig. 2

were 144 and 138 for the memorized and non-memorized delay tasks, respectively.

Very few of the delay errors were made during the first 300 ms of the delay (5 in the memorized and 5 in the non-memorized delay task); most were made during the remaining delay period, that is, after the cue was turned off in the memorized task or after the 300 ms had elapsed in the non-memorized task. The time course of the occurrence of these errors was very similar in the two tasks (Fig. 3).

The directional performance was very good and very similar in all three tasks. The relevant directional statistics are given in Table 1. The pairwise differences between mean directions in the three tasks were not statistically significant (test of Watson and Williams for the equality of mean directions; see Mardia 1972).

The reaction and movement onset times (see Materials and methods) were consistently shortest in the memorized delay task, longest in the non-delay task and in-between in the non-memorized delay task (Table 2). All pairwise differences between tasks in both measures were highly significant ($P < 0.001$, t-test).

Table 1. Directional performance in the three tasks. N is number of trials. Positive and negative signs in the mean direction indicate counterclockwise and clockwise departures from the target direction, respectively

	Non-delay task	*Memorized delay task*	*Non-memorized delay task*
Mean direction	−0.6°	1.29°	−8.3°
Circular standard deviation	28.4°	27.0°	22.4°
Length of the mean resultant	0.884	0.895	0.926
N	2670	2675	2675

Table 2. Reaction time and movement onset time (see Materials and methods) in the three tasks. N is number of trials. SD = standard deviation; SEM = standard error of the mean

	Non-delay task	*Memorized delay task*	*Non-memorized delay task*
Reaction time			
Mean	397.11	303.15	353.56
SD	122.04	90.86	90.43
SEM	2.36	1.76	1.75
N	2670	2675	2675
Movement onset time			
Mean	321.11	223.59	279.23
SD	124.90	92.79	89.04
SEM	2.42	1.79	1.72
N	2670	2675	2675

Single cell activity

In general, cell activity was similar in the non-delay and the delay tasks following the *go* signal. An example is shown in Fig. 4. Consistent changes in cell activity were observed during the delay period in the memorized and

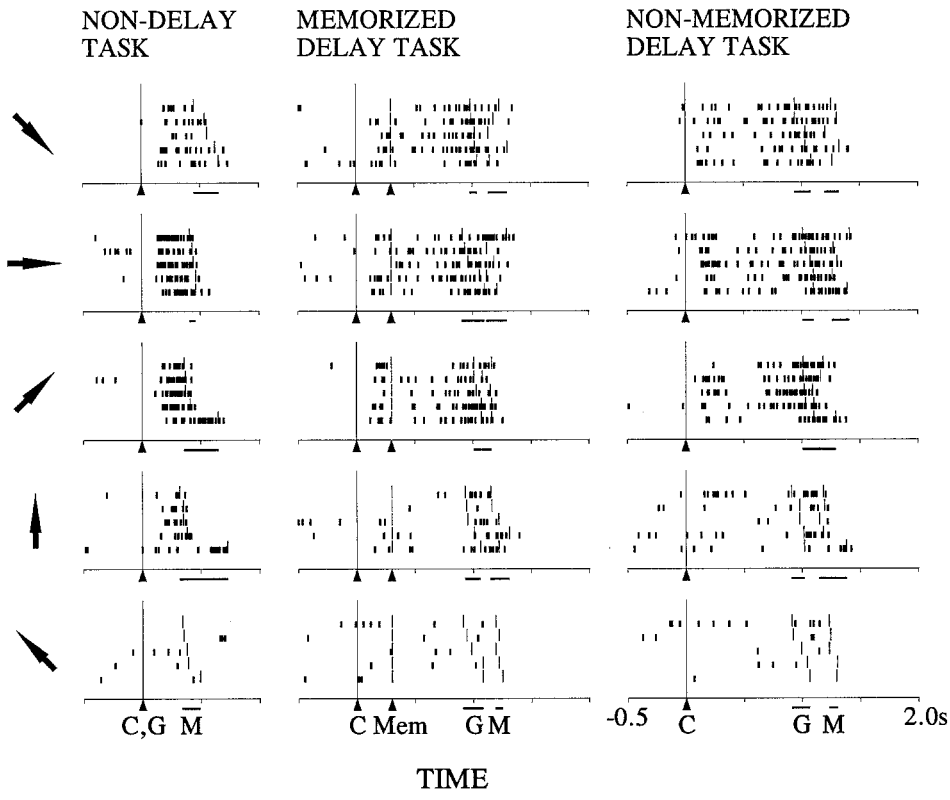

Fig. 4. Impulse activity *(short vertical bars)* of a cell during five trials in the three tasks used. The *arrows* on the *left* indicate the instructed (or memorized) direction. *Longer vertical bars* indicate the time of behavioral events. In all tasks, rasters are aligned to the onset of the *cue* signal (*C*). In the non-delay task, this is also the *go* signal (*G*); *Mem* is the time of *cue* off, and *M* is the onset of the movement. *Horizontal bars* indicate the range of the times above; *filled triangles* indicate fixed events. (Cell Si025/3)

