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RESEARCH ARTICLE

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Sequential movement representations based on correlated neuronal activity

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Abstract We tested the hypothesis that sequential movements are represented in the correlated activity of motor cortical neurons. We simultaneously recorded multiple single neurons in the motor cortex while monkeys performed a two-segment movement sequence. Before any movement began the correlated spike firing between pairs of neurons differed when these sequences were planned as whole (planned) as compared to when they were planned one segment at a time (unplanned) even when the firing rates of these neurons did not distinguish between the two conditions. Moreover, the correlation strength was significantly larger when the directional preferences of the neurons matched the direction of the final segment of the sequence. Our results suggest that spatially distributed groups of MI neurons form dynamic correlation structures that distinguish different forms of sequential action.

Keywords Sequential movements · Correlated activity · Motor cortex · Multi-electrode recording · Population coding

Introduction

The ability to perform a variety of complex motor actions, such as playing the piano, hitting a forehand in tennis, or speaking is ubiquitous in everyday life and is a hallmark of highly encephalized mammals. Most learned motor behaviors can be viewed as complex movement sequences composed of simpler movement elements or primitives (Lindemann and Wright 1998; Mussa-Ivaldi 1999). In speech, for example, an utterance is composed of multiple words which, in turn, are composed of multiple phonemes, while for limb movements complex actions may be decomposed into simpler movement segments.

It is unknown how the nervous system quickly and flexibly combines movement segments and coordinates their execution to produce smoothly generated actions. From behavioral studies, it is known that component movements are sometimes modified when produced in the context of a sequence, as in speech co-articulation (Ostry et al. 1996) and in piano playing (Engel et al. 1997). These results suggest that the neural representation of a motor sequence is not simply a concatenation of movement segment representations, but is a product of their interactions. Single cell recording experiments in primates have shown that neurons in supplementary motor cortex fire selectively for particular sequences and not to others which share the same segments in different orderings (Mushiake et al. 1991; Tanji and Shima 1994). These experiments have also shown that single neurons in primary motor cortex (MI) do not encode movement sequences, firing for particular movement segments regardless of the context in which they are being performed. Although single cells in MI are generally not selective for particular sequences, representations involving groups of MI neurons may carry information about the planning of movement sequences. In this study, we hypothesized that movement sequences are represented by the correlated activity of ensembles of MI neurons.

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Materials and methods

Behavioral task

Two macaque monkeys (one *Macaca fascicularis* and one *M. mulatta*) were trained operantly to perform sequential movements with their contralateral arm. Animals moved a two-joint manipulandum in the horizontal plane to direct a cursor from the bottom target through an intermediate target or "via-point" and then to one of two possible final targets, either to the left or to the right. Each sequence could be performed under one of two possible conditions. In the *planned* condition (Fig. 1A, left), the monkey was given knowledge of the complete sequence before the signal to move (the go signal), allowing it to plan and then execute the entire sequence. Each trial was composed of four epochs: a 500-ms hold period, a 1,000-ms instruction period during which the desired sequence was specified, the go period at which time all targets were blinking, and the movement period. In the *unplanned* condition (Fig. 1A, right), the monkey was initially instructed to move to the intermediate target and was informed of the final target (left or right) as it approached the intermediate target. The instruction regarding the second segment appeared after the cursor moved 4 cm or half the distance to the intermediate target. In this condition, the monkey could not plan the entire sequence before movement onset. In addition, a control condition was added in which the monkey was instructed to move to the intermediate target and stop at that target. This condition was included to insure that the animal was paying close attention to the visual cues indicating which movement to perform. Data from this condition were not analyzed and, therefore, are not further referred to in this work. All five kinds of trials (i.e. leftward and rightward × planned and unplanned plus the control) were intermingled randomly throughout the experiment. Two kinds of instruction signal were used in separate experiments. In four of the seven data sets, the intermediate and final targets appeared in green while all other targets were white ("visually guided"). In the remaining three data sets, all nine targets appeared in the same color, and two circles appeared in the middle of the screen either in "blue" signaling a leftward sequence or in "yellow" signaling a rightward sequence ("non-visually guided"). This was deemed "non-visually guided" because the monkey did not simply move to different colored targets but had to learn a rule or association linking the color of the circles to the final target (either left or right) that had to be reached.

Electrophysiology

We used a silicon-based electrode array developed at the University of Utah to record neural discharge from multiple sites in the arm area of primary motor cortex (see Maynard et al. 1999 for more details concerning the electrode array). During a recording session, signals from up to 50 electrodes were amplified and recorded digitally onto disk at either 20 or 30 kHz per channel (Datawave Technologies, Longmont, CO & Bionic Technologies LLc, Salt Lake City, UT). Only waveforms that crossed a threshold (1.5 ms in duration) were stored and spike-sorted off-line. Autocorrelation functions were computed to verify single unit isolation. A total of seven data sets (four in the first animal and three in the second animal) were analyzed where a data set is defined as all neural data collected in one recording session. An eighth data set was recorded but not analyzed because of kinematic differences observed between planned and unplanned sequences. Each data set contained between 7 to 14 simultaneously recording units. A total of 67 single units were well isolated from which 314 pairs could be analyzed. All of the surgical and behavioral procedures were approved by Brown University's IACUC and conform to the principles outlined in the Guide for the Care and Use of Laboratory Animals (NIH publication no. 86–23, revised 1985).

