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RESEARCH ARTICLE

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Neural activity in prefrontal cortex during copying geometrical shapes

I. Single cells encode shape, sequence, and metric parameters

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Abstract In drawing a copy of a geometrical shape, a sequence of movements must be produced to represent the sides of the object in the proper spatial relationship. We investigated neural mechanisms of this process by training monkeys to draw (using a joystick) copies of geometrical shapes (triangles, squares, trapezoids and inverted triangles) presented on a video monitor while recording single cell activity in prefrontal cortex. The drawing trajectories monkeys produced were divided into a series of discrete segments, varying in direction and length. We performed a stepwise multiple linear regression analysis to identify those copy parameters significantly influencing cell activity. The copied shape (e.g., triangle, square) and the serial position of the segment within each trajectory were the most prevalent effects (in 46% and 43% of cells, respectively), followed by segment direction (32%) and length (16%). Effects of temporal factors (maximum segment speed and time to maximum segment speed) were less frequent. These results demonstrate that prefrontal neurons encode several spatial and sequence variables that define copy trajectories. We also found that specific groupings of significant effects tended to occur together in single neurons. Specifically, single neurons simultaneously processed the serial position of a segment within each trajectory along with the corresponding spatial (but not temporal) attributes of that segment (i.e., direction and length), as well as with the overall shape to which the segments belong. Finally, we discovered that relationships between neural activity and segment serial position were systematic in many instances, described by monotonically increasing and decreasing functions, as well as parabolic functions. These findings indicate that, within the copying task, the serial segment position is a key factor for neural activity in the periprincipalis area of the prefrontal cortex.

Keywords Prefrontal cortex · Constructional apraxia · Copy · Motor performance · Neural activity

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Introduction

Copying objects has been used as a probe to detect brain damage since early in the last century (Poppelreuter 1917). The inability to copy objects accurately is considered a sign of constructional apraxia (Kleist 1934), which can be defined broadly as the "inability to assemble component parts into a coherent whole" (Koski et al. 2002). Although constructional deficits are most often observed following damage to posterior parietal cortex, there is broad consensus that constructional deficits and other forms of apraxia also result from cortical damage that is confined to the prefrontal cortex (Luria and Tsvetkova 1964; Benton 1968; Gainotti 1985; Koski et al. 2002). The fact that lesions in widespread cortical areas can cause constructional deficits led Benson

and Barton (1970) to suggest that, "... drawing, by itself, is a reasonably good test for detecting brain damage." One possible reason that constructional ability is easily disturbed may be that it is a complex task requiring the functional coordination of many different processes.

In this work, we trained monkeys to copy simple geometrical shapes and recorded the activity of single cells recorded simultaneously as small ensembles in the prefrontal cortex while the monkeys performed the task. The shapes were drawn as a series of oriented movement segments and, therefore, could be analyzed at different levels; for example, at the level of drawing single segments, at the level of the ordered sequence of segments, and at the level of the overall shape. The two papers in this series are organized as follows. This first paper describes the results of analyses of the activity of single cells in relation to these various aspects of copying; and in the second paper (Averbeck et al. 2003) we focus on coding and information-theoretic issues using small neuronal ensembles recorded simultaneously. Preliminary results have been presented (Averbeck et al. 2001).

Materials and methods

Animals

Two male rhesus macaques, *Macaca mulatta* (M157 and M555, 8–10 kg BW), were used in the experiments. Care and treatment of the animals during all stages of the experiments conformed to the *Principles of Laboratory Animal Care* (NIH publication no. 86–23, revised 1995). All experimental protocols were approved by the appropriate Institutional Review Boards.

Copy task

During the experiments, monkeys drew copies of visual stimuli projected on a tangent screen using a joystick. The position of the joystick was linked to the position of a visible cursor whose movement left a persistent trace on the screen. To initiate a trial, monkeys maintained the joystick cursor within a start hold circle presented on the left half of the display (Fig. 1A), for a waiting time (WT, 1 s for M157, 2 s for M555). At the end of the WT, a template stimulus appeared on the right half of the screen. The template was a geometrical shape that the monkey was required to copy. Monkey M555 copied triangles and squares and monkey M157 copied triangles, squares, trapezoids and inverted triangles (Fig. 1B). When the template appeared the cursor began to leave a visible trace and the monkey was free to begin drawing. Since all drawings started from the same position and templates were centered, the point at which the movement was initiated was at the top vertex of the upright triangle but at the middle of the top side for the other shapes (Fig. 1B).

To complete a trial successfully, the monkey had to draw an accurate copy; the trajectory had to remain within pre-specified corridors (not visible to the monkey) that bounded the ideal trajectory defined by each template shape, and close the drawn shape by returning to the start circle. Monkeys drew copies that were spatially offset from the templates, and did not trace them. If the monkey produced an accurate copy, it was rewarded once each trial when the joystick cursor reentered the start circle. Monkeys produced a smooth and continuous drawing while executing each copy trajectory in a manner similar to that one might expect of a human subject (although this apparently smooth trajectory was actually composed of a sequence of submovements, see below). Trials were aborted without reward if the joystick cursor strayed beyond the corridor. The monkeys copied shapes within two different trial sequences. M157 drew shapes in a sequence of blocks, each block consisting of six trials drawing a single shape. Six successful copies of each shape were required in each block before the next shape was presented, and the order of blocks (shapes) was randomized within each block sequence. Each set of neurons was examined while the monkey drew five repetitions of the above randomized block sequence, resulting in 30 correct trials for each of four shapes. M555 drew two blocks of 60 trials of each shape; the order of blocks was randomized for each set of neurons. Only correct trials were analyzed.

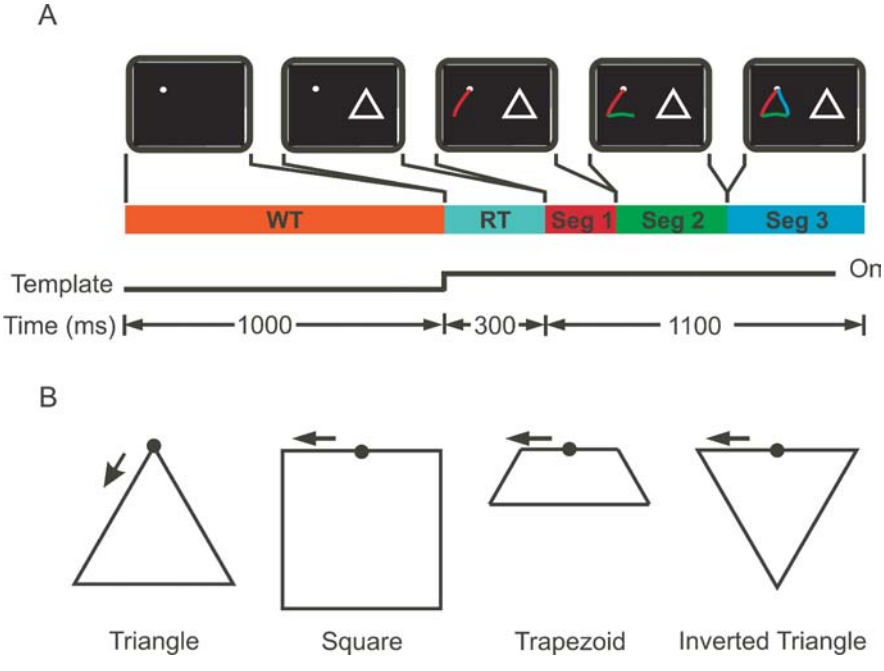

Fig. 1A, B Task diagram and shapes drawn. **A** Task sequence. To initiate a trial the monkey moved the cursor to the start hold circle, indicated by a *white circle* in the WT panel of the task diagram. After the WT a template was presented on the right half of the screen. The monkey then drew the template which had been presented (the segments of the drawn trajectory are depicted in different colors for purposes of illustration; during the task the cursor left a white trace). After returning to the start hold circle the trial was complete. **B** The four shapes drawn by the monkeys

A freely moving joystick (model 541 FP, Measurement Systems, Inc., Norwalk, CT) was used as an input device. A 26-mm excursion of the joystick (equivalent to one side of the square) resulted in a 113-mm (13.4° of visual angle) excursion of the cursor on the screen. The joystick position was sampled at 200 Hz along with the eye-movement data. The joystick was manipulated by the left hand of both monkeys.

An LCD projector (NEC Model MT-820) with a resolution of 480×640 pixels was used to project an image on a tangent screen placed 47 cm in front of the monkey. One centimeter on the screen corresponded to 1.2° of visual angle.