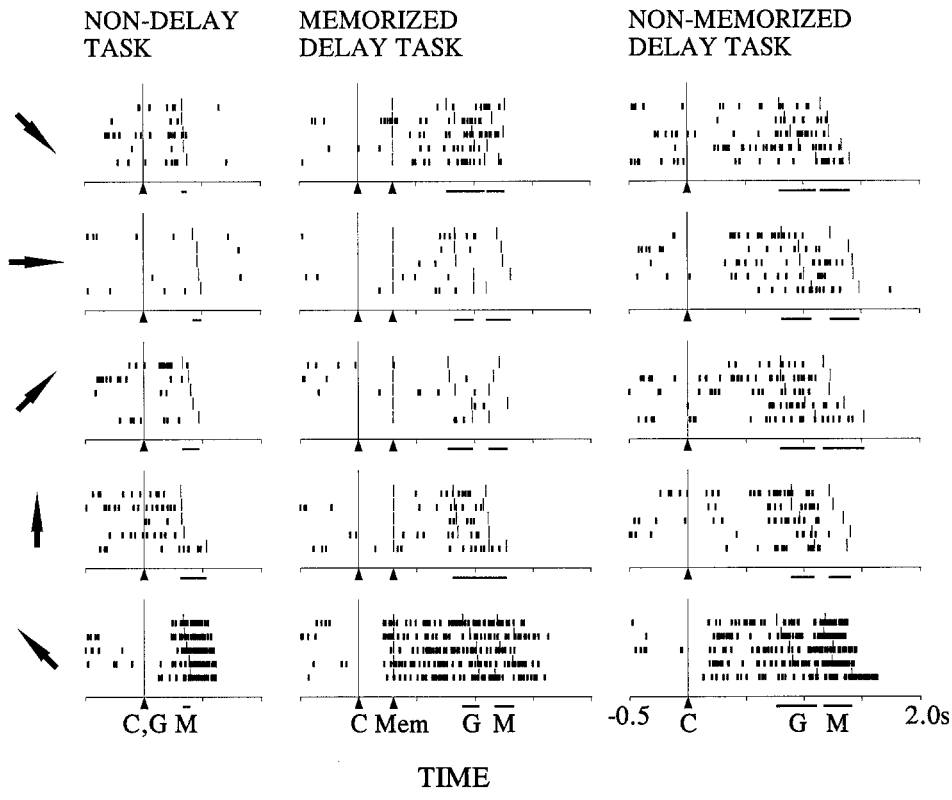

Fig. 5. Impulse activity of another cell in the three tasks used. Conventions as in Fig. 4. (Cell Pi273/7)

non-memorized delay tasks. Examples are shown in Figs. 5–7.

A different question concerns the strength of the association of cell activity to the occurrence of movement. Since in delay errors (see Materials and methods) the animals moved prematurely during the delay period, it was interesting to examine whether the occurrence of movement and change of cell activity were coupled. An inspection of the raster records of delay errors did not reveal a simple association. This can be appreciated from

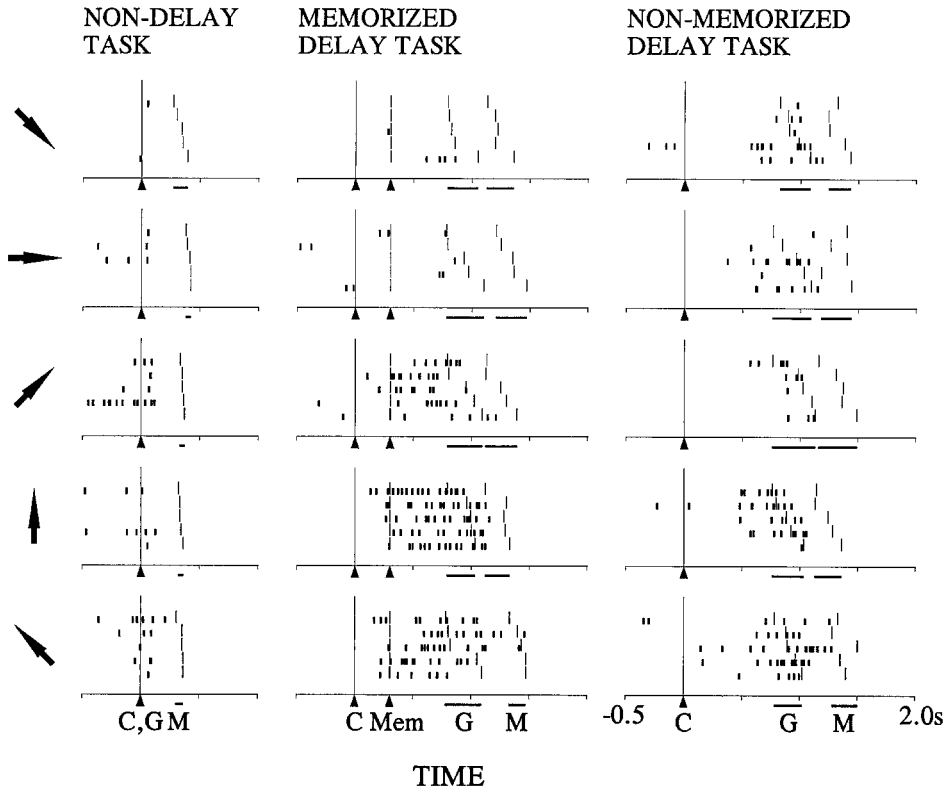

Fig. 6. Impulse activity of another cell in the three tasks used. Conventions as in Fig. 4. (Cell Pi260/0)

