Neuronal Activity in Motor Cortical Areas Reflects the Sequential Context of Movement

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Ben-Shaul, Yoram, Rotem Drori, Itay Asher, Eran Stark, Zoltan Nadasdy, and Moshe Abeles. Neuronal activity in motor cortical areas reflects the sequential context of movement. J Neurophysiol 91: 1748–1762, 2004. First published November 26, 2003; 10.1152/jn.00957.2003. Natural actions can be described as chains of simple elements, whereas individual motion elements are readily concatenated to generate countless movement sequences. Sequence-specific neurons have been described extensively, suggesting that the motor system may implement temporally complex motions by using such neurons to recruit lower-level movement neurons modularly. Here, we set out to investigate whether activity of movement-related neurons is independent of the sequential context of the motion. Two monkeys were trained to perform linear arm movements either individually or as components of double-segment motions. However, comparison of neuronal activity between these conditions is delicate because subtle kinematic variations generally occur within different contexts. We therefore used extensive procedures to identify the contribution of variations in motor execution to differences in neuronal activity. Yet, even after application of these procedures we find that neuronal activity in the motor cortex (PMd and M1) associated with a given motion segment differs between the two contexts. These differences appear during preparation and become even more prominent during motion execution. Interestingly, despite context-related differences on the single-neuron level, the population as a whole still allows a reliable readout of movement direction regardless of the sequential context. Thus the direction of a movement and the sequential context in which it is embedded may be simultaneously and reliably encoded by neurons in the motor cortex.

INTRODUCTION

Animals interact with their environment through a rich repertoire of actions. Numerous actions may be efficiently realized in motor cortical areas by integrating simple elements of action into complex constructs. Even though several approaches to decomposing complex actions have been suggested, it is uncertain which units of decomposition are physiologically meaningful (Mussa-Ivaldi and Bizzi 2000; Sternad and Schaal 1999; Viviani and Cenzato 1985; Viviani and Flash 1995). In contrast, the decomposition is “built-in” using sequential motion paradigms, and indeed such paradigms have guided many psychophysical and physiological studies. A set of imaging (Catalan et al. 1998; Gordon et al. 1998; Hikosaka et al. 1996; Karni et al. 1998; Richter et al. 1997), electrophysiological (Mushiake et al. 1991; Nakamura et al. 1998), and lesion (Shima and Tanji 1998) studies highlighted the role of the frontal cortex in generating action sequences. In a key series of studies it was demonstrated that the activity of neurons in the supplementary and pre-supplementary motor areas is associated with specific sequences of movements (Shima and Tanji 2000). Another set of studies focused on neuronal activity associated with individual motions, rather than motion sequences. Although the coordinate frames in which neurons encode movements are still debatable, these studies have demonstrated a clear correlation between neuronal activity and various kinematic and dynamic aspects of movement (Ajemian et al. 2000; Ashe and Georgopoulos 1994; Cheney et al. 1988; Kakei et al. 1999; Kalaska et al. 1997; Moran and Schwartz 1999). By combining findings from both sets of studies, a simple scheme for generating action sequences emerges. In this hierarchical scheme, high-level “sequential” neurons recruit lower-level “kinematic” neurons in a modular manner to generate complex motions. Although it has been shown that motor cortical neuronal activity is also related to nonkinematic/dynamic aspects of behavior (Carpenter et al. 1999; Georgopoulos 2000), this simple scheme provides a useful framework for investigating motor cortical activity with respect to performance of action sequences.

The present study investigated motor cortical neuronal activity in the dorsal-premotor (PMd) and primary motor cortex (M1) of 2 monkeys during performance of a simple reaching movement in two contexts. In the “single” condition, movements were performed individually, whereas in the “double” condition, movements were performed in the context of a two-segment sequence. Our aim was to compare neuronal activity during preparation for and execution of individual motion segments between these two contexts. However, analysis of the motions themselves revealed frequent subtle yet significant differences between the two contexts. These differences appear to represent an inherent characteristic of the manner by which basic elements are integrated into complex constructs, a finding supported by psychophysical studies in humans as well (Klein Breteler et al. 2003). We therefore used statistical measures to identify and exclude movement-related contributions to context-related neuronal activity. Our results reveal that the sequential context is reflected by the activity of single units, including those with direct correlation to movement parameters, during both preparation and execution. A recent study addressed M1 neuronal activity during execution of single- and double-segment motions (Hatsopoulos et al. 2003), yielding results that at first seem at odds with ours. Specifically, the main conclusion of that study was that the sequential context is represented by pairwise correlations...
rather than by rate changes of individual neurons. However, also in that study approximately one-third of the units revealed differences in firing rates between the two contexts, and were therefore excluded from the analysis. Apparently, our study focuses on a similar population of neurons. Taken together, these results imply that the sequential context is manifest in both individual-unit firing rates and in their correlation patterns. Part of this work was previously published in abstract form (Ben-Shaul et al. 2001).

