Activity in the Supplementary Motor Area Related to Learning and Performance During a Sequential Visuomotor Task

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Lee, Daeeyeol, and Stephan Quessy. Activity in the supplementary motor area related to learning and performance during a sequential visuomotor task. J Neurophysiol 89: 1039–1056, 2003. First published October 30, 2002; 10.1152/jn.00638.2002. Monkeys were trained in a serial reaction time task to produce hand movements according to changing locations of visual targets. In most trials, targets followed the same sequence repeatedly, whereas in other trials targets were presented in random locations or switched unpredictably between two alternative sequences. Single-unit activity was recorded from the caudal supplementary motor area (SMA-proper). Based on the activity associated with random movement sequences, effects of hand position and movement direction were evaluated. Activity was influenced by the hand position in ~60% of the neurons, and the movement direction influenced the activity of 51% of the neurons. In addition, 37 and 71% of SMA neurons displayed nonstationarity in their activity across successive movements within a given trial and across trials, respectively. Such nonstationarity in the ongoing neural activity and the effects of performance-related variables were evaluated using a regression model and separated from learning-related activity changes. About a third of SMA neurons displayed gradual changes in neural activity related to experience with a movement sequence across trials. Furthermore, about a quarter of SMA neurons showed similar changes within individual trials. When the individual movements included in the frequently repeated movement sequences were introduced unexpectedly, learning-related changes in neural activity were reduced, indicating that many SMA neurons changed their activity in relation to the learning of particular movement sequences. These results suggest that the pattern of neural activity in the cortical network involved in the control of movement sequences can be modified continuously by experience.

INTRODUCTION

Much of our daily activity consists of learning new movement sequences and executing those learned previously. Although production of complex movement sequences depends on a broadly distributed network of cortical and subcortical areas (Tanji 2001), the primate supplementary motor area (SMA) appears to play an important role in this process. Originally, the medial portion of Brodmann’s area 6 was designated as the SMA based on the results of electrical stimulation experiments (Penfield and Welch 1951; Woolsey et al. 1952). Since then, numerous lesion studies as well as single-cell recording and metabolic imaging studies have implicated the SMA in various functions, such as voluntary movement initiation (Deiber et al. 1991; Eccles 1982; Goldberg 1985; Kurata and Wise 1988; Okano and Tanji 1987; Rizzolatti et al. 1983; Romo and Schultz 1987; Thaler et al. 1988), sequence learning (Clower and Alexander 1998; Grafton et al. 1995, 1998; Jenkins et al. 1994; Mushiake et al. 1991; Roland et al. 1980; Tanji and Shima 1994), and bimanual coordination (Brinkman 1984; Halsband et al. 1993; Laplane et al. 1977; Tanji et al. 1987, 1988). In addition, anatomical (Luppino et al. 1990, 1993) and physiological (Matsuzaka et al. 1992) studies have identified two distinct subdivisions within the traditional SMA, the rostral presupplementary motor area (pre-SMA or F6) and the caudal supplementary motor area proper (SMA-proper or F3). For simplicity, the SMA-proper is now commonly referred to as the SMA, and this convention is adopted hereinafter.

The SMA and the pre-SMA display several functional specializations (Hikosaka et al. 1999; Picard and Strick 1996; Shima and Tanji 2000). For example, the pre-SMA appears to play a more important role in updating motor plans (Matsuzaka and Tanji 1996; Shima et al. 1996) and coding the serial orders of multiple movements in a given sequence (Clower and Alexander 1998; Shima and Tanji 2000). In addition, these two cortical areas might play a different role in the learning of a new movement sequence than in the execution of a previously learned sequence (Hikosaka et al. 1999). For example, imaging studies have found increased activation in the pre-SMA during the initial stage of learning complex movement patterns (Sakai et al. 1998). This early pre-SMA activation might reflect the acquisition of novel visuo-motor associations (Dassonville et al. 2001; Sakai et al. 1999) or the processes of attention and working memory during the early phase of sequence learning (Hikosaka et al. 1999; Petit et al. 1998). In contrast, the role of the SMA during the learning of skillful movement sequences remains less well understood. The SMA was activated in some imaging studies when subjects performed previously learned movement sequences compared with new sequences (Doyon et al. 2002; Grafton et al. 1998; Jenkins et al. 1994), but this activation was not consistently observed in other studies (Rauch et al. 1995, 1997; Sakai et al. 1999) or the processes of attention and working memory during the early phase of sequence learning (Hikosaka et al. 1999; Petit et al. 1998). The reason for this discrepancy is not known, although it might be related to the differences in the behavioral paradigms.