Kinematics

The position of the hand was monitored by using a digitizing tablet (Numonics Co., Mongomerville, PA, and Wacom Technology Co., Vancouver, WA) placed under the manipulandum, which sampled its position either every 14 ms (72 Hz) or 6 ms (167 Hz). Movement onset was defined as the time at which the cursor left the bottom "hold" target at the bottom of the screen. The mean path was computed by taking the average *x, y,* and velocity signals at even temporal increments of the movement duration. The movement duration for each trial consisted of a period starting when the tangential velocity first surpassed 1 cm/s and ending when the tangential velocity fell below 3 cm/s.

Statistical analyses

The correlation coefficient, ρ, between the number of spikes generated by two neurons in a time bin was calculated as follows:

$$\rho = \frac{\sum_{i=1}^{N} (s_1^i - \bar{s}_1)(s_2^i - \bar{s}_2)}{\sqrt{\sum_{i=1}^{N} (s_1^i - \bar{s}_1)^2 \sum_{i=1}^{N} (s_2^i - \bar{s}_2)^2}}$$

where N is the number of trials, s^i1 and s^i2 are the spike counts from neuron 1 and 2 on trial i, and $\bar{s}_1$ and $\bar{s}_2$ are the average spike counts from neuron 1 and 2 measured over all trials. Unless otherwise noted, all correlation coefficients were transformed according to the Fisher z-transformation to make them normally distributed (Kleinbaum et al. 1988). The formula is: $z_{\text{transf}}=0.5\ln[(1+\rho)/(1-\rho)]$. Significant positive and negative correlations of individual neuron pairs were assessed using a t-test:

$$t = \frac{z_{transf}}{1/\sqrt{N-3}}$$

where N is the number of trials.

A two-sample t-test was used to assess significant differences in correlation strength between the planned and unplanned conditions:

$$t = \frac{z_{transf}^{planned} - z_{transf}^{unplanned}}{\sqrt{1/(N_{planned}-3) + 1/(N_{unplanned}-3)}}$$

To account for multiple statistical comparisons, the number of significant correlations or correlation differences was compared to the number obtained when the trial order in one neuron was randomly shuffled with respect to that of the other neuron. One thousand such random shuffles were performed on all neuron pairs to obtain a distribution of the number of significant correlations (or correlation differences) under the null hypothesis that all neuron pairs were statistically independent. If the number of significant correlations in the unshuffled population exceeded all 1000 shuffled values, it was considered significant at $p<0.001$. A binomial test was also performed to account for multiple comparisons. Spike counts and their pairwise correlation coefficients were measured in time bins anchored to movement onset. The preferred direction of each cell was computed as follows. The average firing rate was computed over a 600-ms interval starting 300 ms before the cursor left the intermediate target for planned left and right sequences. Firing rate modulation was then computed by subtracting the firing rate over the 500-ms hold period at the start of the trial and taking the absolute value of this difference. The preferred direction of the cell was determined by the sign of the difference (i.e. subtraction) in rate modulations between left and right planned sequences. In other words, if the cell's firing rate modulation was greater for planned left sequences than for planned right sequences, the cell's preferred direction was leftward and vice versa. In some cases, a cell's firing rate decreased relative to the hold period. In those cases, a cell was considered to have a preferred direction to