METHODS

Behavioral task

Two monkeys (B and T, female Macaca fascicularis, weight 2.5 kg each) were trained to perform single- and double-segment reaching movements. The monkeys sat in a primate chair and controlled the position of a cursor with a horizontal 2-jointed low-friction manipulandum. The head and nonworking arm were restrained. The cursor and workspace were projected on a horizontal board at chest level in front of the monkeys so that the hand position was mapped directly onto the cursor position. The workspace included a hexagonal array of 19 targets (adjacent target distance: 3 and 2.9 cm for monkeys B and T, respectively; target radius: 0.5 cm, both monkeys, Fig. 1). A trial began when one of the targets (the origin for that trial) changed color from gray to red (origin on, Fig. 1, A and B). Once the cursor was placed within the origin (cursor inside), the origin changed its color to green. After a delay (200–300 ms for monkey B, 400 ms for monkey T), either one (single-segment trials), or 2 (double-segment trials) targets changed their color to red (target on). After an additional interval (300 ms monkey B, 100 ms monkey T) the red target(s) color changed to gray again (target off). The cursor had to be maintained within the origin as long as it remained green. Only when the origin changed its color to gray (origin off, after 700 ms for monkey B, 120 ms for monkey T), the monkey was allowed to move the cursor out of the origin into the first target (target on). The first target changed its color from red (target on) to gray again (target off). The cursor could be moved to the second target, after which the reward was given. Because the 19-target array was dimly displayed throughout the entire trial, the monkeys had to memorize the identity, yet not the exact coordinates of the subsequent targets.

All motion segments were defined by 2 adjacent grid targets, and thus the angle between the first and second segments, denoted henceforth as the “bend” of the sequence, assumed values from the set [−120°, −60°, 0°, +60°, and +120°]. A positive bend corresponds to a clockwise turn from the first to the second segment. Generally, the rule for double motions was that the target closer to the origin was the first target. For those few double sequences with a 120° bend (constituting an equilateral triangle), the correct order was to be determined by the monkey through trial and error. This usually required no more than a few trials (about 10).

In general, we compared neuronal and movement data from the first segment of a double-segment motion (designated henceforth as “first segment”) to that associated with the single motion constituting that segment of the double motion (designated as “single segment”). In each (daily) session, monkeys performed alternating blocks of single-segment and double-segment motions. Within these blocks specific double or single motions were selected randomly. Blocks of single segments constituted 16 to 24 correct trials, and blocks of double segments constituted 8 to 16 correct trials. Because each monkey performed on the order of 1,000–2,000 correct trials per session, this implies that double and corresponding single trials were interleaved and uniformly distributed during the course of each daily session. Most sessions included 4 double segments (range 2–6) and the single segments of which they were composed. Blocks of single segments also included the 6 motions from the central grid target to determine the directional tuning of the units.

To exclude any contribution of temporal drift in the recorded neuronal responses to differences between single- and double-segment neuronal activity we 1) included only sections of stable neuronal activity (see Neuronal database in METHODS) and then 2) accounted for any remaining subtle effects of temporal drift by testing whether the actual trial execution order could account for differences in neuronal activity (see Movement parameters do not account for differences in spike counts in RESULTS).
Surgical procedure and data acquisition

After training, a square recording chamber (27 × 27 mm) was attached to the skull under deep ketamine–xylazine anesthesia in aseptic conditions. Magnetic resonance imaging (MRI, monkey T) was used to position the chamber above the dorsal premotor (PMd) and the primary motor cortex (M1) of the contralateral (left) hemisphere. Positioning of the electrodes within the arm area of PMd and M1 was verified by somatosensory examination (both monkeys) and intracortical microstimulation (ICMS, monkey B). Specifically, at the end of each daily session, the responsiveness of the recording site to passive manipulations of the limbs was assessed, whereas ICMS was applied in preliminary mapping sessions to identify the arm-related region of the motor cortex. ICMS currents were delivered with approximately 0.2-s trains of 300-Hz cathodic current at 20–100 μA with individual pulses of 2-ms duration through individual electrodes.

On each recording session, 8 glass-coated tungsten electrodes (impedance 0.2–1 MΩ at 1 kHz) confined to a guide tube (ID, 1.5 mm) were lowered manually to about 3 mm above the dura matter. Then, a computer-controlled microdrive (EPS, Alpha-Omega Engineering, Nazareth, Israel) was used to advance individual electrodes into the cortex. The output of the electrodes was amplified, band-pass filtered (0.3–6 kHz, MCP+, Alpha-Omega Engineering), and fed to a template-matching device (MSD, Alpha-Omega Engineering) to isolate the extracellular activity of 1–3 units per electrode. Spikes and behavioral events were sampled at 1 kHz and logged on a custom data-acquisition system. Hand position was sampled at 100 Hz, and subsequently low-pass filtered off-line (4 Hz) before analysis. In several sessions (monkey B), intramuscular electromyograms (EMGs) were recorded from the following proximal and distal arm muscles: extensor carpi ulnaris, flexor carpi ulnaris, biceps brachii, triceps brachii, trapezius, extensor digitorum 45, and palmaris longus. EMG was sampled at 24 kHz and its root mean square (RMS, low-pass at 0.3 Hz) was taken. All surgical and animal handling procedures complied with the guidelines of the National Institutes of Health and the Hebrew University.

Surface mapping and histology

Monkeys were killed with an overdose of pentobarbital, and then perfused transcardially with 0.9% saline followed by 4% formaldehyde in 0.1 M phosphate buffer. After fixation, pins were inserted in defined chamber locations to allow reconstruction of chamber coordinates, and the location of penetration sites relative to cortical landmarks was determined. In addition, 50-μm coronal sections were prepared from the left hemisphere of monkey B. Sites where a change in the density of large pyramidal cells occurred were noted in individual slides, and then used to set an approximate border between PMd and M1.