Data acquisition

Eye position was monitored using the scleral search coil technique (CNC Engineering, Seattle, WA; Fuchs and Robinson 1966; Judge et al. 1980). The coil was implanted during an aseptic surgery, performed under general gas (isoflurane) anesthesia. In the same surgery four posts were attached to the skull with stainless steel 4–40 screws, and a titanium recording chamber (7 mm i.d.) was implanted within a small craniotomy. A single chamber was placed over the principal sulcus of the prefrontal cortex in the right cerebral hemisphere of both animals, and was guided using coordinates from a structural MRI. A lightweight halo (Nakasawa Works Co., Tokyo), necessary for holding the head fixed, was attached to the implanted posts. Eye position was sampled at 200 Hz.

We recorded the activity of multiple single cells simultaneously using a 16-microelectrode recording matrix (Uwe Thomas Recording, Marburg, Germany; see Mountcastle et al. 1991; Lee et al. 1998). We isolated and recorded all cells present with no preselection.

Preprocessing of motor data

The copy data were analyzed in several steps. First the hand position and eye position data were filtered (Mottet et al. 1994) and differentiated twice with respect to time (Rabiner and Gold 1975). This provided filtered position, velocity and acceleration data every 5 ms (200 Hz sampling rate) for both the hand and the eye.

We defined the start point (SP) of each trajectory as the point at which the monkey moved the cursor out of the start hold circle, and the reaction time (RT) as the interval between the onset of the template stimulus and the time at which this occurred. Copy trajectories were divided into a series of movement segments—monkeys produced a smoothly linked series of such movement segments in copying each shape. Each movement segment was characterized by its own bell-shaped velocity profile, and the boundaries between them were identified by minima in joystick velocity (Fig. 3). Specifically, we identified points in the trajectory where joystick acceleration switched from negative (decelerating) to positive (accelerating). These positive-going acceleration zero crossings correspond to the transition between the end of one movement (when the joystick decelerates to a velocity minimum) and the beginning of the next (when the joystick accelerates again). The monkeys occasionally made multiple small submovements within a straight segment of a given trajectory and near the vertices; in those cases, we picked the zero-crossing that was closest to the vertex. The various shapes were segmented by the monkeys as follows. The triangle had three segments for both monkeys; the square had three segments for M555 (this monkey tended to round the top corners) and five segments for M157; the trapezoid had three segments (M157), and the inverted triangle four segments (M157).

Copy trajectory averaging

Average trajectories were derived by dividing the copy trajectories produced for each trial into a fixed number of bins, such that the width of the bin scaled with the length of the trajectory. Then, the *x*-*y* position of the trajectory was sampled at each of the points separating adjacent bins, spaced at equal intervals E $(E=D/B)$, where D is the total length of the trajectory and B is the number of bins. The sampled points were averaged across trials to generate an average trajectory. The average firing rate of each neuron was calculated during the intervals corresponding to each bin of the averaged trajectory. Changes in average firing rate were represented in plots of the average trajectory by employing a line width for each bin proportional to the firing rate (Figs. 6, 7, 8, 9, 10, 11).

Analysis of saccades

Saccades were found by locating points of negative going acceleration zero-crossings that also exceeded a speed threshold in the eye movement data. These points correspond to maxima in the speed profile and mark the midpoints of saccades. The speed threshold insured that random fluctuations and noise were not detected as saccades. After the speed maximum was identified, the algorithm searched forwards and backwards until the speed fell below a pre-specified threshold. These points were then marked as the beginning and end of the saccade.

Analysis of the relations between neural and behavioral data

We investigated the relations between neural activity and several parameters defining each segment of the drawn trajectories using a multiple linear regression analysis with a forward stepwise procedure (Draper and Smith 1998). The dependent variable was the frequency of discharge during a given segment, and the independent variables included the following: the serial position of the segment, and the shape of the template (shape factors); segment direction, peak joystick speed, time to peak speed, curvature, length, and joystick position at the midpoint of the segment (segment factors); and, finally, average eye position, and saccade frequency (oculomotor factors). Segment direction was expressed in separate x and y components (direction cosines). Segment curvature was calculated as the sum of the shortest distances between each point in a trajectory segment to the chord anchored at the beginning and end of that segment. Saccade frequency was expressed as the number of saccades made in each of the four direction quadrants (demarcated by the horizontal and vertical meridians). Shape and serial position were coded as dummy variables. Average eye and joystick position were expressed as x and y components. The sequential trial number was also included as a covariate to account for possible time trends in neural activity.

Variables were added or removed from the model during the stepwise procedure in groups, based on the fact that, together, they specified a particular parameter. These sets included: (a) the *x*- and *y*-components of the direction of a segment, (b) the *x*-*y* joystick position of the midpoint of the segment, (c) the average *x*-*y* eye position during the drawing of a segment, and (d) the four variables representing the number of saccades in the four quadrants, (e) shape, and (f) segment serial position. The shape and the serial position of a segment were entered as qualitative factors.

The analysis proceeded in a forward stepping manner. The model began with an intercept. At each step the algorithm assessed all variables that were already in the model for removal and all variables that were not yet in the model for addition. If a variable that was already in the model had a p value that exceeded 0.051, it was removed from the model. If no variables were removed, the variable or variable group not yet in the model with the smallest p value less than 0.050 was chosen for addition to the model. When no variables could be added or removed from the model, the process was terminated. In order to determine which set of variables had the strongest impact on the activity of each neuron, we ranked the factors on the basis of the increment in R^2 gained by the addition of the set to the model, in the presence of the other variables. Finally, a collinearity analysis was performed using the SPSS 10.1 statistical package for Windows (SPSS Inc., Chicago,

IL, 2000) to ensure that possible correlations between the independent variables did not interfere with the results of the regression analysis.

Analysis of frequencies of occurrence of significant effects

The multiple regression analysis above identified the factors that had a significant influence on cell activity. In this analysis we were interested in testing whether certain effects occurred together (for a single cell) more frequently than allowed by chance. Specifically, we wanted to test the significance of co-occurrence of effects of the serial position of the segment with other factors included in the analysis. For this purpose, we constructed a number of 2×2 tables containing data for a pair of factors (e.g., segment serial position and segment direction) and their binary attributes (significant effects present or absent). In all tables, the serial segment position was one factor whereas the other factors included the shape shown, and the direction, length, maximum speed and time to maximum speed corresponding to the particular segment. Chi-square statistics (two-tailed) were used to evaluate the level of statistical significance of association between the effects of a pair of factors (Armitage and Berry 1987). Since each factor could have an effect independently of the other factor, we also wanted to calculate the odds by which the co-occurrence of the two effects exceeded the level of independent occurrence. For this purpose we used the odds ratio. Finally, we were interested in testing the hypothesis that single cells might co-process more than two attributes of the four above (i.e., shape, segment serial position, direction and length). We used loglinear analysis in this case to assess the statistical significance of higher-order associations. Specifically, we applied the loglinear analysis procedure of the SPSS statistical package above using backward elimination. This analysis assessed the statistical significance of the single four-way and all possible three-way associations among the four factors above.

Results

General

We recorded the activity of 560 single cells from the right prefrontal cortex of two monkeys (168 cells from monkey M555 and 392 from M157). During these recordings, monkey M555 drew 2038 triangles and 1178 squares, and monkey M157 drew 778 triangles, 779 squares, 778 trapezoids and 776 inverted triangles. The following results are based on a detailed analysis of these drawings and the corresponding neural activity.

Behavioral results

Figure 2 shows ten single trials for each shape the monkeys drew. Movement trajectories were obviously segmented. Figure 3 shows the kinematic profile for a single trial when the monkey was drawing a triangle. The triangle was made up of three straight segments joined together by vertices. The velocity in the middle of the straight segments was high and decreased systematically as the trajectory approached the vertices. We identified positive-going zero acceleration crossings in the kinematic profiles of individual trajectories. These zero crossings mark the vertices, and the portion of the trajectory between two zero crossings corresponds to the segment of the shape being drawn.