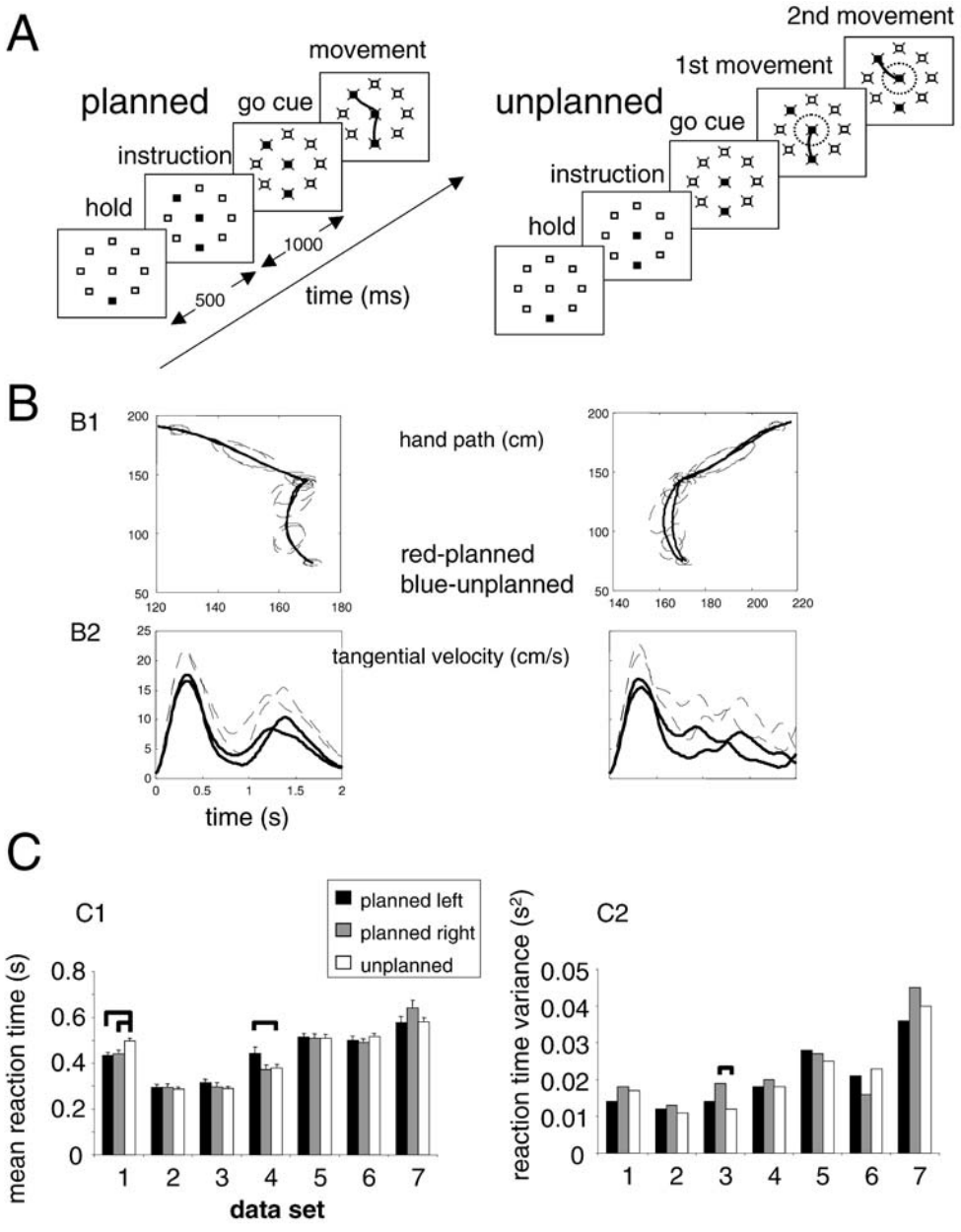

Fig. 1A–C The behavioral task results in no systematic differences in hand kinematics between the planned and unplanned sequence conditions before movement onset. **A** Two sequential movements (leftward and rightward; only the leftward sequence is shown) were performed under two conditions using visual displays updated as shown in the series of squares. In the *planned* condition, each trial is composed of four epochs: a 500-ms hold period, a 1000-ms instruction period during which the desired sequence is specified by colored targets, the go period at which time all targets are blinking, and the movement period. In the *unplanned* condition, only the intermediate target is colored during the instructed period so that the complete sequence cannot be pre-planned. The instruction regarding the second segment appears after the cursor moves 4 cm or half the distance to the intermediate target (*dotted circle*, not visible). **B** Kinematic parameters were directly compared between the planned and unplanned conditions. **B1** The mean paths for planned (*red*) and unplanned (*blue*) conditions are superimposed. The ellipses on the average paths represent the trial-to-trial variability (i.e. 1 SD) in the directions of the eigenvectors of the two-dimensional x and y covariance matrices. **B2** The mean tangential velocities for both conditions are superimposed. The *dashed lines* represent 1 SD above the mean. **C** Reaction time mean and variance under planned left, planned right, and unplanned conditions (pooled unplanned left and unplanned right conditions) for all seven data sets. **C1** A t-test revealed significant differences in mean reaction time between the planned and unplanned conditions in two of the seven data sets (two-tailed, $p<0.05$). **C2** An F-test comparing the reaction time variances revealed a significant difference in one data set (two tailed, $p<0.05$). Significant differences are denoted by *brackets*

the left if its rate decreased (relative to the hold period) more for leftward sequences than for rightward sequences.

Results

We measured the kinematics of the hand under the planned and unplanned conditions to insure there were no differences in the characteristics of the movement early in the trial that might result in firing rate differences of individual neurons. The kinematics of the hand were nearly identical up to the first tangential velocity peak. The average (and standard deviations) paths and tangential velocities from one data set are shown in Fig. 1B1, B2. Average *x*- and *y*-positions of the hand were directly compared at discrete time points along the path. For all data sets except for one, *x*- and *y*-trajectories for planned and unplanned trials were not significantly different until at least 100 ms after movement onset (n.s. at p=0.01 level, two-tailed *t*-test). This one data set that exhibited a significant kinematic difference was not analyzed further. From the remaining seven data sets, mean reaction time (RT) (Fig. 1C1) did not distinguish the two conditions with two exceptions. In one data set (data set 1), mean RT was significantly shorter (p<0.05, two-tailed *t*-test) under both planned conditions relative to the unplanned condition (i.e. pooled unbound left and unbound right conditions), and mean RT was significantly longer in the planned left condition in data set 4. There were no differences in RT variability (Fig. 1C2) under the two conditions except for one data set (data set 3), in which planned right trials showed significantly larger variability as compared to unplanned trials. Thus, for the data sets analyzed here, there were no systematic differences in kinematics up to 100 ms after movement onset except for the cases noted above. On the other hand, there were systematic differences in kinematics later in the movement. Most notably, the average tangential velocity trough near the middle of the sequence measured on a trial by trial basis was significantly shallower (i.e. higher velocity magnitude), and it occurred significantly earlier for planned compared to unplanned sequences for all but one data set (p<0.05, one-tailed *t*-test).