Neuronal database

Criteria for including units in the database were quality of isolation and stable activity across trials. Isolation quality was determined by spike waveforms detected with each of the templates and by histograms of (Euclidean) distances of individual spikes from their respective templates. Our database includes single units, or at most mixtures of 2 well-isolated units. The stability of neuronal activity was inspected off-line with a custom stability analysis package that simultaneously displays raster plots and firing rates for each recorded unit during the entire session. Only sections of data with stable neuronal activity and only correct trials were considered for analysis. As Table 1 shows, the minimum number of correct trials for a given single or double trajectory is 12, but typically is considerably more.

Definition and analysis of movement parameters

On individual trials, motion onset time was defined as the time when tangential velocity reached 15% of its peak for that motion. End of motion was defined as the time when the cursor entered the target. Individual motion segments were characterized by the following movement parameters.

1) Peak velocity: peak tangential velocity.
2) Time to peak: time from motion onset to time when peak velocity is acquired.
3) Motion duration: interval from motion onset to motion end.
4) Start delay: time from dimming of the origin (origin off) to motion onset.
5) Path length: length of actual trajectory traversed by the cursor from start to end of motion.
6) Mean deviation: angle between mean motion direction (defined by line connecting cursor position at motion onset and position at motion end) and the nominal motion direction (defined as the direction between the center of the origin and the center of first target).
7) Deviation at peak velocity: angle between mean motion direction at peak velocity (defined by line connecting cursor position at motion onset and position at time of peak velocity) and the nominal motion direction.

Single and corresponding double motions were compared by applying a nonparametric ANOVA [Kruskal–Wallis test (Sokal and Rohlf 2000)] to the distributions of each of these movement parameters. For all applications of the Kruskal–Wallis test we used the MATLAB “kruskallwallis” function supplied with the MATLAB statistical toolbox.

Note that the start delay parameter is not a reaction time in the conventional sense. This is because the origin was dimmed (origin off), following a fixed interval after the targets were dimmed (trace on), allowing anticipation of origin off. Consequently, motion onset occasionally occurred before the origin was dimmed. Nevertheless, by design of the task, in correct trials the cursor never left the origin before it was dimmed.

Testing the correlation between first and second segments of a double motion

To check for interdependency between execution of the first and second segments we tested the correlation between specific movement parameters describing the first and second segments of a double motion. This was accomplished by calculating Spearman’s rank correlation coefficient. The rank correlation coefficient (rather than the standard product–moment correlation coefficient) was used because parameter distributions are not necessarily distributed in a bivariate normal fashion. Additionally, to rule out the potential correlating

<table>
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<th>Monkey</th>
<th>Number of Sessions</th>
<th>Number of Double Trajectories</th>
<th>Number of Units</th>
<th>Number of Observations</th>
<th>Number of Double Trials (min/median/max)</th>
<th>Number of Single Trials (min/median/max)</th>
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<tbody>
<tr>
<td>B</td>
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<td>145</td>
<td>351</td>
<td>1284</td>
<td>20/65/125</td>
<td>12/54/143</td>
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<tr>
<td>T</td>
<td>18</td>
<td>83</td>
<td>79</td>
<td>339</td>
<td>54/113/229</td>
<td>66/131/189</td>
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Distributions of the number of trial repetitions for individual double and single trajectories (performed on a single session) are summarized by the minimum, median, and maximum values.
effects of trial time on parameter values (e.g., first- and second-segment motion duration may correlate simply because movements become slower as the session proceeds and the monkey loses motivation), we actually calculated the partial correlation coefficients with respect to the trial time parameter. Thus for each parameter we obtained a set of correlation coefficients, each corresponding to a specific double motion (i.e., all repetitions of a given double motion performed by one monkey on a single session). Then, these correlation coefficients were z-transformed ($z = 0.5 \ln ((1 + R)/(1 - R))$, where $R$ is the rank correlation coefficient), and then normalized by the factor $1/(n - 4)^{1/2}$ where $n$ is the number of trials for that double motion. After this normalization, the $z$-transformed values follow a standard normal distribution under the null hypothesis of zero correlation. Having obtained this distribution of transformed correlation coefficients, we used a 2-tailed $t$-test to check whether its mean is different from 0. All statistical procedures used for this analysis and the rationale for adopting them are described in the standard statistics textbook, *Biometry* (Sokal and Rohlf 2000).

**Characterization of neuronal activity**

Neuronal activity was characterized by spike counts in the following behaviorally defined epochs (Figs. 1 A and B):  
*Presentation*: interval during which the targets were presented (300 ms for monkey B, 100 ms for monkey T).  
*Preparation*: interval after target presentation but before arm muscle activity was apparent, as measured by EMG (300 ms interval ending 100 ms before motion onset, defined for monkey B only).  
*Execution*: interval constituting actual motion (beginning at motion onset and ending 300 ms later for monkey B, or 250 ms later for monkey T).

Note that all epochs are included in the first part of a double motion (first segment) or the corresponding single-segment motion. Differences in epoch definitions for the 2 monkeys were required because of the differences in the temporal intervals used for their tasks. Specifically, because of the shorter interval between target presentation and motion onset, presentation and execution were shorter for monkey T, whereas preparation was altogether inapplicable.