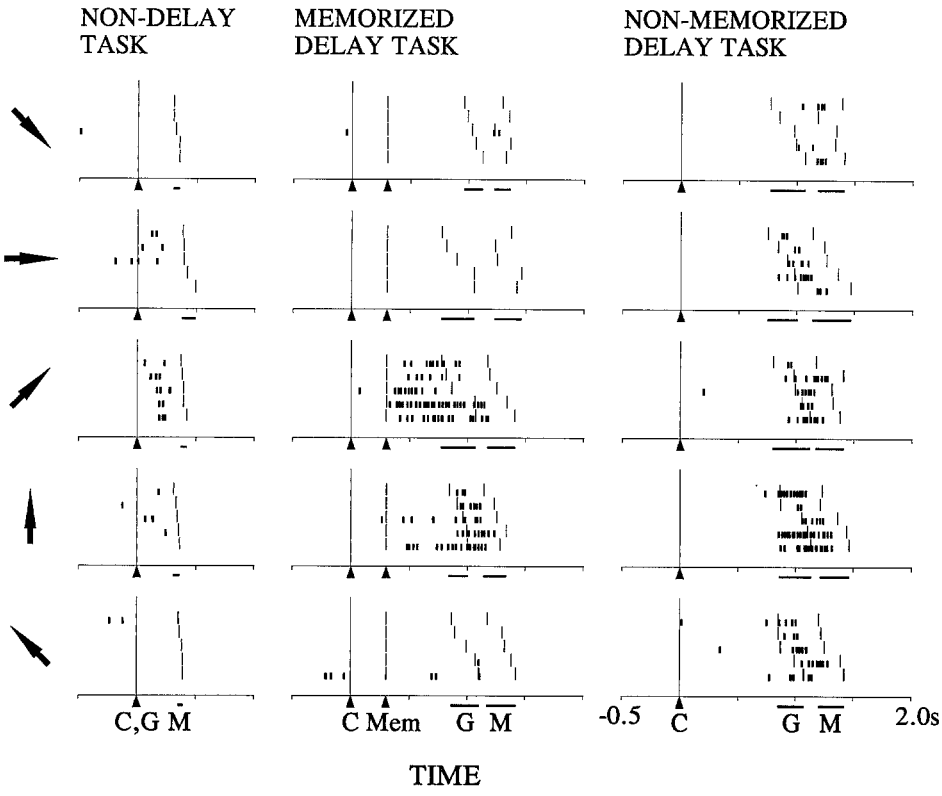

Fig. 7. Impulse activity of another cell in the three tasks used. Conventions as in Fig. 4. (Cell Pi237/2)

the examples illustrated in Fig. 8A–F, and the summary of cell activity shown in Table 3. Figure 8A, B illustrates cases of cells that (a) were active in the non-delay task, (b) were inactive during the memorized delay, (c) were activated following the *go* signal in the memorized delay task, and (d) were activated during memorized delay errors. These patterns of activation are consistent with the idea that these cells are related to movement initiation. However, Figure 8C–F illustrate cases of cells the activity patterns of which cannot be explained easily