To evaluate differences in neural activity in planned and unplanned trials, we recorded simultaneously from up to 14 MI arm area neurons. Responses were typical for MI neurons; they began modulating their firing rate before movement onset and exhibited directional tuning, which was revealed during the execution of the second movement segment to the left or to the right (Fig. 2A). By contrast, in most cells the firing rate associated with the first movement segment did not differ under the planned and unplanned conditions during the 400-ms pre-movement period ending at movement onset (Fig. 2A, see gray horizontal bar). By considering the entire population of 67 recorded neurons as a whole, the differences in firing rates (measured during the 400-ms pre-movement period) under the planned (left or right) versus unplanned conditions were not significantly different from zero

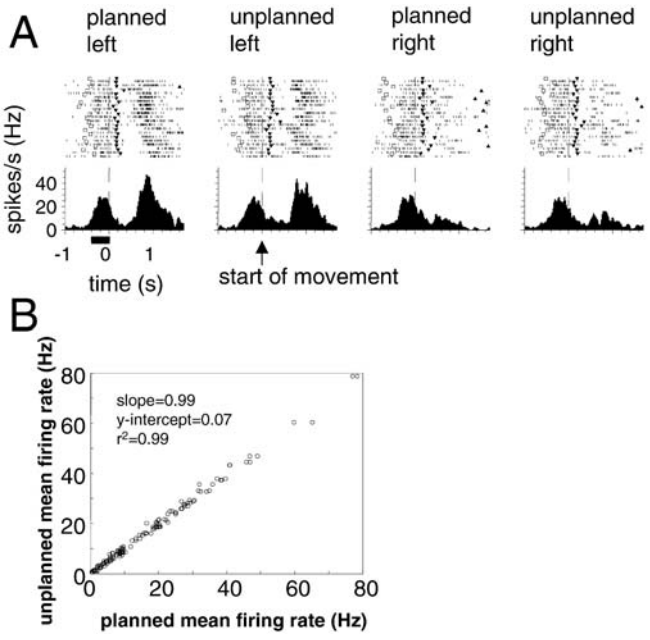

Fig. 2A, B The firing rates of single neurons do not differ under the planned and unplanned sequence conditions before movement onset. **A** Rasters and peri-event time histograms aligned on movement onset show neural firing patterns for one neuron over all four conditions. On each raster, the go signal is denoted by a *hollow square*, the time when the cursor moves half the distance to the intermediate target is denoted by an *inverted solid triangle*, and the reward by a *right-side up, solid triangle*. The *reward symbol* does not appear on every trial because of the limited time interval shown in the figure. Note that the firing of the cell is similar for all four conditions before movement onset. Also, it is evident that this cell is directionally tuned, firing more strongly for leftward segments than rightward segments. The *black horizontal bar* marks the period of time (400 ms before the start of movement) during which all further data analyses were performed. **B** Considering all 67 neurons recorded as population, the difference in mean firing rates (measured over the 400-ms interval before movement onset) between the planned (both left and right) and unplanned conditions was not statistically different from zero (n.s., two-tailed, paired *t*-test)

(p<0.05, paired, two-tailed *t*-test) (Fig. 2B). However, despite a lack of difference over the entire population, 13 cells did exhibit small but marginally significant firing rate differences between planned left and unplanned conditions (two-tailed *t*-test, p<0.10). Twenty-three cells, many of which were the same as the original 13, exhibited marginally significant firing rate differences between planned right and unplanned conditions. These cells were removed from further analysis.

The role that coordinated neural activity might play in representations of sequential actions was examined by comparing the correlated firing of neuron pairs under the planned and unplanned conditions. The number of spikes generated by pairs of neurons during a 400-ms interval before movement onset often exhibited significant positive and negative correlations on a trial by trial basis. This interval of time before movement onset was chosen because significant correlations tended to appear more often during this time (see Fig. 3C), and firing rates were