Figure 4 shows a histogram of the angles at the zero-acceleration crossings for the triangle of M555 and the square of M157. The angles clustered around the ideal angles for the shapes: the modes were near 60° and 90°

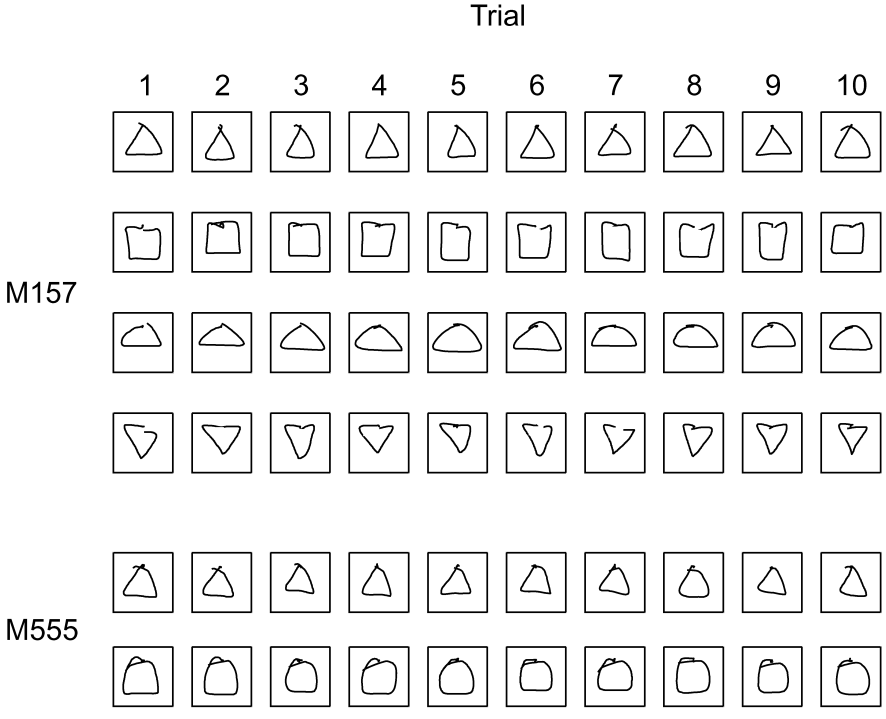

Fig. 2 Examples of copy trajectories. Each trajectory is from a single trial. Monkey M157 copied four shapes, triangle, square, trapezoid, and inverted triangle. Monkey M555 copied two shapes, triangle and square

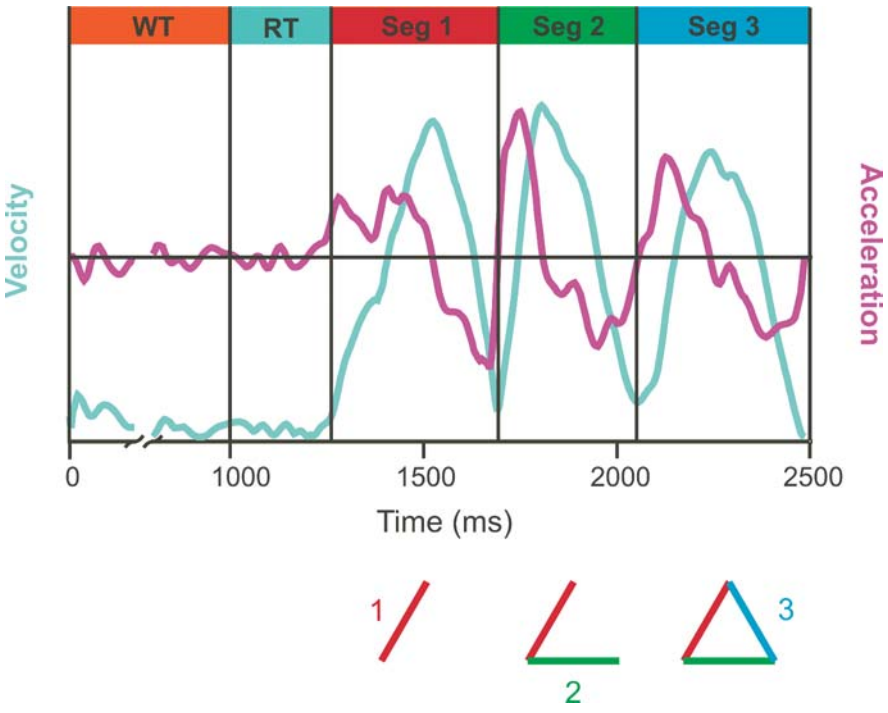

Fig. 3 Kinematics of drawing. Velocity and acceleration profiles for a single triangle trajectory. The *purple line* shows acceleration and the *blue lines* show velocity for one trial of the triangle. Standard bell shaped velocity profiles are evident for each segment of the triangle. The velocity minimums correspond to positive-going acceleration zero-crossings

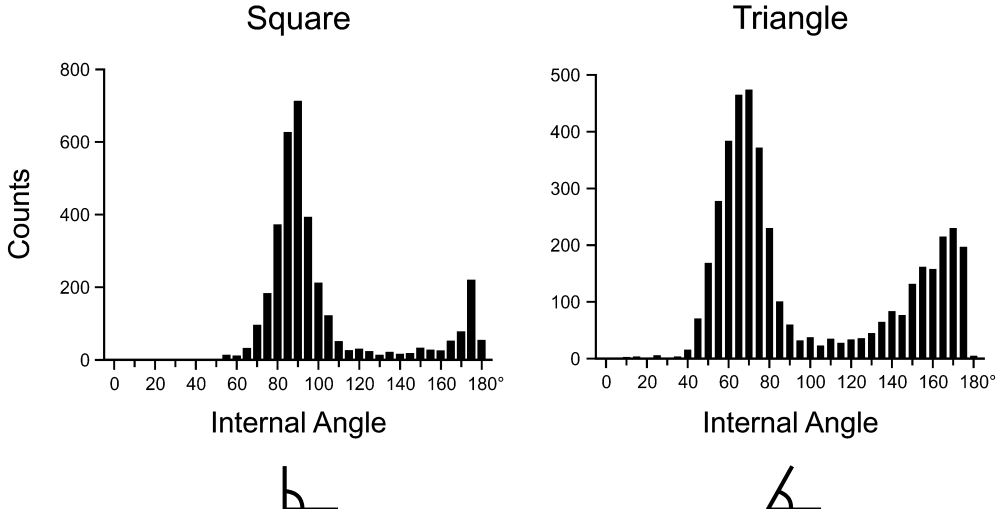

Fig. 4 Histograms of angles formed between successive segments bounding vertices in the trajectory (positive going acceleration zero-crossings). Modes are close to 60° for the triangle and at 90° for the square. Additional mode at 180° corresponds to submovements in straight segments

for the triangle and the square, respectively. There was an additional mode near 180° for both shapes. This was due to the fact that the monkeys often made small submovements as they were approaching the vertices. Such submovements are frequently observed in movements requiring endpoint precision (Keele 1981; Milner and Ijaz 1990).

Figure 5 shows a plot of the time sequence of eye positions, the trajectory drawn, and their relation to the template for a representative trial. In general the monkeys made a saccade to the template when it was presented, and then made a saccade back to the drawing area as they began to draw. The monkeys then typically made several saccades near the copy trajectory as it progressed.

Neural results

In a first attempt to organize the visualization of the neural activity, we constructed spike histograms by summing spikes across trials for individual shapes. These histograms were then plotted on the average trajectory for each shape. Thus one can see how the neural activity varies within an individual shape, as a function of the part of the shape being drawn, as well as how the neural activity varies across shapes. Figures 6, 7, 8, 9, 10, and 11 show examples of histograms and corresponding rasters from six different cells. The thickness of the line of the average trajectory plotted is proportional to the average spike rate for a bin.

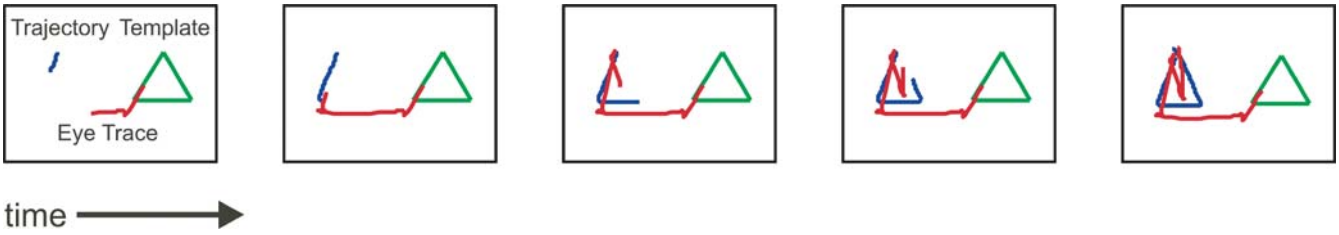

Fig. 5 Eye trajectory (*red*), hand trajectory (*blue*) and template shape (*green*) shown at a sequence of successive time points in a representative trial. The monkeys generally made a saccade to the template after it appeared. They then made a saccade back to the drawing area as drawing began, followed by a sequence of smaller saccades falling near the advancing copy trajectory as this progressed