**Comparison of neuronal activity between the double and single conditions**

Henceforth, we use the term “observation” to denote the activity of one unit during the first segment of a particular double motion and in the corresponding single segment. Two measures were applied to quantify the differences in activity of individual units between particular double and corresponding single motions. The first is the “double-single index” ($DS_{index}$), defined as

$$DS_{index} = \frac{S - D}{S + D}$$

where $S$ and $D$ are the mean spike counts in the first part of the single and the corresponding double motion, respectively. An observation with a $DS_{index}$ of zero indicates identical mean activity in both conditions, whereas values approaching 1 and −1 are associated with increased activity during the single or double conditions, respectively. As a second measure, we considered the $P$ value obtained from a nonparametric one-way ANOVA (Kruskal–Wallis test), denoted as the DSp (for “double single P value”). Observations associated with double and single spike counts, significantly different at the 1% level ($DSp < 0.01$), were designated as “double-single sensitive.” Note that because spike count comparisons are invariably applied to epochs of identical durations, our procedure is equivalent to the comparison of firing rates.

**Accounting for spike count differences by movement parameters**

Double-single–sensitive observations ($DSp < 0.01$) were further analyzed to reveal movement-associated contributions. Two conditions must be fulfilled for a given movement parameter to account for the double-single sensitivity of a particular observation. First, the parameter’s distribution must differ between the double and single conditions; and second, the unit’s activity should be correlated with the values of that parameter. The basic procedure used here is illustrated in Fig. 2. First, the distribution of each movement parameter is compared between the double and corresponding single trials (nonparametric one-way ANOVA, Kruskal–Wallis test). If the distributions are significantly different at the 0.05 level, thus fulfilling the first condition, the second condition is tested. The relatively relaxed threshold (0.05) was used to increase the ability to detect differences in the movements. To test the second condition, the pooled set of single and double trials (and associated spike counts) is partitioned according to the parameter’s value. After this partitioning, one group includes spike counts from trials with low parameter values, whereas the other group includes spike counts of trials associated with high parameter values. The numbers of trials in these groups are equal to those of the original single- and double-trial groups, which are not necessarily identical to each other. Following the same procedure used for the original double- and single-trial groups, we compare the distributions of the spike counts of the regrouped trials. This process is repeated for each of the movement parameters, and the minimum $P$ value obtained over all parameters is denoted as the “motion-related $P$ value” ($P_{MV}$) for that observation. The $P_{MV}$ is thus a measure of how well individual movement parameters account for the observed double-single sensitivity. The $P_{MV}$ is then used to classify double-single–sensitive observations into one of 3 categories. Observations are labeled as “context”-related if the $P_{MV}$ is larger than 0.01, indicating that differences in spike counts are not accounted for by changes in any of the movement parameters considered. If the $P_{MV}$ is equal to or smaller than 0.01, yet larger than the DSp, the observation is designated as “mixed,” implying that it is associated with both movement- and context-related effects. Finally, if the $P_{MV}$ is smaller than the DSp, and thus by definition also smaller than 0.01, the observation is designated as “movement”-related, indicating that individual movement parameters can fully account for the observed DSp.

**Directional tuning and construction of population vectors**

We used the directional population vector (Georgopoulos et al. 1986) to compare ensemble activity associated with first segments of double motions with those of the corresponding single motions. Preferred directions (PDs) were calculated from neuronal activity associated with the 6 center-out (single segment) motions using vector summation for each epoch separately. Significance of tuning was assessed by bootstrapping as described by Crammond and Kalaska (1996). Only units for which the length of the observed directional vector exceeded 99% of those obtained in 4,000 shuffles were considered as well-tuned units. Population vectors for single and first parts of double motions were computed from well-tuned units using the standard expression

$$PV = \sum_{i} PD_{i} W_{im}$$

where $i$ is an index over units, $m$ is an index over motions, $PD_{i}$ is the preferred direction of unit $i$, and $W_{im}$ is the weighting function of unit $i$ for motion $m$ (being either a single segment or the first part of a double). The weighting function (corresponding to Eq. 4 in Georgopoulos et al. 1988) is given by

$$W_{im} = [(R_{im} - R_{i})/\text{range (R)}]$$
where $R_{im}$ is the mean spike count of unit $i$ during execution of motion $m$, $R_i$ is the mean count of unit $i$ over all center-out motions, and range $(R_i)$ is the range of counts of unit $i$ during center-out motions. To increase the sample size, all double trajectories with a given bend were rotated to align all first segments to the right (0°), and then pooled together. Variability in direction and magnitude of population vectors was estimated with random resampling (10,000 trials) of the population as described in Georgopoulos et al. (1988). We also tested the null hypothesis that population vectors of corresponding double and single motions originate from a single underlying distribution. Thus in each of the bootstrapping trials we calculated the directional differences between the single- and double-condition population vectors. If the 99% (2-tailed) limits of this bootstrapped distribution spanned the value of zero, this null hypothesis was not rejected.

**RESULTS**

The numbers of sessions, units, trajectories, observations, and trial repetitions are given in Table 1 (recall that an observation refers to the activity of one unit during a first segment of a double and the corresponding single motion). Figures 3–5 provide examples of neuronal and movement data for 3 observations, illustrating different patterns of responses. Each of these figures shows (from top to bottom) averaged tangential velocities and paths, spike and event raster displays, and mean firing rates. Figure 3 represents an observation with higher spike count during the preparation and the execution epochs in the single-segment condition. Marked differences in the movements (reflecting the fact that double motions, by definition, constitute an additional segment) are evident only at around 1.5 s after the earliest apparent spike count differences. In the example of Fig. 4, the spike count during presentation is higher in the double than in the corresponding single. Figure 5 shows an example where the spike counts during the execution epoch are similar in both conditions. All subsequent analyses of neuronal and movement data are applied to the first segment of the double motion and the corresponding single segment motion. Thus the behavioral constraints are identical for both conditions under comparison.