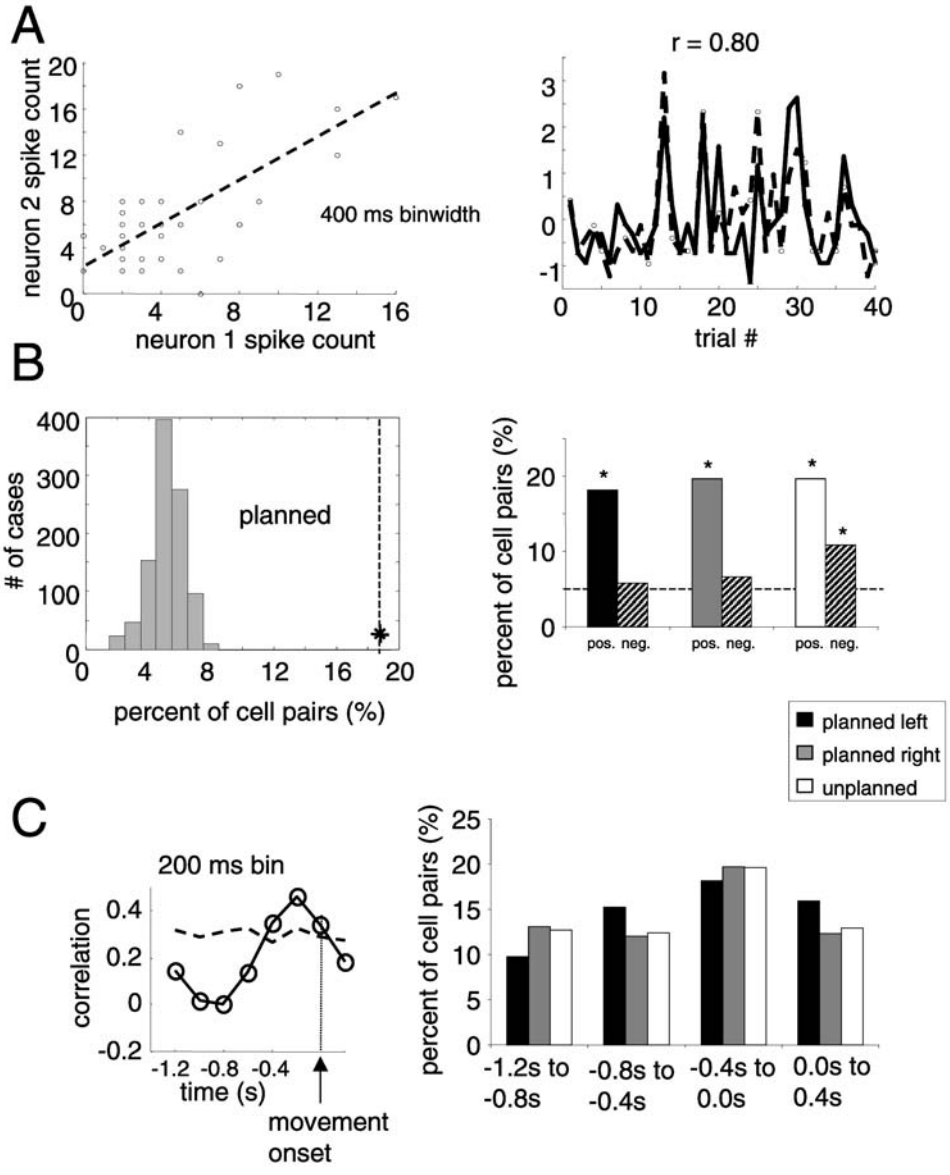

Fig. 3A–C Pairs of MI cells engaged in broad correlated activity. **A** (*left panel*) Scatter plot of spike counts from two neurons during planned rightward trials reveals significant positive correlation. Spike counts were measured over a 400-ms interval ending at movement onset. *Each point* represents the pair of spike counts measured on one trial. A least squares linear fit is also shown with a correlation coefficient of 0.8. **A** (*right panel*) The normalized firing rate of the same two neurons (*solid and dashed lines*) shown in the left panel as a function of trial number. Normalization was performed by subtracting the mean firing rate and dividing by the standard deviation. **B** (*left panel*) To account for the problem of multiple comparisons, the percentage of significant positive correlations for planned sequences was compared to a distribution created by shuffling the trial order of one neuron relative to another. This trial-shuffle was performed 1000 times. **B** (*right panel*) The percentage of significant (*t*-test, $p<0.05$) positive and negative correlated cell pairs during the planned left, planned right, and unplanned conditions. *Dashed line* is the expected percentage (5%). *Asterisks* denote percentages that are significant using the trial-shuffle test. **C** (*left panel*) The time course of the spike count correlation coefficient (Fisher *z*-transformed) between two neurons during a planned rightward sequence. The *black dashed line* indicates a significance level of 0.02 based on a normal approximation of the trial-shuffled data (100 shuffles). A 200-ms binwidth was used. **C** (*right panel*) Percentages of significant positive correlations under four different time intervals indicate that the largest percentages occur during the 400-ms interval before movement onset

not significantly different under the different behavioral conditions (see Fig. 2). Movement onset was used as an anchoring event as opposed to the go signal because neurons in MI modulate their responses at a fixed time with respect to the beginning of movement (see Fig. 2A). Therefore, the observed correlations in spike counts cannot be due to correlated latencies that do occur when the go signal is used as an anchoring event. An example of significant positive correlation between two neurons for planned left trials is shown in the scatter plot in Fig. 3A (left panel). The normalized firing rates (*z*-transformed) of the two neurons (solid and dashed lines) over the