The results from the previous single-unit recording studies suggest that the SMA plays a role in executing previously
learned movement sequences because many SMA neurons become active only when the animal produces a particular sequence of movements (Nakamura et al. 1998; Shima and Tanji 2000; Tanji and Shima 1994). In these studies, however, the animals were required to memorize movement sequences explicitly, and therefore it is not known whether such sequence-specific neural activity reflects the encoding and retrieval of a movement sequence or its working memory representation. In addition, how the activity of SMA neurons changes as the animal becomes familiar with a given movement sequence has not been examined. To address these issues, we examined the activity of SMA neurons during sequence learning in monkeys performing a serial reaction task (Nissen and Bullemer 1987). In this task, target locations repeatedly followed a simple pattern, and the animals were required to produce hand movements accordingly. Explicit memorization of movement sequence was not required because all individual movements were visually specified. In addition, activity was monitored during random movement sequences to evaluate nonstationarity in ongoing neural activity and also to determine how movement parameters are specified in the SMA. Learning-related activity was separated from nonstationarity and other changes in neural activity related to the variability in behavioral performance. The results show that many SMA neurons displayed gradual changes in activity specifically related to experience with a particular movement sequence, suggesting that activity patterns in the SMA are dynamically reorganized by experience.

METHODS

Animal preparation

Two male adult monkeys (Macaca mulatta; 6–8 kg. body wt) were used. After each animal was fully trained on the behavioral task, a set of four titanium posts were attached to the skull, and an eye coil was placed around the orbit of one eye in a sterile surgery. On recovery, the animal received additional training in which it was acclimated to control the cursor position. The computer screen was located 18 mm) was implanted above the supplemental motor area (SMA). All of the surgical and behavioral procedures were approved by the University of Rochester Committee on Animal Research and conformed to the principles outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, revised 1985).

Behavioral task

The animal was seated in a custom-built primate chair with its head fixed, and it was trained to produce a series of visually guided movements with its right hand on a touch screen. The touch screen was installed horizontally in front of the animal and therefore did not block the view of the 17-in computer monitor on which visual stimuli were presented. The spatial resolution of the touch screen was 0.5 mm. The animal’s hand position on the touch screen was displayed as a feedback cursor (white disk, rad = 0.47°) on the computer screen. Both animals consistently used their index and middle fingers to control the cursor position. The computer screen was located ~57 cm from the animal’s eyes, and the touch screen was calibrated so that a 1-cm displacement on the touch screen corresponded to the same displacement (1° visual angle) on the computer monitor. Targets (red disk, rad = 1.4°) were presented in a 4 × 4 grid (Fig. 1), and the center-to-center distance between the neighboring target locations was 4.2° (4.2 cm). The animal was required to acquire 10 successive targets in a given trial to receive a drop of apple juice. The interval between the acquisition of a given target and the presentation of the next target (response-stimulus interval) was 250 ms. The animal was required to acquire each target within 1 s from its onset, except for the first target in each trial. The first target was presented after 1-s inter-trial interval, and the animal was allowed to acquire it at any time. The animal’s hand and eye positions were sampled with the sampling rates of 100 and 500 Hz, respectively. The animals used in the present study were extensively trained with the same behavioral task for a period of several months, and their hand movements during the recording sessions were relatively stereotyped.