Many observations reveal differential activity in double and single motions

The double-single index ($DS_{\text{index}}$) and the double-single $P$ value ($DS_p$) for the examples in Figs. 3–5 are indicated at the bottom of these figures, demonstrating that these measures match the qualitative impression obtained by a visual inspection of the data. Distributions of the $DS_{\text{index}}$ for all observations from each monkey in each of the epochs are shown in Fig. 6A. All histograms assume symmetrical unimodal forms and are thus not consistent with several separate populations associated with distinct distributions of double-single sensitivity. Under the null hypothesis of equal spike count distributions for single and double motions, the expected fraction of $DS_p$s less than 0.01 is 1%. Figure 6B shows that the fraction of rejections in each of the epochs for both monkeys far exceeds the expected values. The figure also demonstrates a consistent increase in the fraction of significant observations as the task proceeds from presentation to execution.

Double and single motions are similar but not identical

Direct comparison of neuronal activity between the double and single motions entails the assumption that physical aspects of the movements are identical during both conditions. A gross comparison of EMGs, hand paths, tangential velocities, and
movement parameter distributions suggests that this assumption is justified (Fig. 7). However, direct comparison of parameter distributions reveals that movements are not the same under the 2 conditions. Figure 8 shows that the fraction of trajectories with significant differences ($P < 0.05$) in individual movement parameters are invariably higher than expected by chance. A higher fraction of significant differences are associated with the motions of monkey T. This is consistent with the briefer intersegment interval imposed on this monkey, presumably affecting a more pronounced interaction between the second and the first segment during performance of the double motion. Overall, comparison of double and single trajectories is associated with frequent small, yet significant, differences. The prominence of differences between single and double motions on individual trajectories could potentially reflect general characteristics of single versus double execution, and is therefore interesting from a psychophysical point of view (Klein Breteler et al. 2003). A brief account of this aspect is given in the DISCUSSION.

Interdependency of the first and second segments of a double motion

In the previous section we have shown that first segments of a double motion and the corresponding single motions often differ. A related phenomenon is the potential interdependency in execution of the first and second segments of a particular double motion. To test for such interdependency we studied the correlation coefficients between the following 6 movement parameters characterizing the first and second segments of a double motion: peak velocity, time to peak, motion duration, path length, mean deviation, and deviation at peak velocity. The start delay parameter could not be analyzed in this manner because it is not defined for the second motion segment. For simplicity, we did not study the correlation between distinct movement parameters in the first and second motion (e.g., peak velocity on the first segment with mean deviation on the second motion). Thus we calculated correlation coefficients (see METHODS for details of the calculation) for each double motion, constituting all trials of a specific sequence performed by one monkey on a specific session. A 2-tailed $t$-test was then used to test whether the mean of the distribution of (transformed) correlation coefficients associated with the entire set of double motions was significantly different from zero. This analysis was performed for each monkey separately, either for all double motions, or only for double motions of a 60° bend. Several conclusions can be drawn from this analysis, the results of which are shown in Table 2. First, at least 2 of the 6 parameters are significantly correlated for each case studied.
Second, the pattern of correlations is not identical for both monkeys, either when all movements or when only 60° motions are studied. This implies that each monkey adopted a different “motor strategy” to execute the double motions. One possible cause for these differences is attributed to the differences in intersegment intervals imposed on each. Indeed, for monkey T (for which this interval was shorter), there are more instances of correlated parameters, implying that the shorter interval is associated with a stronger interdependency of the 2 motion segments. However, differences in the pattern of correlations also emerge when the set of all movements is compared with only those of 60° for each monkey separately. This implies that the pattern of correlations depends not only on the individual subject, but also on the precise nature of the double segment. These results are consistent with previous results conducted with human subjects (Klein Breteler et al. 2003). As Table 2 shows, motion duration was positively correlated for monkey B but not for monkey T. This positive correlation suggests a global planning of sequence execution. It is interesting that a positive correlation was found only for the monkey with the longer intersegment interval (monkey B). Finally, note that the actual mean values of the correlation coefficients (Table 2) are small, being invariably less that 0.2. This implies that, although statistically significant, the interdependency of execution of the first and second motions is a subtle phenomenon.

Movement parameters do not account for differences in neuronal activity

We next addressed the contribution of individual movement parameters to the observed differences in spike counts using the procedure illustrated in Fig. 2. Specifically, we examined whether any of the 7 previously defined movement parameters and an additional parameter, “trial time,” can account for observations of double-single sensitivity (DSp < 0.01). Trial time is simply the starting time of trial, and was incorporated to account for subtle nonstationarities in the neuronal response attributed to recording artifacts or physiological/psychophysical changes associated with movement repetition. For simplicity, we hereafter refer to all 8 parameters, including the trial-time parameter, as “movement parameters.”

An example of how our procedure applies to actual data is shown in Fig. 9. The top row shows spike counts, paths, and trajectories for the single segment and first segment of double trials. The bottom 3 rows show the same data, but with trials partitioned according to peak velocity, deviation at peak velocity, and time to peak. In this example, significant differences in spike counts occurred after partitioning by single versus double...
(top panels: “context”). Given that in this case neither of the movement parameters yielded a significant P value (not shown), the observation is classified as “context” related.