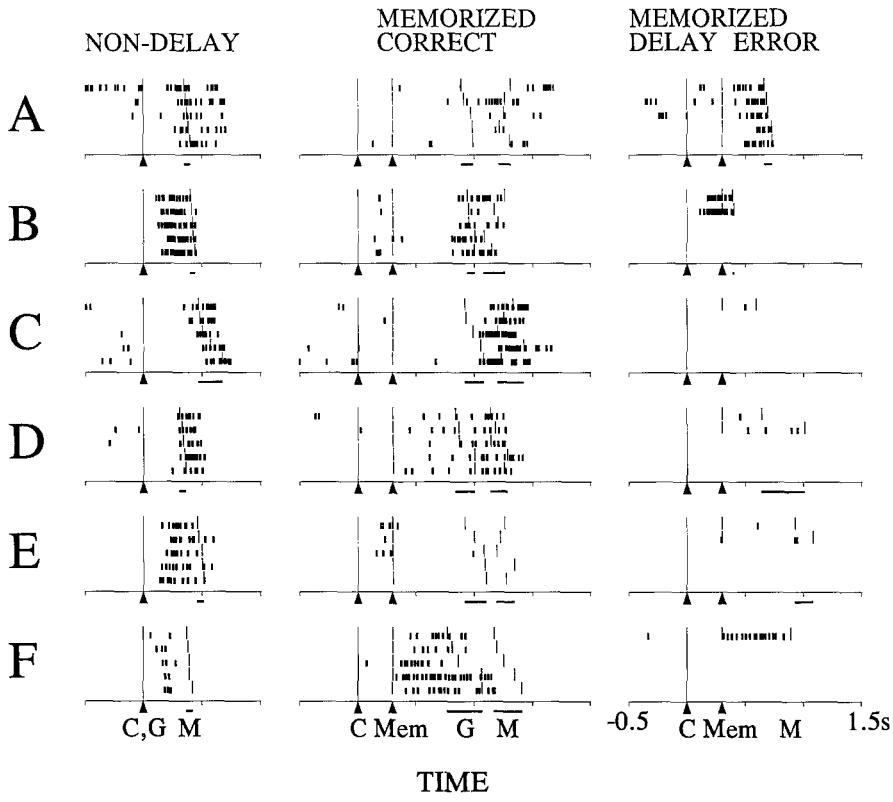

Fig. 8. Examples of activity of six cells (*A–F*) during delay errors. Three sets of rasters are shown for each cell, as indicated at the top. All rasters for a given cell are from the same direction (not shown), in which delay errors were committed; these directions differed among different cells. Numbers of trials in the "delay error" column vary, depending on how many errors were made. Conventions as in Fig. 4. A qualitative summary is given in Table 3. (*A* cell Pi221/5; *B* cell Si004/2; *C* cell Si001/5; *D* cell Pi258/1; *E* cell Si029/3; *F* cell Pi237/2)

Table 3. Qualitative summary of cell activity during delay errors for the cells illustrated in Fig. 8

Cell in Fig. 8	Non-delay task	Memorized delay task		
		Delay period	Reaction time	Delay error
A	Present	Absent	Present (weak)	Present
B	Present	Present (late)	Present	Present
C	Present	Absent	Present	Absent
D	Present	Present (weak)	Present	Absent
E	Present	Absent	Absent	Absent
F	Present (weak)	Present	Present (weak)	Present

under a simple idea. For example, the lack of cell activity during the delay errors in Fig. 8C–E defies a simple relation to movement, as does the presence of an activation during delay errors in Fig. 8F. Thus relations between cell activity and behavioral events can be quite complex. Therefore, the memory process and the movement triggering process seem to be dissociated at the neuronal level.

Changes in cell activity during the delay period

The numbers of cases in which an increase, decrease or no change in activity were detected in the first part (300 ms) and the remaining of the delay period in the two delay tasks used (see Materials and methods) are shown in Table 4. The differences between the two tasks were not significant in the first 300 ms of the delay

Table 4. Changes in cell activity during the delay period in the tasks used. Values are numbers of cases in which the indicated change (or no change) in activity was observed. (Total $N = 171$ cells × 5 directions × 2 tasks = 1710)

	Memorized delay task	*Non-memorized delay task*
First 300 ms		
Increases	74 (8.7%)	67 (7.8)
Decreases	37 (4.3)	23 (2.7)
No change	744 (87.0)	765 (89.5)
Total	855 (100.0)	855 (100.0)
Remaining delay period		
Increases	138 (16.1%)	113 (13.2)
Decreases	57 (6.7)	33 (3.9)
No change	660 (77.2)	709 (82.9)
Total	855 (100.0)	855 (100.0)

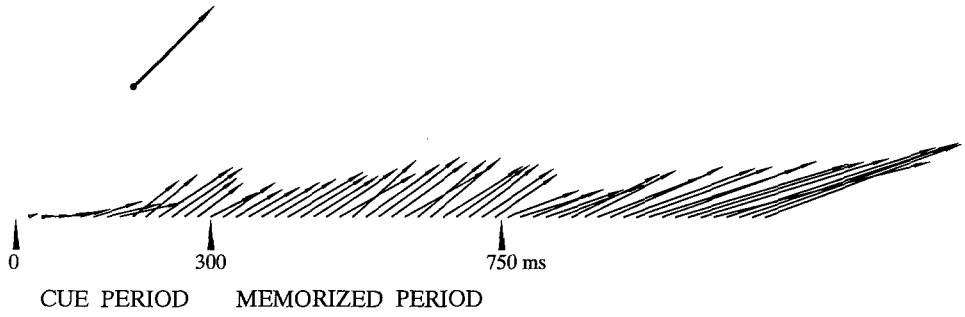

Fig. 9. Population vectors in the memorized delay task for the direction indicated are plotted every 20 ms. The *arrow* on top indicates the direction of the *cue* signal present during the first 300 ms of the delay period

(χ^2 (2) = 3.906, $P < 0.14$) but were highly significant in the remainder of the delay period ($\chi^2(2) = 10.644$, $P < 0.005$). The latter was due to a larger proportion of increases (1.22 times) and decreases (1.73 times) observed in the memorized vs the non-memorized delay.

Task-related differences in cell activity

The levels of mean cell activity were compared for the following time periods between the memorized and non-memorized delay tasks.

Control period. Statistically significant task differences were observed in 20/171 (11.7%) cells. In order to avoid influences from factors other than those related to the presentation of the cue or the memorization process, the analysis below was restricted to those cells ($N = 151$) for which significant task effects in the control period were not observed.

First 300 ms of the delay period. As mentioned in Materials and methods above, in the memorized delay task this period is demarcated from the period that followed by the fact that the cue was turned off. However, this was not true for the non-memorized delay task in which the cue stayed on until the end of the trial. Nevertheless, it is useful to examine the differences between the two tasks for this time period even if it is arbitrarily defined for the second task above. The results of the ANOVA were as follows. Statistically significant ($P < 0.05$, F-test) Task, Direction and Task × Direction interaction effects were observed in 17/151 (11.3%), 38/151 (25.2%) and 17/151 (11.3%) of cells, respectively. The presence of a significant task effect means that the overall cell activity differed between the two tasks; the presence of a significant direction effect means that cell activity differed among directions; and the presence of a significant interaction effect indicates that cell activity among various directions differed between the two tasks.