Sequence of target locations

For each daily recording session, 5 target locations were randomly selected from 16 possible locations (Fig. 1). Denoting these five locations with letters A through E, two triplets of target locations, ABC (primary triplet) and DEC (secondary triplet), were created. For five pseudo-randomly selected trials in a block of eight trials, target locations followed the sequence consisting of the primary triplet (ABCABCABCA, primary condition). For another trial in each block, target locations followed the secondary triplet (DECDECDEC, secondary condition), whereas in a third type of trial (once per block), target locations switched from the secondary to the primary triplet for the seventh target in a given trial (DECDECABC, switch condition). Because the first six targets were identical in the secondary and switch conditions, the animal could not predict whether the switch would occur in a given trial. In addition, the movement required immediately after the switch (C→A) also occurred in the same serial position during the trials in the primary condition. This makes it possible to determine whether the animal extracted any high-order information (e.g., 2nd-order conditional probability) about the primary triplet in addition to the difference in the frequency of targets and doublets in the primary triplet. For the remaining trial in each block, target
locations were randomly determined with the exception that two consecutive targets in the same locations were avoided (random condition).

**Neural recording and anatomical localization**

Single-unit activity was recorded using an Eckhorn 16-channel microelectrode manipulator (Thomas Recording, Giessen, Germany) and a Plexon multi-channel acquisition processor (Plexon, Dallas, TX). Spikes were isolated using two separate boxes set by the users in terms of time and voltage. In most cases, multiple neurons were recorded simultaneously from different electrodes (mean = 5.25), and only one neuron was recorded from a given electrode. Although multiple neurons were isolated from the same electrode occasionally, this was rare and the average number of neurons recorded from the same electrode was 1.08. The arrival times of spikes were originally stored with 25-μs resolution and later binned with 1-ms resolution. All the neurons were recorded in the SMA of the left hemisphere, which was contralateral to the hand the animal used to perform the task. Localization of neurons in the SMA was based on anatomical MR images and physiological criteria. All neurons included in the analysis were recorded in a region posterior to the facial representation located in the border between the SMA proper and the pre-SMA (Matsuzaka et al. 1992; Mitz and Wise 1987). Throughout the recording session, stability of spike isolation was thoroughly monitored by the visual display that superimposed multiple waveforms. Only the neurons that maintained stable spike isolation throughout the recording session were included in the analysis. Because the main goal of this study was to determine the pattern of changes in the neural activity during the learning of a movement sequence, our strategy was to record the activity of a given set of neurons for as many trials as it was practically possible. Only the neurons for which the data were collected for ≥200 trials were included in the analysis. This corresponds to 1,800 movements (200 trials × 9 movements/trial).

**Analysis of behavioral data**

For each movement, reaction time was defined as the interval between target onset and the time when the hand exited the previous target, and acquisition time was defined as the interval between target onset and the time when the new target was acquired. Movement time was defined as the difference between the two. Eye position data were smoothed by a 5-point median filter followed by a Gaussian filter (σ = 10 ms), and the onset of saccade was detected with a velocity threshold of 20°/s (Lee and Malpeli 1998). Although each trial included 10 target presentations, the movement to the first target was excluded from the analysis because in this case the initial hand position was not controlled. The behavioral data and the neural data were obtained from the same recording sessions.

**Analysis of neural data**

To examine learning-related changes in neural activity, it is necessary to exclude the possibility that the observed changes are related to other confounding factors. First, neurons might display different types of nonstationarity in their activity unrelated to learning. For example, some neurons may display changes in their activity according to the serial positions of targets within each trial (within-trial nonstationarity) regardless of whether a particular target sequence is repeated or not. Furthermore, neurons can display nonstationarity in their overall excitability across multiple trials (cross-trial nonstationarity). In this study, these two different types of nonstationarity were estimated from the neural activity during the trials in the random condition in which the target sequence was always random. However, the activity in the random condition was highly variable because the required movements varied. Therefore to obtain more reliable estimates of nonstationarity, we first estimated the effects of different movement parameters, such as target position and movement direction, and nonstationarity was evaluated using the residuals from this model. Second, as shown in RESULTS, the animal’s behavioral performance improved in the primary condition as the target sequence was repeated, and the activity of some neurons may have been altered merely as a result of such changes in the animal’s behavior. The effects of performance-related variables, such as reaction time and movement time, were therefore factored out from the activity during the primary trials, before the effects of experience were tested. The following sections describe these procedures in detail.