Classification of observations with double-single sensitivity into “context,” “mixed,” or “movement” categories is shown in Fig. 10. The figure shows that also after excluding observations accounted either entirely (“movement”) or partially (“mixed”) by the movement parameters, the fraction of observations exhibiting significant context-related differences (i.e., “context”) is still well above the expected chance level of 1%.

**FIG. 5.** Example of double-single “insensitivity” during the execution epoch. Same conventions as in Fig. 3. Data are from Monkey T.

**FIG. 6.** Distribution of the DS\_index and fraction of significant comparisons. A: DS\_index distributions are shown for each monkey and each epoch separately. Within each panel, the distribution of all observations is represented by the white histogram, whereas the shaded area denotes the corresponding distribution for double-single–sensitive observations (DSp <0.01). B: fraction of double-single–sensitive observations in each epoch (P < 0.01). Top: monkey B. Bottom: monkey T. Dotted lines mark the expected fraction (0.01) under the null-hypothesis. pres, presentation; prep, preparation; exec, execution.
Because the 8 movement parameters considered here are not strictly independent, testing their capacity to account for the single-double sensitivity of a given neuron may be redundant with each other. To potentially increase the power of the original movement parameters to account for double-single sensitivity, we transformed them to a set of 8 orthogonal parameters. The transformed parameter space was obtained by applying a principal-component analysis for the set of 8 movement parameters obtained in all repetitions (trials) of a specific double motion and the corresponding single. The principal components obtained from this analysis then served as a new space on which to characterize that set of double and corresponding single trials. Each individual trial was thus characterized by projecting the 8 original parameters onto the corresponding 8 principal components. Finally, we tested the capacity of each of these transformed parameters to account for double-single differences using the same procedure described above for the original parameters. The number of "context-related observations after this transformation was virtually identical to the number obtained with the original set (not shown).

**Both directionally tuned and nontuned units display double-single sensitivity**

We next investigated the relationship between double-single sensitivity and one commonly studied kinematic property, directional tuning. For this analysis an observation was defined as double-single sensitive if "context" related or "mixed." Units were considered as tuned if the directional preference during the execution epoch was significant at the 1% level. We then studied the interaction between these 2 properties with a $G$-test for independence. Contingency tables for this analysis are shown in Table 3. Results of the $G$-test, indicated above each table, show that independence is not rejected for any epoch for monkey B, but is rejected during execution for monkey T (nr, null hypothesis of independence not rejected; *, rejected at the 0.05 level). Thus overall, double-single-sensitive observations are associated with tuned as well as nontuned units, with some tendency for observations of monkey T to be...
well tuned. The population of directionally tuned units is therefore neither distinct nor identical, but rather partially overlapping with that of double-single–sensitive units.

**Movement direction is reliably specified by the population under both contexts**

In light of the differences on the single-unit level, it is interesting to investigate whether the population as a whole still provides a reliable readout of movement direction. For example, if differences in context-related activity are attributed to preparation for the upcoming second segment, the population vector (PV) associated with the double motion should rotate in the direction of the second motion. We therefore compared the PVs during execution of the first segment of double trajectories with those of the corresponding singles. For this analysis, we included all directionally tuned units, regardless of whether they showed a context-related effect. To increase sample size, all double trajectories with a given bend were rotated and pooled together (separately for each monkey). Results for the execution epoch are shown in Fig. 11A for trajectories of −60° (monkey B) and +60° bends (B and T). Other classes (bends) of trajectories are not shown because the small sample sizes did not yield statistically reliable PVs.

For each of the cases in Fig. 11A, the double PV is included within the confidence limits of the single, and vice versa, with respect to both direction and magnitude. Moreover, the confidence intervals of the double PV never span the expected, nor the actual direction of the second single-segment PV. An additional test was conducted on the differences between the single and double PVs with the result that for all cases, the 99% confidence limits spanned 0°. Thus during execution, the PV does not rotate in the direction of the second segment and thus provides a reliable prediction of movement direction, regardless of the double-single context.

Calculation of PVs during presentation is not reliable, mainly because of the small number of well-tuned units. However, for...
monkey B we can also study the PV during preparation. This comparison is especially interesting in the light of previous studies (Ashe et al. 1993; Kettner et al. 1996), suggesting that during preparation, the vector should initially point toward the direction of the second segment, and only then to the first. The PVs during preparation are shown in Fig. 11B. In both examples, the single and double PVs are still confined within each other's confidence intervals. However, for trajectories with a +60° bend, the double PV does somewhat rotate toward the second segment, and in this case the 99% confidence interval for the difference does not span 0° (not shown). In the other example (−60° bend), the double PV practically coincides with the single PV. Thus also during preparation, there is no consistent rotation of the PV toward the second segment.

For all calculations of the population vector, the preferred directions of each of the units were determined by trajectories beginning at the center of the workspace, whereas double motions and corresponding singles often began at noncentral positions. Thus the positional effect on the tuning functions was neglected in our analysis. This may partly account for the (small) directional deviation of the single and double PVs from the expected directions. Nevertheless, this does not invalidate the comparison, given that double motions and corresponding singles began from the same locations and so were subject to the same positional effects.