Remaining delay period. The results of the ANOVA obtained for this period were as follows. Statistically significant ($P < 0.05$, F-test) task, direction and task × direction interaction effects were observed in 45/151 (29.8%), 88/151 (58.3%) and 68/151 (45.0%) of cells, respectively.

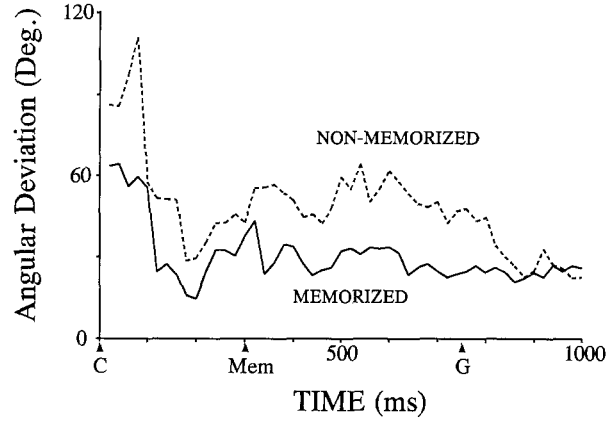

Fig. 10. Angular deviation of population vector is plotted against time for the two delay tasks. *G* Minimum time of onset of the *go* signal

Directional analyses

Ninety-four cells were directionally tuned (see Materials and methods). Their preferred directions ranged throughout the directional continuum. The length of the mean resultant of the preferred directions was 0.076; therefore, the null hypothesis that preferred directions are uniformly distributed is not rejected ($P > 0.5$, Rayleigh test).

Population vector analysis. There are two aspects of interest regarding the population vector. One concerns its direction, which can be interpreted as the directional information carried by the directional signal; the other aspect concerns the length of the population vector, which can be regarded as the strength of the directional signal carried.

The *direction* of the population vector during the memorized delay period was close to the direction of the target. An example is shown in Fig. 9. The directionality of the population vector became statistically significant 120 ms following the *cue* onset and remained so throughout the memorized delay period and until the end of the movement ($P < 0.05$–$P < 0.01$ for different time bins, modified Rayleigh test for vectors).

The direction of the population vector was closer to the target direction in the memorized than in the non-memorized delay task. This is illustrated in Fig. 10, which plots the angular deviation of the population vector from

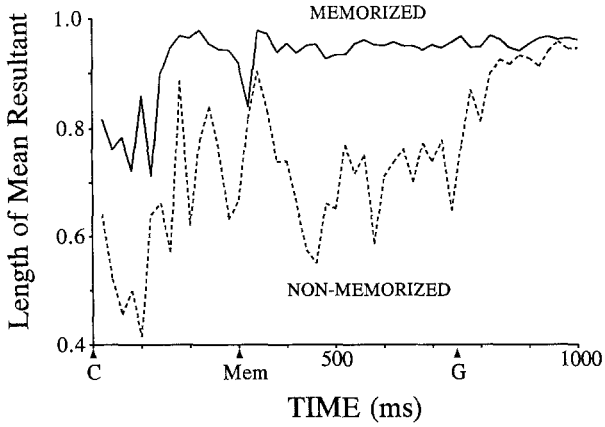

Fig. 11. Length of mean resultant of the population vector is plotted against time for the two delay tasks. Conventions as in Fig. 10

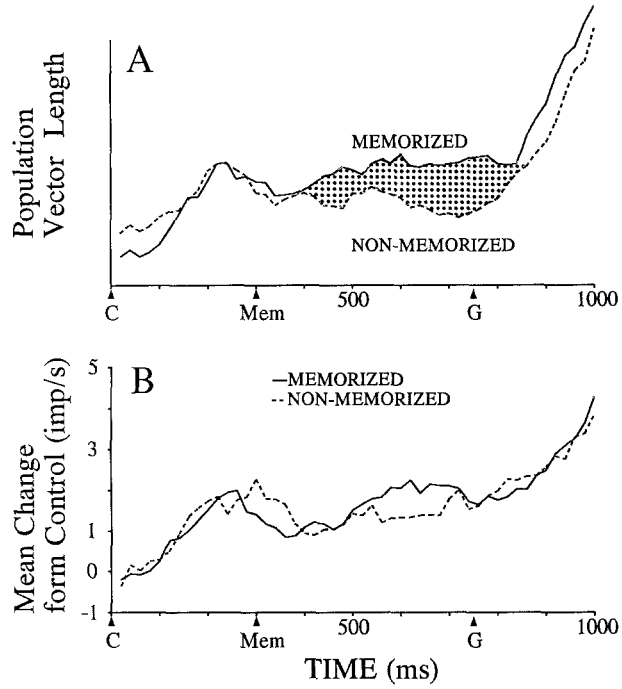

Fig. 12. Length of the population vector (**A**) and mean change in cell activity (**B**) from the rate during the center hold time are plotted against time for the two tasks used. Conventions as in Fig. 10