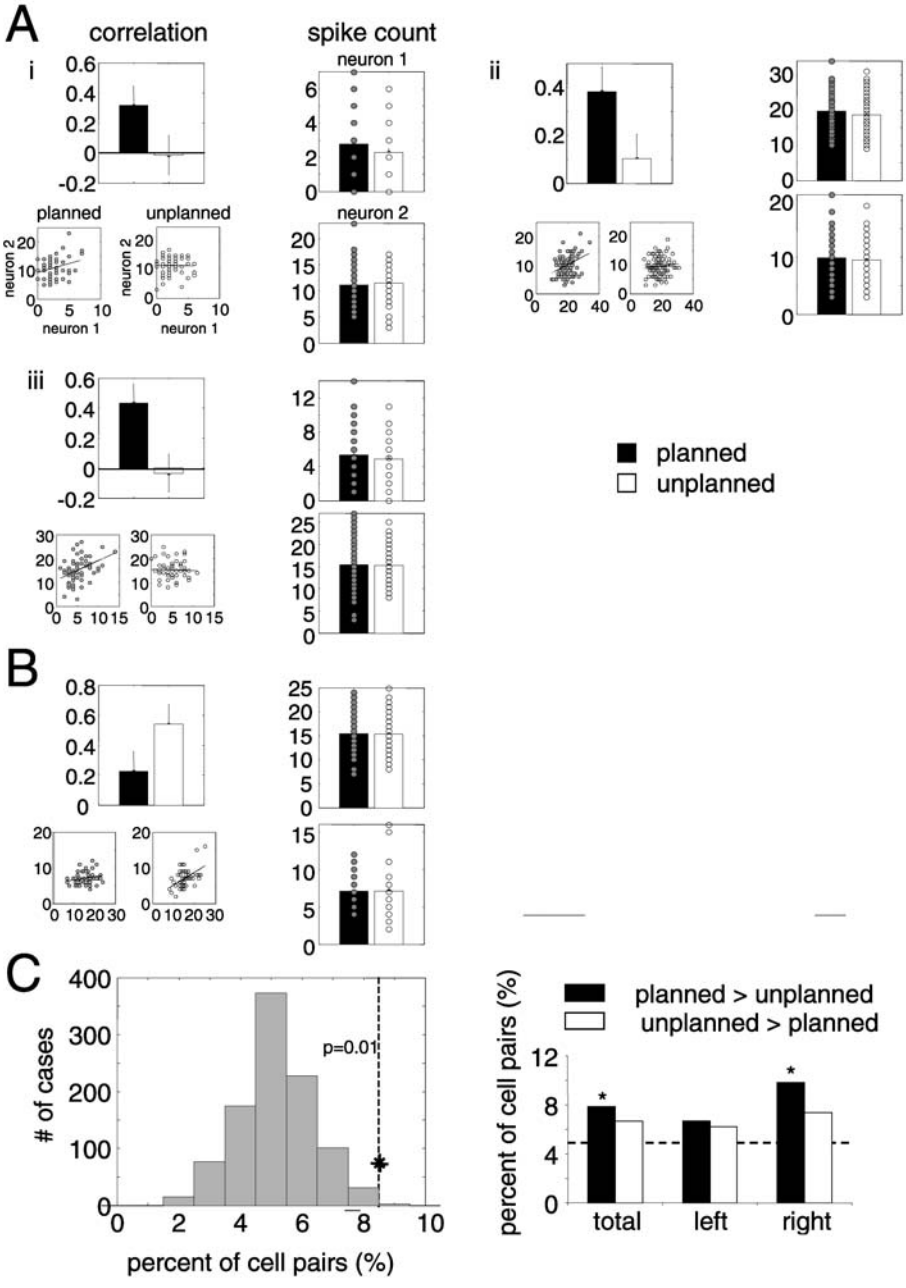

Fig. 4A–C Cell pairs showing significant differences in correlation strength under the planned and unplanned sequence conditions even when their firing rates do not. **A** Three examples of cell pairs that exhibited stronger correlations during the planned condition (*black*) as compared to the unplanned condition (*white*). Scatter plots below the mean correlation values display the trial-by-trial spike counts of one cell versus another under the two conditions. Mean spike counts are shown to the right along with the trial-by-trial scatter. *(i)* Planned right vs. unplanned from one cell pair in a "non-visually guided" experiment. *(ii)* Planned left vs. unplanned from a second cell pair again in a "non-visually guided" experiment. *(iii)* Planned left vs. unplanned from a third cell pair in a "visually guided" experiment. **B** An example of a cell pair that exhibited a stronger correlation during the unplanned condition as compared to the planned right condition. Display conventions are the same as in **A**. **C** (*left*) The percentage of cell pairs that showed a significant difference ($p<0.05$, two sample, *t*-test) between the planned and unplanned conditions is compared to a distribution generated by trial shuffling 1000 times. **C** (*right*) A breakdown of cell percentages that showed significantly stronger correlations for planned (*black*) as compared to unplanned and unplanned (*white*) as compared to planned conditions. The *dashed line* represents the expected percentage (5%). *Asterisks* denote percentages that are significant using the trial-shuffle test

course of the experiment are shown in Fig. 3A (right panel). The percentage of all cell pairs that exhibited significant correlations for planned sequences was 18.7% (62 out of 331 cases) and 6.0% (20 out of 331 cases) for positive and negative correlations, respectively ($p<0.05$, one-tailed *t*-test). To account for multiple comparisons, a trial-shuffle test was used to estimate the percentage of significant correlations that would occur by chance (see

"Materials and methods"). The percentage of positive correlations fell to the right of the null distribution and, therefore, was highly significant (Fig. 3B, left panel). The percentages of cell pairs that exhibited positive correlations under the planned left, planned right, and unplanned conditions were all highly significant using the trial-shuffle test (Fig. 3B, right panel). The results using a binomial test were consistent with those using the trial-shuffle test and, therefore, are not reported. The percentages of negative correlations were not significant under either the planned left or planned right conditions but was significant under the unplanned condition. This correlation strength was observed to be a temporally dynamic property which varied throughout the trial (Fig. 3C, left panel). The largest percentage of cell pairs that engaged in significant positive correlations occurred in the 400-ms interval immediately before movement onset.