Each table corresponds to one epoch for one monkey. Each cell shows the observed (expected) number of observations for the corresponding class. Results of a G-test for independence are indicated above each table (nr, independence not rejected; *, independence rejected at the 5% level). See RESULTS for definitions of tuned and double-single–sensitive observations.
Recording sites and spatial distribution of context-related units

Recording sites location relative to cortical surface landmarks are shown in Fig. 12. For both monkeys, neuronal activity from virtually all recording sites responded to passive manipulation of either elbow or shoulder, and a minority to distal arm-joint manipulation (wrist or hand). ICMS in monkey B confirmed that recording sites were confined to the arm area (bordered laterally and medially by palm/digit and leg responses, respectively). However, because of technical difficulties with our stimulus generator, only sparse sampling of the recording site regions could be obtained. We therefore could not identify a clear transition of threshold intensities representing the border between M1 and PMd. However, we did study the distribution of large layer 5 pyramidal neurons (Betz cells) to infer a tentative border (Fig. 12). This tentative border suggests that for monkey B almost all recording sites were positioned within PMd rather than M1. Although a border has not been determined for monkey T, it seems, based on the location of the cortical landmarks, that also for this monkey most recording sites were located within PMd.

We next investigated the frequency of “context-related units” in individual recorded sites. We define context-related units as those associated with context-related activity on at least one trajectory. The diameters of the circles in each of the panels in Fig. 12 represent the fraction of context-related units at the indicated recording site within each epoch. As expected, circle diameters increase from presentation to execution. For monkey B, anterior-lateral sites include higher fractions of context-related units than do posterior-medial, and this trend increases from presentation to execution. Data from monkey T do not reveal a similar gradient.

DISCUSSION

In this work we compared motor cortical neuronal activity during execution of a reaching movement when performed individually to that occurring when the same movement constitutes the first part of a double-motion sequence. Our main finding is that a substantial fraction of observations display context-related neuronal activity. A smaller fraction of observations could be explained either fully or partially by differences in movement execution under the 2 contexts, and were designated as movement related or mixed, respectively. Nevertheless, a reliable readout of the movement direction is attained in both contexts by applying a population vector. Therefore the direction of a movement and the sequential context in which it is embedded may be simultaneously and reliably encoded by neurons in the motor cortex. The fact that similar results emerged from analysis of both monkeys data, despite the temporal differences in task execution, confirms that our findings are not an outcome of using one particular temporal regime.

Analysis of movement parameters

Although in this work we have largely treated differences in movements as confounding variables, patterns of differences between single- and double-motion performance is interesting from a psychophysical point of view. For example, the isochrony principle states that velocities on double motions should be higher than those of single motions (Viviani and Flash 1995). In our experiments, although consistent patterns emerged for the motions of each monkey separately, these patterns generally differed between monkeys. For example, whereas for monkey B the peak velocity was often larger during the double motions, the opposite was true for monkey T (not shown). These results are consistent with psychophysical studies of human-performed sequences (Klein Breteler et al. 2003), where it was shown that different subjects may use distinct patterns of coarticulation, as reflected by hand paths. However, our comparisons are also confounded by the fact that the composition of trajectories of different bends was not identical for the 2 monkeys. Thus when only trajectories with
60° bends were analyzed, we observed that for both monkeys, the first segments of the double motions tended to rotate toward the second segment (e.g., compare single and double paths in Figs. 3–5). Although this effect is small (the median difference being <1 and 2° for monkeys B and T, respectively, during both peak velocity and motion end), its existence implies that the second motion influences the first in a predictable way. Furthermore, and in agreement with other studies (Engel et al. 1997; Klein Breteler et al. 2003), we observed interdependence between execution of the first and second segments of double motions. Together, these findings imply that from a behavioral point of view, the temporal composition of basic elements is not simply a linear process. This suggests that differences in motion execution under the 2 contexts studied here reflect an intrinsic property pertaining to the process of motor composition.

**Accounting for spike count differences by movement parameters**

In this study the experimentally controlled variable was the sequential context. However, interpretation of neuronal activity critically depends on the extent of context-driven changes in motor performance. We therefore made extensive efforts to test whether individual movement parameters could account for the differences in neuronal activity. A limitation of this approach is that differences explained by *combinations* of parameters may be overlooked. Potentially, other methods such as multiple linear regression or multidimensional ANOVA could have been used to test the extent to which movement parameters account for the double-single sensitivity. However, the crucial variable in our study, context, is not suitable for analysis using linear regression (or any other form of regression) because it can assume only one of 2 possible values. Moreover, spike counts are generally not a linear function of parameter values (e.g., cosine tuning for direction), thus rendering linear regression inappropriate. Multidimensional ANOVA is also not practical for our data, given that 9 dimensions (8 parameters and context) correspond to $2^9 = 512$ possible categories (assuming each parameter is partitioned into 2 classes). Although the numbers of trials in our data are relatively large, the data set is far too small to enable reliable testing using such a high-dimensional ANOVA. The major advantage of the procedure we have used here is that it tests the effects of each parameter on the spike count distributions using the same measure with which double-single sensitivity is determined. Moreover, defining the MVp as the *minimum* $P$ value over the 8 movement parameters results in a conservative test.