the target direction as a function of time in the two delay tasks. During the first 300 ms of the delay period this deviation was $19.8 \pm 3.47°$ (mean ± SEM) more in the non-memorized vs the memorized delay task ($P < 0.0007$, Wilcoxon paired signed ranks test, $N = 15$, 20 ms time bins); it was higher by $23.1 \pm 5.49°$ during the subsequent 440 ms of the remaining minimum delay period ($P < 0.0001$, Wilcoxon paired signed ranks test). Moreover, the directional signal was tighter in the memorized delay task. This is illustrated in Fig. 11, which plots the length of the mean resultant of the population vector vs time in the two delay tasks: it can be seen that it is consistently higher in the memorized delay task. (The length of the mean resultant is inversely related to the directional variance: the longer the mean resultant, the less the variance, and the tighter the distribution.) During the first 300 ms of the delay, the mean resultant was longer by 0.239 ± 0.028 (mean ± SEM) in the memorized vs the non-memorized delay task ($P < 0.0007$, Wilcoxon paired signed ranks test) and by 0.227 ± 0.019 during the subsequent 440 ms of the remaining minimum delay period ($P < 0.0001$, Wilcoxon paired signed ranks test).

The *length* of the population vector reflects the strength of the directional signal: it was highest during the reaction time and smaller during the two parts of the delay period. Concerning the comparison between the two delay tasks, it is interesting that the population vector length was similar in the cue period but it was longer during the memorized vs the non-memorized part of the delay. This is shown in Fig. 12A, which illustrates the time course of the length of the population vector in the two delay tasks. Three phases can be distinguished in this time course. First, there is an initial increase of the vector length during the 300 ms of the delay period; this increase is similar for both tasks. Second, this increase subsides during the rest of the non-memorized delay period but continues at a somewhat higher level during the memorized delay period; the latter difference is indicated in Fig. 12A by stippling. Finally, there is a steep increase in the population vector length following the *go* signal, at the end of the delay period. Thus the memorized task is distinguished from the non-memorized one by the higher population signal during that part of the delay period during which the instructed direction had to be kept in memory.

These differences between the two tasks with respect to the length of the population vector were not statistically significant for the first peak (0–400 ms; $P < 0.1$, Wilcoxon paired signed ranks test) but were highly significant for the remaining delay period ($P < 0.0003$, Wilcoxon paired signed ranks test). In contrast to this difference in the length of the population vector, the average changes of cell activity from the preceding control period (Fig. 12B) were very similar in the two tasks. Overall then, the strength of the directional signal is higher and its information content more accurate and more tight in the memorized delay task. All of this evidence above suggests that the memory process possessed a distinctly directional aspect, which differed to some extent from that in the non-memorized task. This was further evaluated by examining the directional properties of the cells recruited over time during the delay period.

Engagement of cells with specific preferred directions during the delay period. The analyses above dealt with the neuronal population vector. However, a different insight into the memory process can be gained by analyzing the directional properties of cells engaged during the delay period. In particular, we wanted to know whether cells with specific preferred directions (e.g. in the memorized direction) were preferentially recruited at different intervals during the delay period. We investigated this problem as follows: (a) For each of the two delay tasks, it was determined whether the cell changed activity during the first 300 ms of the delay period and, if so, its preferred

Table 5. Directional contributions of cells recruited during the delay period in the memorized and non-memorized delay tasks. See text for details

	Memorized delay task	*Non-memorized delay task*
First 300 ms		
Target direction ± 45°	21 (38.9%)	23 (46.0)
Other directions	33 (61.1)	27 (54.0)
Total	54 (100.0)	50 (100.0)
Remaining delay period		
Target direction ± 45°	27 (42.2)	13 (22.4)
Other directions	37 (57.8)	45 (77.6)
Total	64 (100.0)	58 (100.0)

direction was noted; if the cell activity increased, the cell was taken to exert a directional influence in its preferred direction; if the activity decreased, the opposite direction was taken. The same procedure was followed for the remaining part of the delay period, if the cell activity did not change during the first 300 ms. (b) Frequency distributions of cells exerting directional influences were constructed for the first 300 ms and the remainder of the delay period. (If a cell did not change activity, it did not contribute to the distribution.) (c) Data from all five directions were pooled after they were normalized with respect to the target direction.

The results are given in Table 5. It can be seen that, during the first 300 ms of the delay period, very similar proportions of cells with directional contributions in and around the target direction (±45°) were engaged in both delay tasks (38.9% in the memorized vs 46.0% in the non-memorized delay task); these differences were not statistically significant ($\chi^2(1) = 0.54$, $P < 0.46$). However, during the remaining delay period, this proportion was almost twice as high in the memorized (42.2%) than in the non-memorized (22.4%) delay task; this difference was statistically significant ($\chi^2(1) = 5.4$, $P < 0.02$). This finding indicates (a) that the memory process engages cells in the direction memorized and (b) that this is not necessary when memorization is not required.