We tested the hypothesis that the correlation strength of cell pair firing could distinguish planned from unplanned sequences. Figure 4A shows three examples in which there was significantly stronger correlation for planned sequences as compared to unplanned sequences. The first two examples are from "non-visually guided" experiments and the third is from a "visually guided" experiment. Scatter plots displaying the spike counts of one cell vs. the other over all trials for both conditions are presented below each bar graph. The average spike counts of each of the cells were not statistically different and are shown on the right along with the scatter of the data points. There were instances in which the correlation strength was stronger for the unplanned sequences as compared to the planned sequences (Fig. 4B). We found that correlation strength differed significantly ($p<0.05$, two-tailed t-test on Fisher z-transformed correlation coefficients) under the planned and unplanned conditions in 8.5% of the cell pairs (28 out of 331 cases), which was significant using the trial-shuffle test ($p=0.01$) (Fig. 4C, left panel) and the binomial test ($p=0.005$). The percentage of significant correlation differences was roughly the same for both instruction signals used (8.7% for "visually guided" data sets and 8% for "non-visually guided" data sets). Figure 4C (right panel) shows total percentage of cell pairs with stronger correlations in the planned condition (7.9%, 26 out of 331), stronger correlations in the unplanned condition (6.7%, 22 out of 331) and a breakdown by sequence type.

Given that any particular cell pair did not manifest the same correlation structure for planned left and planned right sequences suggested that correlation strength may be related to the directional preferences of the constituent neurons. We hypothesized that neuron pairs would exhibit stronger correlation when the sequence direction matched the neurons' preferred directions. We crudely categorized all our neurons as having a left, right, or no preferred direction (PD) based on their firing rate modulation during initial execution of the second segment of the planned sequences (see "Materials and methods"). According to this hypothesis, left-tuned neurons would exhibit stronger correlations for planned left sequences as compared to planned right sequences. The opposite result should occur for right-tuned neurons. Significantly stronger correlations were evident when the directional preferences of the two cells matched (i.e. were congruent with) the direction of the second segment (Fig. 5A, left panel). The average correlation strength was 0.073 when the directional preferences of the constituent cells matched the direction of the second segment as compared to 0.001 when they did not match. This result suggests that cells that will be engaged during the execution of the second segment are linked early on by becoming transiently correlated before any movement begins.

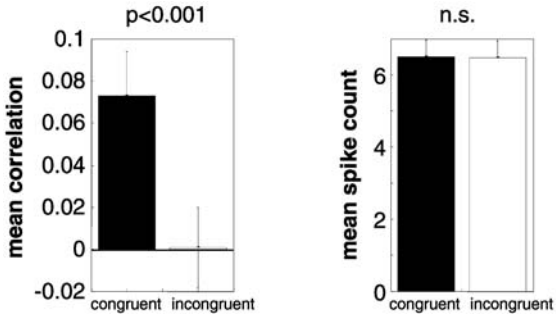

Fig. 5 The spike count correlation strength is stronger when the preferred directions (*PD*) of the constituent neurons match the direction of the sequence's second segment. *Left panel* The average correlation strength of cell pairs during planned leftward and planned rightward trials was directly compared for neuron pairs whose PDs were both either leftward or rightward. The mean (±1 SE) correlation coefficient over all cell pairs whose PDs matched (i.e. were congruent with; *black bar*) the direction of the second segment was significantly greater than that for those cell pairs whose PDs did not match (i.e. incongruent; *white bar*) ($p<0.001$, paired t-test). *Right panel* The mean spike counts of the congruent and incongruent cells were not statistically different

Discussion

Although previous studies have demonstrated the existence of broadly defined spike count correlations in visual (Nelson et al. 1992), parietal (Lee et al. 1998), and temporal cortex (Gochin et al. 1991), the present experiment is unique because it demonstrates that second-order interactions between cortical neurons carry information about movement sequence planning that is not available from the first-order firing rates of individual neurons.

The lack of firing rate differences that we observed seems to be inconsistent with results from Georgopoulos and colleagues, who found evidence that the firing rate over a large neural population carried directional information about the second segment of a two-segment sequence before movement began (Ashe et al. 1993). This inconsistency may be explained by noting that in their task the monkey was required to plan only one sequence repetitively. Perhaps, a default plan was used that contained information about the second segment. In our task, however, such a default plan was less likely to have

been generated because on each trial the upcoming action was uncertain; one of three options could occur.