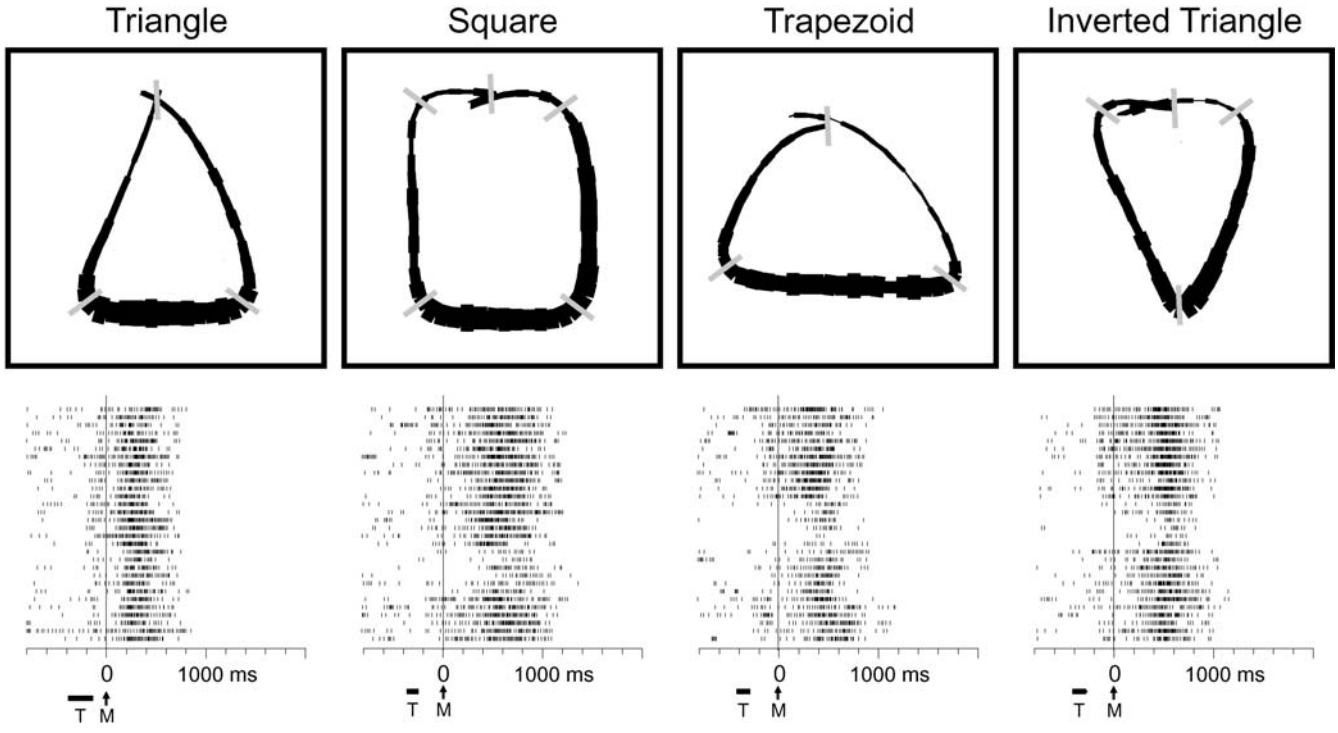

Fig. 6 The activity of a single prefrontal neuron during copy performance. Average spike activity is plotted on the average trajectory, above (the width of the line is proportional to firing rate, vertices indicated by perpendicular line segments). Trials in rasters below are aligned to the initiation of the drawing movement (*M*). The range of template onset times is indicated by a *horizontal bar (T)*. Drawing proceeds in a counterclockwise direction from the start dot. Neural activity increased markedly at specific points in the copy trajectory of each shape, during the drawing of the lower segments of the triangle, square, and trapezoid, and the last segment of the inverted triangle

The histograms and rasters in Figs. 6, 7, 8, 9 (M157) and 10 and 11 (M555) show that the neurons were clearly modulated during the drawing of the shapes. For example, Fig. 6 shows a cell that increased its firing rate while drawing the bottom segment of the triangle, the square and the trapezoid. This cell was apparently modulated by the direction of a segment, and preferred the rightward direction. The example in Fig. 7 illustrates other aspects of the modulation of neural activity during copying. This cell was active for the last segment of the square, the inverted triangle, and the last portion of the trapezoid. It had very little activity for other segments of the shapes. It is interesting to note that despite the fact that the first and last segments of the square and inverted triangle have the same directions, this neuron was only active for the last segment of each shape. This indicates that neural activity in this case was related to the serial position of the segment in the sequence and not to movement direction alone. Another example is shown in Fig. 8. This cell was highly active for the first segment in multiple shapes. In another example (Fig. 9) there was activity for the bottom side of the square and the trapezoid but hardly so for the bottom side of the triangle, despite the fact that all of these segments were highly similar in direction and position. Figure 10 shows an example of a cell that preferred the first segment of the square and the triangle. Finally, Fig. 11 shows a cell that has a strong increase in

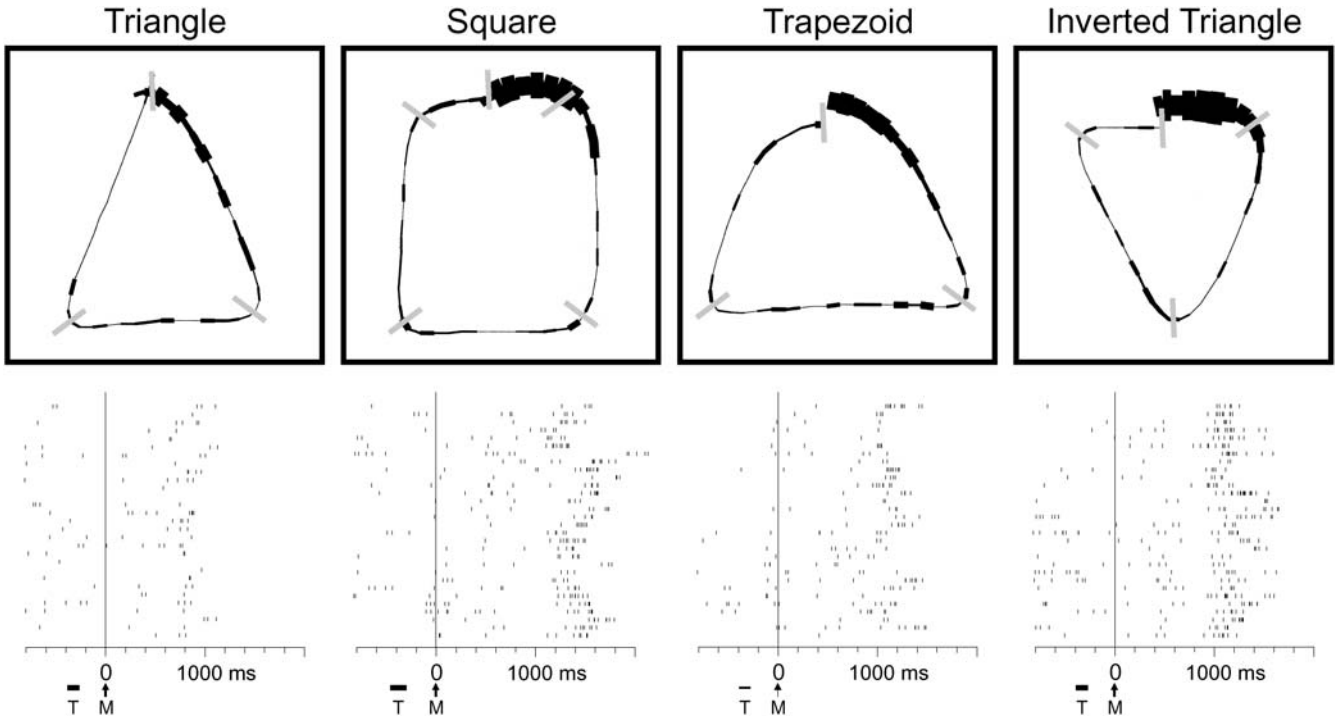

Fig. 7 The activity of a prefrontal neuron that increased during the drawing of the last segment of the square, the trapezoid and the inverted triangle. Little activity was seen while drawing segments of the same direction but different sequential order in the copy trajectory (e.g., the first segments of the square, trapezoid, and inverted triangle). Conventions as in Fig. 6