By design of our experiment, we could explain differences in neuronal activity based only on cursor-associated parameters. In principle, additional muscles (e.g., postural muscles) that do not correlate with cursor position may also be differentially active under the 2 contexts, and may thus account for the observed differences. These considerations are especially relevant given the previously mentioned findings showing that sequential segments made by humans reveal consistent patterns of coarticulation at the level of arm posture, but not by hand paths (Klein Breteler et al. 2003). Although this explanation cannot be altogether discounted with respect to our findings, several considerations argue against a substantial influence of such an effect here. First, comparison of the data from both monkeys argues against the contribution of muscles that do not correlate with cursor motion. Thus as apparent from Fig. 8, the single and double motions differed more for monkey T than for monkey B. However, the fraction of context-related observations (i.e., after correcting for cursor-associated parameters) is not higher for monkey T. This would not have been the case had additional motor variables, unrelated to cursor position, been contributing to the raw differences between single- and double-neuronal activity. Second, the human study (Klein Breteler et al. 2003) involved 3D motions, whereas in our study, hand movements were confined to a horizontal plane. This implies that in our study movements are more constrained, and in addition, gravitational effects that may play a significant role in 3D motions are minimized. Finally, the task used here incorporated a pause between the 2 motion segments, a feature that would tend to decrease the differences between single- and double-motion performance.

**Implications for functional organization of the motor cortex**

Although we have determined a tentative border between PMd and M1, we cannot unambiguously distinguish these 2 regions (Fig. 12). Not only would slight changes in the definition of the border alter the exact fraction of units assigned to each region, but it is now recognized that such a clear border does not really exist (Geyer et al. 2000). A continuous change in histological and physiological properties is a more accurate description of the transition one encounters progressing from one area to another. Nevertheless, it does seem safe to conclude that the majority of recording sites were confined to the caudal premotor cortex (PMd-c). Our results are consistent with the observation that the caudal PMd is associated with limb-related activity, in contrast to the rostral PMd (PMd-r), which is predominantly related to eye movements (Fujii et al. 2000; Geyer et al. 2000). Moreover, PMd-c, but not PMd-r, is directly and strongly connected with M1 (Geyer et al. 2000). Various studies have shown that neurons in PMd encode not only the direction of motion but also that of the targets (Hoshi and Tanji 2002; Lebedev and Wise 2001; Mason et al. 1998; Shen and Alexander 1997). Because in our experiments target and movement direction were not dissociated, these variables cannot be distinguished. Thus a certain fraction of the context-related observations during *presentation* may reflect the visual stimulus (target presentation), which provides information about the target’s location. Our interpretation of double-single sensitivity for target-direction–related neurons would be that activity related to the direction of the immediate target is also modulated by the location of the subsequent target. Inspection of the spread of double-single–sensitive units over the cortical surface (Fig. 12) reveals a larger fraction of context-related units during *preparation* and *execution* in the anterior-lateral region of the recorded sites for monkey B. This finding fits with the notion that, roughly, more anterior motor-cortical regions are associated with more complex aspects of motor control. Such a transition, however, is not apparent in the data from monkey T. This could be ascribed to sampling-related reasons (e.g., the smaller number of sites) or failure of the set of penetrations to significantly span the medial-lateral aspect.

In a recent study (Hatsopoulos et al. 2003), it was concluded that information concerning the sequential aspect of a motor task is conveyed by pairwise correlations, but not by firing...
rates of individual neurons. However, also in that study, about 20–30% of the neurons exhibited “marginally significant firing rate differences.” It seems that such neurons correspond with the population we have focused on here. Integrating findings from both studies, it seems that sequential aspects of the task are reflected both by firing rate modulation of individual neurons and by the correlated discharge of multiple neurons. In contrast to the various types of sequential-related activity in the SMA and pre-SMA (Shima and Tanji 1998, 2000; Shima et al. 1996), the neuronal activity described here seems to encode motor-related and sequential-related aspects of action concurrently. Our results are therefore in line with a series of studies showing that motor cortical neurons are responsive not only to overt physical aspects of motor behavior (Alexander and Crutcher 1990; Georgopoulos 2000; Hocherman and Wise 1991).

What then do the differences in the activity of individual units reflect? Although we have used the term “context,” we are not implying that the sequential context per se is encoded by the activity of the neurons described here. More likely, the observed differences, rather than reflecting some abstract characteristic of the motion, are mechanistically related to the act of complex motion execution. Thus the differences may be attributed to memorization, preparation, or suppression of the upcoming motion. Another possibility is that the differences in activity reflect the probabilistic nature of the functional connectivity. Higher-level neurons (like those found in the SMA and pre-SMA) encoding distinct sequences may have access to a common pool of “kinematic neurons” in PMd and M1. Random factors might cause the neurons that are actually recruited to vary for different groups of higher-level neurons. Thus one such group would hypothetically recruit neurons to execute the first segment of a double, whereas another would recruit neurons for the same motion when executed individually. If the recruitment pattern is probabilistic, some neurons will be equally active during both contexts, whereas others may show higher activation during one of the contexts.

Assuming that the observed differences in neuronal activity are not merely random factors raises the idea of information multiplexing both at a network and on a single-neuron level. For example, one set of neurons may encode various aspects of the first motion segment, whereas another could encode various aspects of the second (Kettner et al. 1996). If these sets are not disjoint, a certain population would then simultaneously encode 2 distinct aspects of the entire motion. At the single-neuron level, such multiplexing could take place at the level of individual spikes. Thus whereas here we summed all spikes of a given neuron in a certain interval, not all spikes may convey the same type of information. Testing this hypothesis would require a finer temporal characterization of the neuronal activity (Abeles and Gat 2001). Such an analysis might reveal that patterns of neuronal activity conveying information about kinematic aspects of the task are distinct from those that convey information about its sequential context, or about the upcoming motion.

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