**CODING OF MOVEMENT PARAMETERS.** Trials in this study consisted of periods in which the animal maintained its hand position at a particular target location (i.e., response-stimulus interval) and those in which the animal prepared (i.e., reaction time) and executed (i.e., movement time) a particular hand movement according to the change in target location. Accordingly, the activity of SMA neurons was influenced by multiple parameters, such as the starting and final target positions as well as the movement direction. In previous studies, the relative importance and time course of different movement-related parameters have been studied using a sliding linear regression model (Fu et al. 1995, 1997; Johnson and Ebner 2000). Similarly, the following regression model was applied to the spike density functions of SMA neurons in the random condition

\[ F_{a,n}(t) = a_0(t) + a_1(t)x_{a,n-1} + a_2(t)y_{a,n-1} + a_3(t)\cos \theta_{a,n} + a_4(t)\sin \theta_{a,n} + a_5(t)(x_{a,n} + y_{a,n}) + e_{a,n}(t) \]

where \( F_{a,n}(t) \) denotes the spike density function at time \( t \) from the onset of the \( n \)th target in trial \( m \) in the random condition, \( a_0(t) - a_5(t) \) the regression coefficients at time \( t \), \( x_{a,n} \) and \( y_{a,n} \) are the horizontal and vertical position of the \( n \)th target in trial \( m \), \( \theta_{a,n} \) the direction of the corresponding movement, and \( e_{a,n}(t) \) an error term. The spike density function was calculated by convolution of the original spike train with a Gaussian kernel (σ = 40 ms) (MacPherson and Aldridge 1979). The preceding regression model was applied to the spike density function during the interval between −400 and 600 ms from target onset in 20-ms steps. It should be noted that the location of a given target in the random condition was somewhat correlated with the direction of the following movement due to the limited number of target locations used. For example, movements initiated from the targets in the uppermost positions were always downward. This problem is often referred to as multicollinearity, and it could increase the variance of the regression coefficients (Stevens 1996). Although this might introduce some uncertainty in the exact values of the regression coefficients of the preceding model, this is unlikely to affect the pattern of nonstationarity in the residuals from such a model and consequently the estimates of learning-related effects.

**ANALYSIS OF NONSTATIONARITY.** The activity associated with individual movements of the primary triplet often displayed gradual changes as the triplet was repeated within a given trial and/or across multiple trials. To determine whether such changes were specifically related to experience with a given triplet or whether they were the results of other time-dependent factors, such as tissue damage or fatigue of the animal, it was necessary to characterize the nonstationarity of neural activity in the random condition. Because the trials in the random condition served as the baseline condition in the present study, the nature of the nonstationarity found in the random condition could not be determined. Nevertheless, such nonstationarity was eliminated from learning-related activity estimated from the primary condition. This provides a relatively conservative estimate of learning-related activity because some changes in neural activity in the random condition might also be a result of learning. To evaluate the pattern of nonstationarity in the random condition, the residuals in the preceding regression model (Eq. 1), \( e_{a,n}(t) \), were averaged for \( -200 \leq t \leq 200 \) and plotted as a function of trial number and target number (i.e., serial order of a given target within a trial) separately. This particular
400-ms interval was chosen for the remaining analyses of learning-related activity because this was the time period in which the animal could prepare for the generation of the next movement based on its prior experience with the primary triplet. To determine whether the activity in the random condition was significantly affected by trial number, the following third-order polynomial regression model was applied

$$E_{m,n} = b_0 + b_1 m + b_2 n + b_3 m n + \mu_{m,n}$$

where \(E_{m,n}\) denotes the mean residual from the regression in Eq. 1 during the 400-ms interval starting from 200 ms before target onset, \(m\) is the trial number, and \(\mu_{m,n}\) is an error term. Similarly, to determine whether the activity was affected by the target number, a second-order polynomial regression model was applied