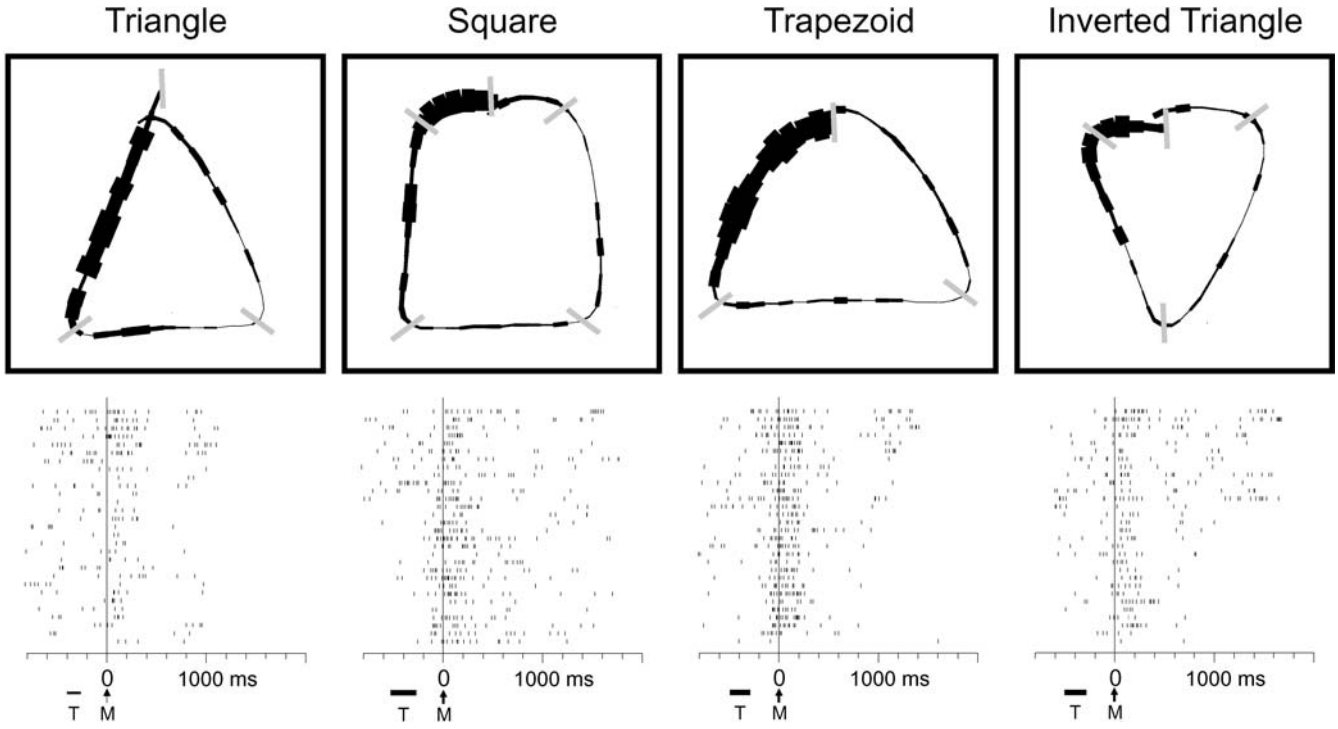

Fig. 8 The activity of a prefrontal neuron that increased during the drawing of the first segment of all shapes. Conventions as in Fig. 6

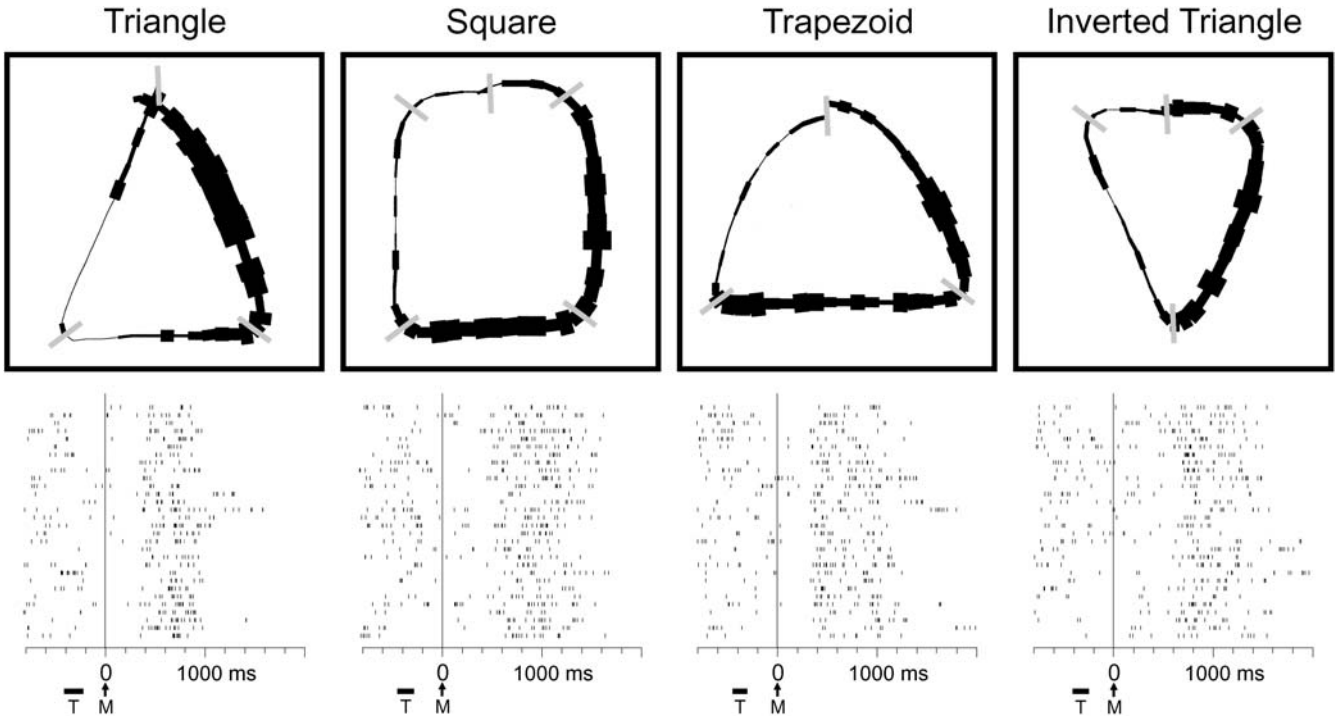

Fig. 9 The activity of a prefrontal neuron that increased during the drawing of the bottom and right segments of most shapes. This cell showed a clear dissociation between direction and neural activity in that it was active for the bottom segment of the square, but not the bottom segment of the triangle. Conventions as in Fig. 6

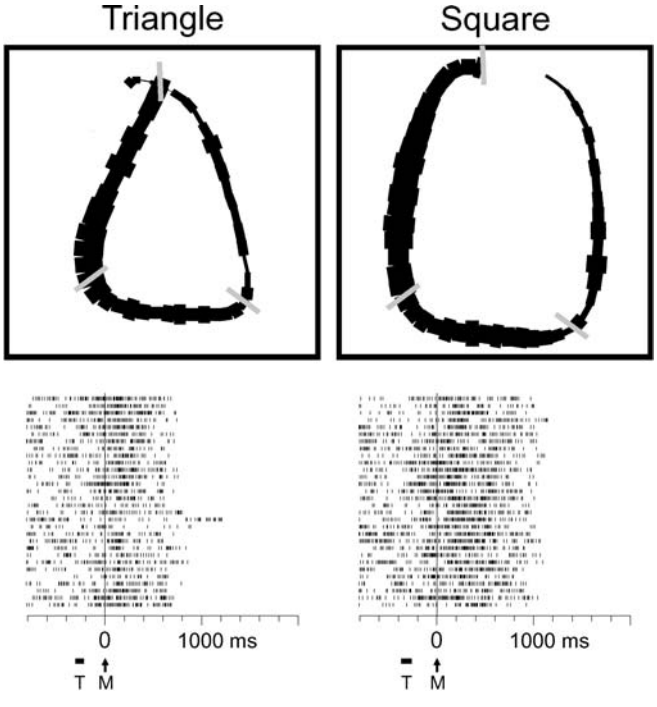

Fig. 10 The activity of a prefrontal neuron that increased during the drawing of the first segment of the square and the triangle. Conventions as in Fig. 6