A final point concerns the magnitude of the directional contributions made by the cells engaged during the two epochs of the delay period (see Materials and methods). These data are shown in Fig. 13A and B for the first 300 ms and the remaining delay period, respectively. The numbers in the abscissa indicate the center of the directional interval of 45° width used to bin the directional contributions, with zero at the normalized target direction; the ordinate indicates the average magnitude of the contribution; and filled and open bars indicate the memorized and non-memorized delay tasks, respectively. It can be seen that (a) for both epochs the directional contributions are highest at the target direction and relatively symmetric, (b) the magnitude of the directional contributions is very similar for the two tasks in the first 300 ms of the delay period (Fig. 13A), but (c) it is higher for the directions in and around the target direction in

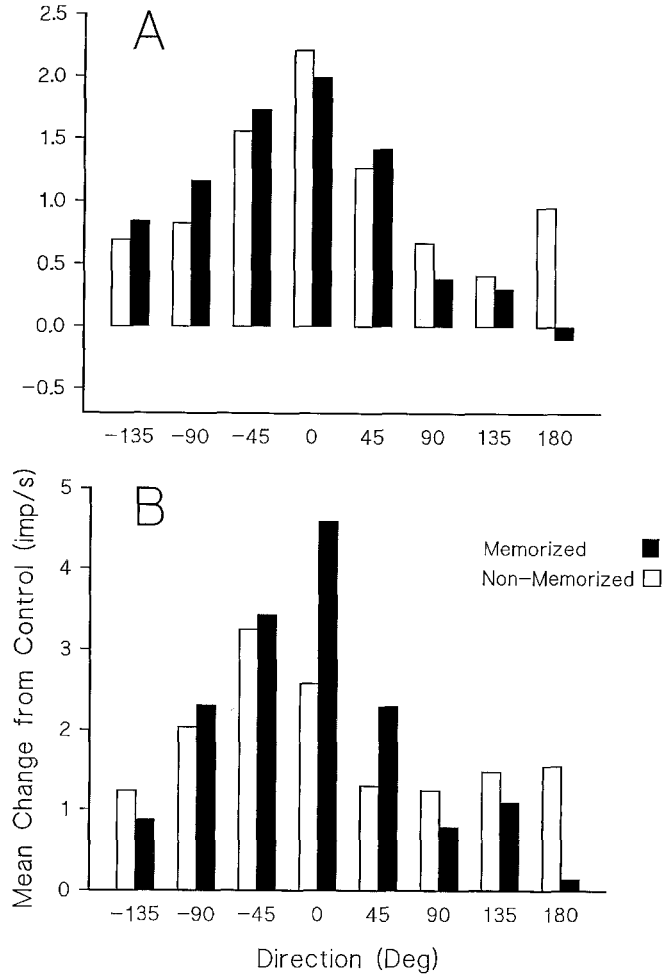

Fig. 13A, B. Bar graph of magnitude of directional contributions. **A** First 300 ms of the delay period. **B** Remaining delay period

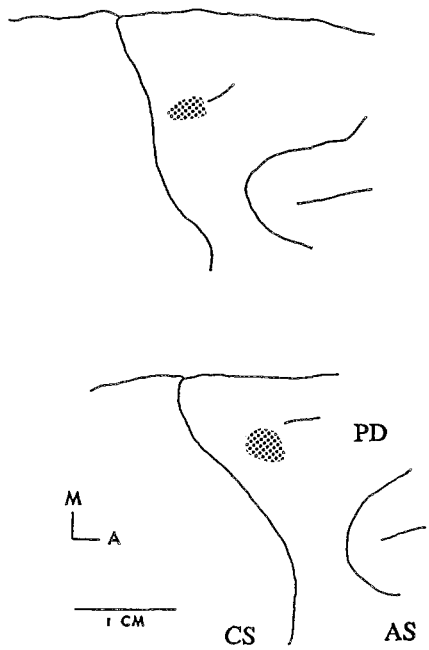

Fig. 14. Recording areas. *CS*, Central sulcus; *AS*, arcuate sulcus; *PD*, precentral dimple

the memorized than in the non-memorized delay task, in the remaining delay period. These results indicate that the memory process not only engages the directionally appropriate cells but also engages them at higher intensities of changes in activity.

Location of penetrations

All recordings were made in the motor cortex (Fig. 14). Most of the recordings were done in the crown and exposed part of the motor cortex but posterior to the precentral dimple.

Discussion

The results of the present study extend previous observations obtained using an instructed delay paradigm (Georgopoulos et al. 1989a) to a memorized delay task. There were four main findings of this study. First, motor cortical cell activity was engaged during the memorized delay period. Second, the neuronal population vector analysis identified the information kept in memory as the direction of the memorized target and/or upcoming movement. This was also confirmed using an analysis of the directional properties of the cells engaged during the delay period, without using the population vector analysis. Third, two processes were suggested by the data, one related to the encoding of the directional information and the other related to its retention in memory. Fourth, these processes were differentially engaged in the memorized and non-memorized tasks.