Functional role of correlations

Although particular cell pairs we observed exhibited very large correlation strengths that were highly significant, the average correlation strength over all pairs was quite low (~0.05). This is consistent with other experimental studies in cortex (Zohary et al. 1994). Nevertheless, even weak correlations may have a profound effect on the response of neurons that receive inputs from these correlated neurons. Recent theoretical work has demonstrated that biophysically realistic neurons are highly sensitive to the second-order statistics of their synaptic inputs (Rudolph and Destexhe 2001a, 2001b). In particular, these studies have shown that a neuron's output response (i.e. its firing rate) can be modulated when the average pairwise correlations of its synaptic inputs are extremely weak, i.e. r~0.0005.

Theoretical and experimental work has demonstrated that spike count correlations may not be detrimental to population codes and may, in fact, improve neural decoding (Oram et al. 1998; Abbott and Dayan 1999; Chapin and Nicolelis-M. 1999). We have shown experimentally that broad correlations (on a 600-ms time scale) between pairs of cells can be used to carry more directional information in motor cortex than do average firing rates of single cells alone (Maynard et al. 1999). In the present study, we extend these results by demonstrating that these correlations carry information about the nature of a sequential movement even when firing rates do not.

The correlations reported in this study were observed over broad time scales and should be distinguished from synchronous discharge defined on a fine time scale (5 ms or lower) that also occurs between motor cortical neurons (Riehle et al. 1997; Hatsopoulos et al. 1998). Using an information theoretic analysis, we examined whether millisecond synchrony might provide information about the planning of sequential movements in these data. This preliminary analysis did not provide significant evidence that synchrony constitutes a code that carries such information. Future work will examine this issue in more detail.

Unlike synchrony, which can be measured *within* a trial, broad spike count correlation is measured at particular time intervals *across* trials. That is, such a correlation indicates that the spike counts (and, therefore, firing rates) from two cells covary about a mean on a trial-by-trial basis. Nevertheless, these correlations could be used to carry information on a single trial if one assumes that the nervous system possesses statistical models relating the behavior of the animal to the joint firing rates of neuronal ensembles. For example, suppose a subcortical target "expects" two particular motor cortical neurons' spike counts to be highly correlated for planned movements and negatively correlated for unplanned movements. These expectations can be viewed as two different joint probability distributions for the pair of motor cortical neurons conditional on either a planned or unplanned movement and could be realized in the population firing of two subcortical groups of neurons (one representing planned movements and the other representing unplanned movements). On any particular trial, one group would fire more strongly if both motor cortical neurons fired above or below their mean rates (i.e. positive correlation) whereas the other group would fire more strongly when one motor cortical neuron fired above its mean rate while the other fired below its mean rate (i.e. negative correlation). This example is not meant to be particularly realistic but rather should be viewed as an illustration as to how it would be theoretically possible to extract information carried in these broad correlations on a single trial.

It is possible that these dynamic correlations may not form a code that is read out by another part of the nervous system but rather are manifestations of functional connectivity that may serve some other purpose. Our findings are consistent with a synergistic theory of motor control that postulates the existence of transiently occurring muscle linkages or synergies for action (Bernstein 1967; Turvey et al. 1978). According to this theory, the nervous system constrains the large number of degrees of freedom in the motor system by creating muscle synergies that are dynamic and flexible, changing for each particular motor action. Muscle synergies manifest themselves as correlated activity between muscles and could be molded by cortical correlations described here. This is not unreasonable given that axons from primary motor cortex are a major contributor to the corticospinal tract, and, moreover, 5–10% of these axons form monosynaptic connections with motor neurons that drive muscles. Recent electromyographic and behavioral experiments support the view that muscle synergies in the hand are flexible and vary with the nature of the task (Hepp-Reymond et al. 1996; Sharp and Newell 2000). In the context of sequential movements such as the two-step movements described in this work, different muscle synergies may be set up by cortex depending on whether the complete sequence can be planned in advance. Whether dynamic muscle synergies are directly related to the dynamic correlations between cortical neurons is not known. Based on post-spike facilitation effects of cortico-motoneuronal neurons onto muscles of the hand, Lemon and colleagues have recently demonstrated that motor cortical neurons that engage in synchronous interactions tend to facilitate similar or synergistic muscle groups (Jackson et al. 2002). However, it is still unclear what the relationship is, if any, between synchrony measured on a fine time scale and the broad spike count correlations that have been characterized in the present study.

The transient nature of these correlations before movement onset, however, seems to be at odds with the synergy hypothesis described above because the correlations sometimes disappear after the movement begins (see

Fig. 3C, left panel). If these correlations are responsible for establishing particular muscle synergies, it is unclear why they would sometimes vanish after movement begins. Correlations before movement onset could reflect early priming of functional connectivity used during the execution of the sequence. This is further supported by the observation that cells with PDs that match the direction of the second segment exhibit stronger correlation before movement onset, again suggesting that these cells are being primed to work cooperatively because they will be active together later in the sequence.

Regardless of whether this particular hypothesis is true, our study demonstrates that statistical interactions between motor cortical neurons differentiate movement sequence plans, when the mean firing rates do not, and, therefore, may indicate functional connectivities at the cortical level that are important for planning and execution of motor actions.

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