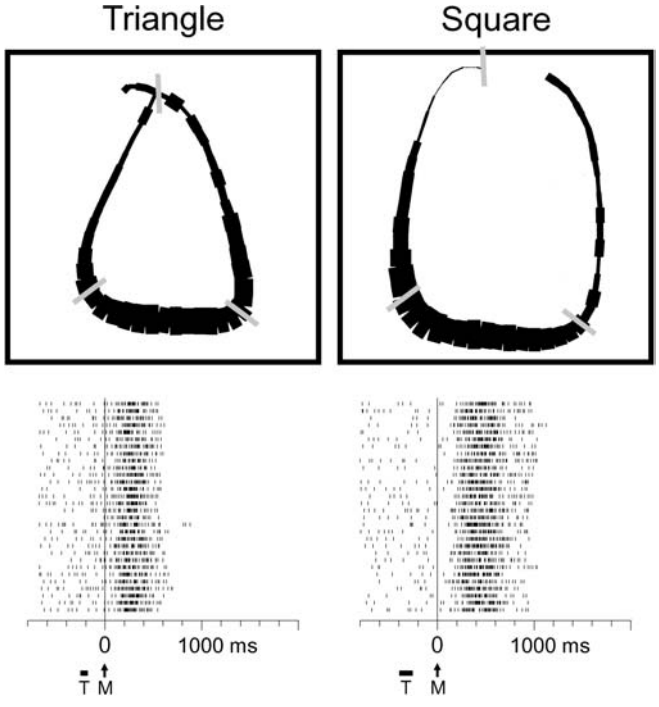

Fig. 11 The activity of a prefrontal neuron that increased during the drawing of the bottom segment of the triangle and the square. Conventions as in Fig. 6

Table 1 The number of prefrontal cells significant for each variable in the stepwise multiple linear regression expressed as a percentage of the 560 prefrontal cells recorded in two monkeys

	Factor	Total
Shape factors	Segment serial position	240/560 (43%)
	Shape	257/560 (46%)
Segment factors	Maximum speed	83/560 (15%)
	Time to max speed	58/560 (10%)
	Curvature	65/560 (12%)
	Direction	179/560 (32%)
	Average position	163/560 (29%)
	Length	91/560 (16%)
Oculomotor factors	Average eye position	241/560 (43%)
	Saccades	86/560 (15%)

neural activity for the bottom of the square and the triangle.

Altogether, Figs. 6, 7, 8, 9, 10, and 11 show that the phenomenology of the neural activity is complex. It is reasonable to suppose that many factors are involved in the drawing of an individual shape, including factors related to arm movements, eye movements, and the shape as well as the segment of the shape being drawn. These factors could differently affect the activity of single cells. In an attempt to clarify this issue, we assessed the relative effect of an exhaustive list of factors on the activity of single cells using regression analysis.

Regression analysis

Given the pervasive segmentation of the movement trajectories, we used the neural activity during individual segments as the basis of the stepwise regression analyses. Specifically, we sought to evaluate the effect on the neural activity of several factors, including the shape drawn, the serial position of the particular segment, and a number of variables related to the movement of the hand and the eyes. Table 1 shows the independent variables used in the regression, and the numbers and percentages of cells for which a specific variable had a statistically significant effect. Overall, many factors had significant effects on cell activity. However, the most frequently significant factors were those that reflected more abstract properties, including the shape being drawn (46% of prefrontal cells) and the serial position of the segment in that shape (43%) ("shape factors"). Of the variables related to the specific execution of the trajectory ("segment factors"), the direction was significant in 32% of cells, average position of the segment was significant in 29%, and segment length was significant in 16%. Finally, eye position was also a prominent factor (43%), but not saccadic eye movements (15%) ("oculomotor factors"). These findings indicate that several factors during copying can influence neural activity. A different question concerns the number of factors influencing the activity of a single cell. This is shown in Fig. 12. It can be seen that single cells most commonly related to three to four

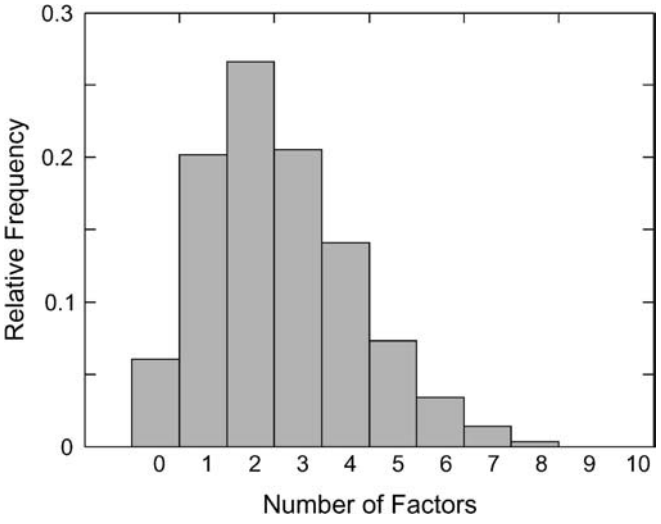

Fig. 12 Distributions of the number of cells whose activity was related to a given number of variables in the stepwise multiple linear regression ("Materials and methods")

factors, and rarely to more than six. Overall, the activities of 94% (525/560) of the prefrontal neurons included in this analysis were related to at least one of the trajectory segment or shape factors included in the analysis.

The importance of a particular set of factors on cell activity was assessed by calculating the gain in R^2 achieved by the addition of the variable or set of variables (see "Materials and methods") to the model, in the presence of the other variables. The variable (or set of variables) that produced the largest increment in R^2 when added to the model was considered to have the greatest explanatory power. Figure 13 shows the percentage of neurons for which each factor was the highest ranked across the population. It can be seen that segment serial position had the highest explanatory power in the most cells (31%), followed by shape (17%), eye position (16%), movement direction (15%), and hand position (11%). All remaining variables were of most importance in 4% of cells, or less. Finally, the collinearity analysis documented the independent contributions of the predictor variables. Observed tolerances substantially exceeded the tolerance threshold of 0.01.

Concurrent processing of copying parameters

Given that copying involves sequential drawing of shape segments with a certain direction and length, we hypothesized that the serial position of the segment might be processed concurrently with the other parameters above. We assessed the merit of this idea by analyzing the results of the regression analysis above by categorizing the result of the significance testing for each variable in a dichotomous fashion (presence or absence of effect). This yielded counts of binary outcomes for each pair of variables the association of which we were interested in testing. We then constructed 2×2 tables to test the significance of a

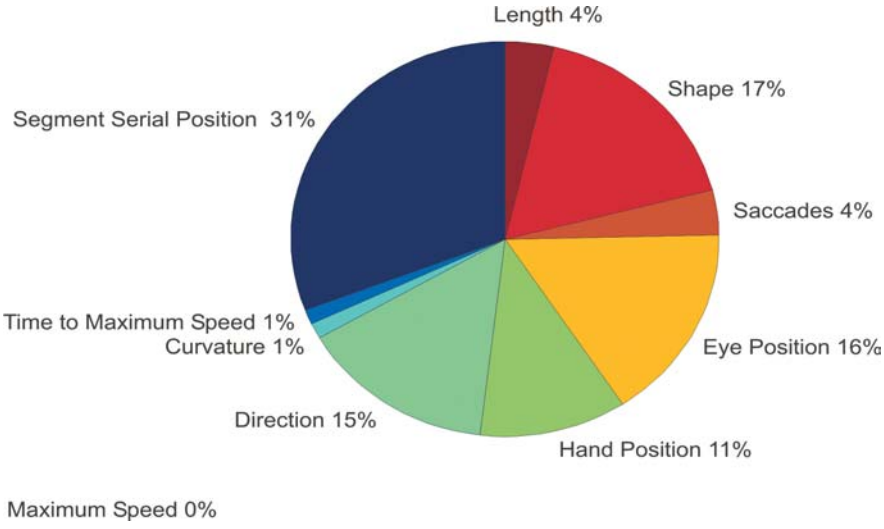

Fig. 13 Percentages of prefrontal cells for which each variable entered the stepwise regression equation first (i.e., accounted for the largest proportion of variance in the model). Segment was the most powerful explanatory variable in 31% of prefrontal neurons, more often than any other variable in the analysis. Shape was the most powerful explanatory variable in 17% of prefrontal neurons