Evidence for memory-related activation of motor cortical cells was provided recently in two different tasks, one of which required the animal to make a previously performed movement (Alexander and Crutcher 1990), whereas the other required the animal to trace a previously outlined trajectory (Hocherman and Wise 1991). In both studies a good number of motor cortical cells showed changes in activity during the preparatory period preceding the triggering of the movement. An effect on motor cortical activity was also observed preceding a delayed movement sequence (Clark et al. 1991; Kettner et al. 1991; Marcario et al. 1991). We employed a paradigm introduced by Hikosaka and Wurtz (1983) and used by those and other investigators (Bruce and Goldberg 1985; Gnadt and Andersen 1988; Funahashi et al. 1989) for the study of memorized eye movements. In all of those studies it was found that cells in subcortical (Hikosaka and Wurtz 1983) and cortical (Gnadt and Andersen 1988; Funahashi et al. 1989) areas showed changes in activity during the memorized delay period. This finding has been interpreted as evidence for the involvement of these areas in "holding in memory" the signal for the upcoming movement. The results of the present study are very similar to those above obtained for eye movements. Thus, we found that a good number of motor cortical cells changed activity during the memorized delay period; therefore, the motor cortex seems to be involved in this memory process. The delay period used in our experiments was 450–750 ms. However, we do not know for how long the motor cortex would sustain the memory signal and whether in that respect it may differ from the prefrontal cortex where the memorized signal can be apparently maintained for at least 3 s (Funahashi et al. 1989). An additional finding of our study was that cells were occasionally (i.e., for some directions) exclusively activated in the memorized delay task (see, for example, Figs. 6, 7). However, most cells were engaged in all three tasks, although frequently at different rates. Judging from the almost ubiquitous presence of memory cells in the eye movement system, it is reasonable to predict that cells such as those found in this study will be found in other motor areas as well. This would imply that these memory operations are distributed in these various areas. In fact, such cells have been observed in the premotor cortex (Mauritz and Wise 1986). In the present experiments the direction of the upcoming movement and the direction of the visual cue were the same, and therefore the discussion below does not differentiate between these two possible signals. We will be referring below to "movement direction", but in that we mean visuomotor processes in general. Finally, the results of this study provide a cellular basis for the psychophysical independence of the processes involved in the planning and triggering of motor responses (Ghez et al. 1990).

The fact that cells are active during a memorized delay period suggests their involvement with memory, but does not reveal the content of the information they carry. The directional tuning observed during the delay (Funahashi et al. 1989) indicates that this information concerns the direction of the movement and/or the location of the target. We obtained a more clear visualization of the information carried by the neuronal ensemble during the memorized delay period by using the neuronal population vector analysis (Georgopoulos et al. 1983). This analysis provides an unambiguous measure of the directional tendency of the ensemble. Moreover, the signal can be followed in time (Georgopoulos et al. 1984), and thus it can provide an ongoing evaluation of the information processing in the directional domain. The directional information carried by the population vector in the memorized task identifies the memorized information in a direct fashion. Moreover, this analysis provided an insight concerning the time course of encoding and holding directional information. For that purpose we used the length of the population vector, which can be regarded as reflecting the strength of the directional signal in the neuronal ensemble. The population vector length showed an initial increase, which started approximately 100 ms following the cue onset and peaked at 250 ms. This increase was very similar in both the memorized and the non-memorized delay tasks (Fig. 12A). We interpret this initial peak as reflecting an *encoding* process. A second phase followed that differed in the memorized and the non-memorized tasks in that a higher, sustained signal was present during the memorized delay period, but not during the non-memorized delay (stippled area in Fig. 12A). We interpret this as reflecting a *holding-in-memory* process. Following the onset of the "go" signal, the

population vector length increased similarly in all tasks used. These findings are interesting because the increase in the signal during the memorized delay period was observed *in the absence* of the target; however, one would have expected that the signal would be stronger in the presence rather than in the absence of the visual stimulus. This finding strengthens our interpretation of this increase as a memory signal, in contrast to a sensory one and raises the more general possibility that the motor cortex may be particularly involved when only part of the visual information about an upcoming movement is provided. The process for generating a movement in a visually specified direction can be thought of as a process that translates the visual information into motor information. In the case of directed movements, the visual information about direction is best given by two stimuli that specify the axis of the movement. (The information about direction is usually provided from the context and training, e.g., center → out movements in the present experiments.) This arrangement should provide all the information and should require the least processing in the translation process. However, when, for example, only the target is presented, without a stimulus at the starting point, the direction of movement will have to be derived from other visual sources (e.g., if one sees one's own hand) or from proprioceptive information about the position of the hand in space. In either situation, more processing will be required. Evidence for a gain of directional information with time when a directed motor output has to be generated has been provided by the results of behavioral studies (Massey et al. 1991), which also showed that the manipulation of the amount of visual information provided is reflected in the amount of information gained. In neural recordings, Riehle and Requin (1989) found an effect on motor cortical cell activity of precueing information concerning the direction of movement. In fact, they observed a reduction in cell activity when requisite information was precued. This is in accord with the present findings of lower activity in the non-memorized delay task in which all the information was present throughout the delay period.

Finally, there are two more aspects of the present results that are noteworthy. The first is that differences between the memorized and non-memorized delay tasks could be identified and interpreted within a directional framework as ongoing, time-varying processes, in spite the fact that the time course of the changes in cell activity (Fig. 12B) was almost identical in the two tasks. This indicates that different messages can be carried by similar changes in cell activity. The second point is that the analysis of the distributions of the directional contributions of cells recruited in the active population during the delay period was sufficient to reveal the relevant directional characteristics of the memory process. A similar analysis was successfully used to provide crucial evidence for a rotation process when other alternative explanations were possible (Lurito et al. 1991).

In summary, the present findings of increased motor cortical activity during the memorized delay period suggest the intriguing possibility that the motor cortex might be specifically involved in these circumstances of partial information, in addition to its involvement in generating a movement under usual conditions; in other words, it may be more involved the more uncertainty there is. In a way, this makes sense because then motor cortical involvement can be regarded as resulting in the reduction of uncertainty, that is, in an increase of information that is only an appropriate function for the brain.

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