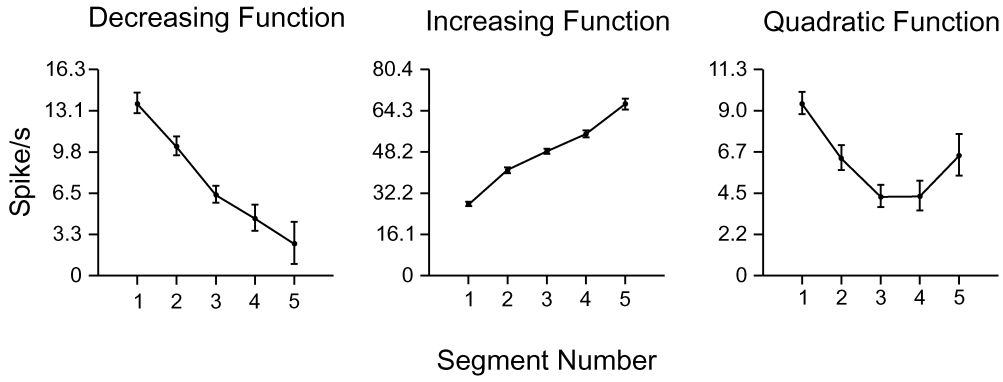

Fig. 14 Adjusted mean firing rate (±SEM) as a function of segment order (estimated effects of all other variables have been subtracted). Data are from three prefrontal neurons showing monotonic decreasing (*left*), monotonic increasing (*center*), and quadratic (*right*) segment tuning functions. Segment number is indicated *on the horizontal axis*, firing rate *on the vertical axis*

possible association using a chi-square test and quantified the magnitude of each significant effect by calculating the odds ratio. First, the serial segment position was significantly associated with shape ($\chi^2_{(1)}$=10.44, p=0.001; odds ratio=1.74), segment direction ($\chi^2_{(1)}$=12.47, p=0.0004; odds ratio=1.90), and segment length ($\chi^2_{(1)}$=5.36, p=0.021; odds ratio=1.70). It is noteworthy that the effects were strong, as shown by the high values of the odds ratio. (In the absence of an effect, the odds ratio would equal 1.) For example, in the case of shape and segment serial position, the odds ratio of 1.74 means that the likelihood of these two factors being processed together was 74% higher than being processed separately. Interestingly, segment direction and length were not significantly associated ($\chi^2_{(1)}$=0.21, p=0.6). As a control, we tested the association of the serial segment position with temporal parameters, such as maximum segment speed and time to maximum segment speed; neither of these parameters was significantly associated with segment serial position (maximum speed: $\chi^2_{(1)}$=1.13, p=0.29; time to maximum speed: $\chi^2_{(1)}$=1.17, p=0.28). These results show that serial segment position is processed more frequently together with other spatial attributes but not with temporal parameters, which, in turn, suggests that serial order and spatial aspects of copying are processed together by single cells in prefrontal cortex.

The results of the loglinear analysis provided a clear result with respect to higher-order associations among the factors of shape, segment serial position, segment direction and segment length. We found that the single four-way association among all factors above was statistically significant (p=0.025). This finding suggests that single cells also process all four factors concurrently. In contrast, none of the four possible three-way associations were statistically significant (range of p values: 0.28–0.88).

Encoding of the serial position of segments

In this analysis, we wanted to investigate whether or not the neural activity related to segment followed any systematic pattern. For this purpose we calculated adjusted means for the segments, that is, adjusted for the effects of all other main effects, except the segment.

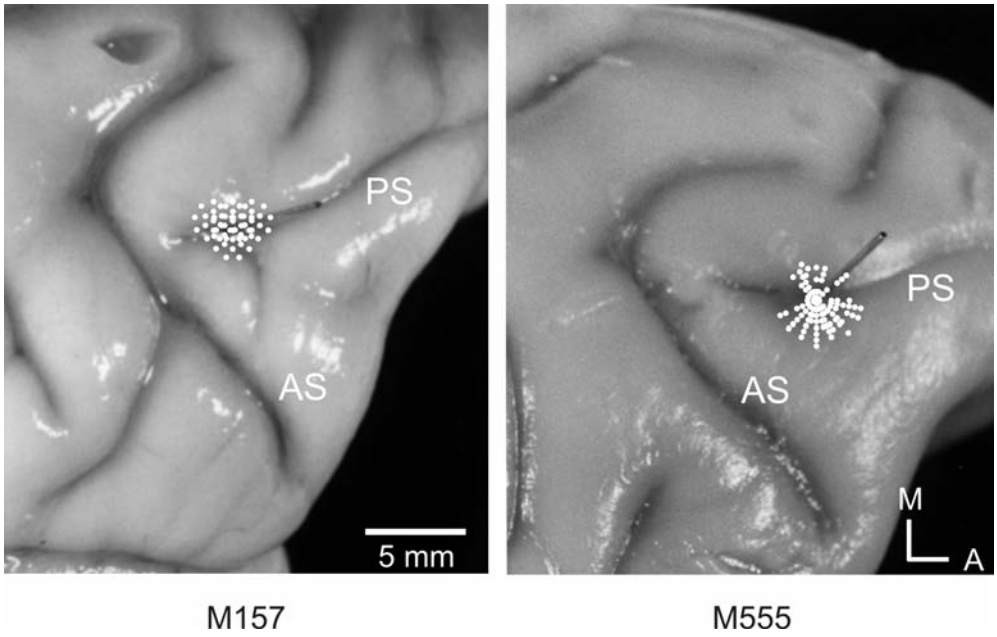

Fig. 15 Recording sites in the prefrontal cortex of both monkeys. *Dots* indicate points of entry of electrode penetrations into the brain. *Pins* mark centers of 7-mm i.d. recording chambers

This was done for all cells whose activity was significantly related to segment serial position in the regression analysis above. Representative results are shown in Fig. 14. Monotonically increasing and decreasing functions were found in 38/245 (16%) and 89/245 (36%) of cells, respectively. Other functions (a quadratic example is shown in Fig. 14, right) were found in 118/245 (48%) of cells.

Recording sites

Both chambers were located in the periprincipalis region of the prefrontal cortex (Fig. 15). The estimated entry points of all penetrations are also shown.

Discussion

The function of copying

To make a copy of a figure, one has to translate a visual pattern to a closely corresponding motor pattern. It is useful to contrast copying with tracing. In the case of tracing, the movement trajectory is on the visual template, whereas in the case of copying the movement trajectory is at a different spatial location. In tracing there is a close spatial proximity of the hand movement to the visual shape, and this enables the continuous visual guidance of the hand. In copying, on the other hand, the movement trajectory describes, ideally, the same figure as the visual template but is not superimposed on it, i.e., there is a separation in space between the template and its copy: this means that, although there is a spatial correspondence between the visual figure and the movement, this correspondence is not immediately given but has to be imparted to the movement trajectory so that it conforms to the template shown. Therefore, there are at least three key aspects of the function of copying. The first aspect concerns general topological characteristics of the shape, in the sense defined by Bernstein (1967). For example, triangles are closed shapes composed of three sides. The second aspect deals with the spatial characteristics of the components of the specific template to be copied, e.g., the orientation and length of the sides of a particular triangle. Triangles, as a general topological class, comprise a large variety of particular cases composed of sides with different length and orientation. Although all of these cases belong to the topological class of "triangle," specific instantiations of this class may differ substantially with respect to the orientation and length of their component sides. Finally, the third aspect in copying is the actual implementation of drawing the template. Since movements unfold in time, two additional points need to be considered. First, the hand traverses a line in a direction, and, therefore, a given orientation of a line is implemented uniquely in one of two possible directions; in contrast, the length of the line is invariant with respect to the movement. And, second, figures can be drawn at different speeds, accelerations, etc., which comprise the dynamic aspects of copying.

Bernstein (1967) drew attention to what he described as the topological versus the metric properties of movements such as drawing a geometrical shape. In Bernstein's formulation, topological properties are related to spatial invariances embedded within movement trajectories, whereas metric properties are related to the specific execution of the movements. Spatial invariances are those properties that must be preserved for a shape to be recognized. Examples of topological properties include the number and relative positions of vertices, whereas the metric properties would include the size of the shape, the

part of space in which it is reproduced, and its dynamic characteristics, such as speed. The metric factors ultimately refer to the muscles used and the forces they exert. For example, the topological properties of an individual's signature are invariant across different workspaces, e.g., a sheet of paper and a chalkboard, whereas the metric properties vary greatly (Wright 1990). Bernstein concluded that the highest-level motor engram for a given shape must be stored with respect to its topological, not its metric, parameters.

In the present study we studied the neural mechanisms of copying geometrical shapes. For this purpose, we trained monkeys using a paradigm which: (a) instructed the overall movement trajectory (by showing a shape to be copied) and (b) constrained the spatial pattern of the ensuing movements by specifying their sequence, direction and length, while (c) leaving unconstrained the speed of execution. Thus monkeys emitted, at different speeds, well-practiced, spatially constrained, good copies of the shape templates. Within the Bersteinian framework above, the instructed shape can be considered as a topological variable; the direction, length and speed of the segment movements as metric variables; and the serial order of the segments as a more abstract, relational variable. During performance of this task, we recorded cell activity in the periprincipalis area of the prefrontal cortex, an area considered to be integrated into a distributed system for arm motor control (Goldman-Rakic et al. 1992; Lu et al. 1994). We thought that the multifaceted task above would be well matched to the rich complexity of the prefrontal cortex.

There were four major findings in this study. First, cell activity was influenced by a number of factors relating to arm and eye movements. Second, the strongest effects on neural activity came from factors more specific to the copying process, namely the serial position of the segment in the copy and the shape template. Each one of these two factors had a significant effect on more than 40% of the cells. Third, there was clear evidence that copy-specific factors tended to be processed together by single cells. The central factor in that respect was the serial position of the segment which was coupled both with the more general factor of the shape of the template as well as with more specific factors relating to spatial segment attributes, i.e., direction and length. Finally, there was a systematic variation in discharge rate emitted during a segment with the serial position of the segment in the copy. These findings document the involvement of the prefrontal cortex in copying and provide an insight into the neural mechanisms underlying various stages in this process, from the global factor of the shape shown, to the relational factor of serial segment order, to specific factors related to specific spatial attributes of the segments (i.e., direction and length). We discuss these findings separately below.

Effects of metric properties of copying: movement parameters

We found a variety of significant effects of movement parameters on single cell activity (Table 1), including direction, length, position and speed. These findings are in accord with, and extend, the results of other studies which have documented changes in prefrontal neural activity during arm movements (Niki 1974a, 1974b; Kubota and Funahashi 1982; Boussaoud and Wise 1993a, 1993b; di Pellegrino and Wise 1993; Hoshi et al. 2000) and studies which link prefrontal cortex with motor areas anatomically (Goldman-Rakic et al. 1992). The existence of these spatial effects in dorsolateral prefrontal cortex is also in accord with studies showing this area to be involved in spatial processing (Goldman-Rakic 1987; Levy and Goldman-Rakic 1999). With respect to our study, it is possible that some of the effects observed could be due to the visual stimulus produced by the joystick feedback on the screen. As the arm trajectory and the persistent trace left by the cursor were isomorphic, variables such as position and direction were necessarily correlated in extrinsic space, just as they are in normal drawing. In fact, visual and motor variables are generally correlated whenever a subject sees their arm move. However, the arm trajectory and the corresponding visual stimulus on the retina were decoupled because eye position was not fixed and the monkeys viewed the advancing trajectories from variable fixation points while drawing (Fig. 5). Therefore, we assume for this discussion that the movement effects observed predominantly related to the movement of the arm. The eye position effect, however, could partially be accounted for by considering the change in retinal input which would result with the change in eye position.

Neural mechanisms of serial order

Drawing a shape in a continuous trajectory implies that oriented segments of this trajectory are drawn piecemeal and in a prescribed sequence determined by the starting point of the movement. Given that the shapes copied by our monkeys were angular, it is not surprising that the drawn trajectories were segmented (Figs. 2, 3). It is noteworthy that segmentation seems to be a general property of more complex movement trajectories, including curved and isometric force trajectories (Morasso 1983; Viviani and Cenzato 1985; Soechting and Terzuolo 1987a, 1987b; Massey et al. 1992; Pellizzer et al. 1992). In the present study, monkeys drew shapes as a sequence of movements, each movement corresponding to a segment. In that respect, this task can be considered a movement-sequence task. The neural mechanisms underlying movement sequences and/or serial order information have been studied in various brain areas, including prefrontal cortex (Barone and Joseph 1989; Petrides 1991; Funahashi et al. 1997), the supplementary eye field (Lu et al. 2002), the basal ganglia (Kermadi et al. 1993; Kermadi

and Joseph 1995; Mushiake and Strick 1995), the supplementary motor area (SMA) and pre-SMA (Mushiake et al. 1990, 1991; Clower and Alexander 1998; Nakamura et al. 1999; Shima and Tanji 2000), and motor cortex (Mushiake et al. 1990, 1991; Kettner et al. 1996; Carpenter et al. 1999). Selectivity for the sequence in which visual stimuli are presented has been reported in prefrontal neurons (Barone and Joseph 1989; Funahashi et al. 1997). We found an orderly relation between cell activity and serial segment position (Fig. 14). To our knowledge, this is the first study to show a dependence of prefrontal neuronal activity on the serial position of a movement in a sequence. These data may provide a positive image of the deficits in sequence processing that follow prefrontal damage in humans (Luria 1966; Shallice 1982; Schwartz et al. 1991), and support the hypothesis that sequence processing is an integral part of praxis (Koski et al. 2002).

Combining topological and metric attributes in copying

As mentioned above, the shape of the template can be regarded as a topological attribute. We found that the shape of the template object significantly affected neural activity during drawing in a large percentage of prefrontal cells (46%). It should be noted that this effect was obtained within a multiple regression model, that is when the effects of all other factors were accounted for. The ventrolateral portion of prefrontal cortex receives projections from inferotemporal cortex (IT) (Barbas 1988), which may convey visual information about the shape of the template object. Visual object information from IT may then be used in prefrontal cortex to specify the topology of the copy trajectory.

It is interesting that cells that showed a significant shape effect also tended to show a significant segment serial position effect. In fact, this association was both highly statistically significant and of a substantial magnitude (odds ratio = 1.74). The segment serial position effect of single cell activity, in turn, was significantly associated with the effects of the spatial segment attributes of direction (odds ratio = 1.90) and length (odds ratio = 1.70), whereas the effects of direction and length were not significantly associated. Remarkably, the segment serial position effect was not associated with dynamic motor parameters, such as maximum segment speed or time to maximum segment speed.

The present data support the conclusion that the activity of single neurons in prefrontal cortex is influenced by several parameters controlling the copy process simultaneously. In itself, this is an interesting result as it indicates that prefrontal neurons are part of a multiplexed system, in which several signals (metric and shape variables for example) are transmitted in the same channel (neuron) concurrently. The validity of this conclusion depends upon the independence of the detected effects. The stepping procedure employed in the current regression analysis ("Materials and methods") ensured that additional factors were added to the model only when they explained a distinct portion of the variance in neural firing rate above and beyond that already accounted for by other factors included in the model. In the present analysis, independent variables were correlated (direction and segment serial position, for example). In the hypothetical case where this correlation rendered a pair of variables redundant (so that the two did not account for more variance in firing rate together than either considered alone), one would not expect that both would enter the stepping procedure and be included in the model. Rather, a significant relationship to one variable would be detected at the expense of the other. This is contrary to the present result. Direction and segment serial position, for example, co-occurred as significant factors in single neurons more frequently than expected by chance (and not less frequently), substantiating the independence of these effects. The same increased frequency of co-occurrence was true of length and segment serial position, and also shape and segment serial position. These results corroborate the simultaneous and independent effects of both higher order (e.g., shape, serial segment position) and lower order (e.g., movement direction and length) variables on the activity of single prefrontal neurons.

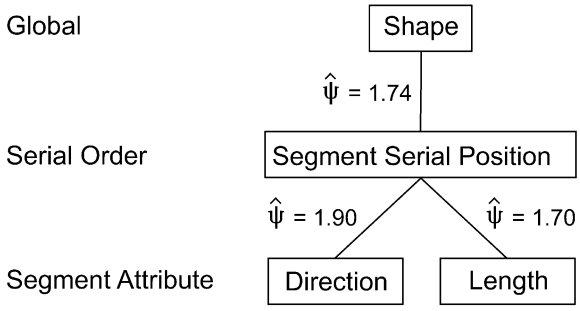

Fig. 16 Concurrent processing of multiple copying parameters by single prefrontal neurons. The effects of shape, segment serial position, and either direction or length tended to have significant effects together on the activity of single neurons. The *numbers adjacent to each line* connecting pairs of factors represent the odds ratio of their association

These results indicate the following with regard to single cell activity: (a) individual cells in prefrontal cortex process multiple copying-related variables (i.e., shape, serial segment position, direction and length), both pairwise and as a group; (b) processing of dynamic variables seems to be dissociated from processing spatial variables; (c) within the domain of specific spatial attributes of a segment, direction and length seem to be processed independently; (d) processing of shape as a global factor is widespread; and (e), finally, processing of the segment serial position information is pervasive among prefrontal cells and is bundled with processing of general (shape) and segment-specific spatial parameters (direction and length). These considerations lead to the hypothesis that prefrontal cortex may play a special role in combining topological and spatial-metric aspects

of the template and its implementation during copying, as follows. Given that all shapes were drawn from a fixed starting point, the combination of segment direction and length, together with the prescribed serial order in which they were drawn, completely constrained the reproduction of the template object. In this scheme, segment serial order information can be thought of as a link between the most general (shape = all segments) and the most specific (segment direction and length) spatial factors involved in the copying function (Fig. 16). Indeed, individual cells in our sample seemed to capture these interactions as evidenced by the observed significant co-processing of segment serial position information with these other factors.

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