$$E_{m,n} = c_0 + c_1 n + c_2 n^2 + \nu_{m,n}$$

where \(n\) denotes the target number and \(\nu_{m,n}\) an error term. Compared with the model for cross-trial nonstationarity (Eq. 2), a simpler model was applied for this within-trial nonstationarity (Eq. 3) to prevent overfitting because the number of movements in each trial (9) was much smaller than the total number of trials (>200). In addition, as shown in RESULTS, estimates of within-trial nonstationarity were unaffected by the order of regression models. Based on these regression models (2 and 3), the following two functions were defined. First, the cross-trial nonstationarity function, \(T_{CT}(m)\), was defined as the following

$$T_{CT}(m) = b_0 + b_1 m + b_2 n + b_3 m n$$

Similarly, the within-trial nonstationarity function, \(T_{WT}(n)\), was defined as the following

$$T_{WT}(n) = c_0 + c_1 n + c_2 n^2$$

Each of these two functions was used as a template to model the time course of nonstationarity related to the target number or the trial number.

ANALYSIS OF PERFORMANCE-RELATED VARIABLES. Although the pattern of hand movements during the neurophysiological recordings in this study was relatively stable, the activity of SMA neurons could be affected by subtle changes in movement kinematics, such as the initial hand position and the movement direction. To exclude the possibility that changes in neural activity related to movement kinematics were confounded with learning-related activity, the effects of these variables were examined in a regression model. In addition, activity of SMA neurons might also be related to changes in the reaction time (RT) or movement time (MT) as well as reaction times and movement times for targets of different movement directions. Finally, RT\(_{m,n}\), MT\(_{m,n}\), and SRT\(_{m,n}\) denote the reaction time, movement time, and saccadic reaction time for the \(n\)th target in trial \(m\), respectively. As in the regression model for \(F^m_{m,n}(t)\) (Eq. 1), this model was applied to the spike density function from −400 to 600 ms from target onset in 20-ms steps. For each time step, the signs of the regression coefficients for within-trial and cross-trial nonstationary functions, \(d_1(t)\) and \(d_2(t)\), were examined. These coefficients should be positive if they reflected the same type of nonstationarity found in the random condition. If either of these coefficients was negative, the corresponding term was eliminated and the new regression model was applied to the same spike density functions.

ANALYSIS OF LEARNING-RELATED CHANGES IN NEURAL ACTIVITY. To determine whether experience with a particular movement sequence influenced the activity of a given neuron, the error term from this regression model (Eq. 6) was averaged for the 400-ms interval starting from 200 ms before the onset of each target. Denoting this mean residual as \(G_{m,n}\), the following regression model was then applied

$$G_{m,n} = b_0 + h_1 m + h_2 n + \lambda_{m,n}$$

where \(m\) and \(n\) again denote the target number and the trial number, respectively, and \(\lambda_{m,n}\) an error term. The regression coefficients associated with \(m\) and \(n\) were taken as measures of the effect of experience with the primary triplet within a given trial (referred to as priming effect) or across multiple trials (referred to as practice effect), respectively. Because these two effects were estimated after potential contributions of variables included in Eq. 6 were eliminated, they reflect learning-related changes in neural activity unrelated to the nonstationarity in the ongoing activity and performance-related activity changes. It is possible that this model might underestimate the extent of transient learning-related activity because it was applied to the average activity during the 400-ms interval surrounding target onset. To examine this possibility, the same model was also applied separately to the 200-ms intervals immediately before and after target onset. In addition, to test whether there was any interaction between the practice and priming effects, the following model was also tested

$$G_{m,n} = b_0 + h_1 m + h_2 n + h_3 m n + \lambda_{m,n}$$

One limitation of the preceding models is that they are all linear functions of the trial number, and therefore it may not detect practice effect with a more complex time course (e.g., exponential). Nevertheless, the use of linear model can be justified in two ways. First, it is simple and parsimonious. Second, as shown in RESULTS, the pattern of behavioral improvement during the sequence learning was relatively linear, suggesting that at least a subset of neurons might display linear changes in their activity. Statistical significance of the regression models and individual regression coefficients was determined by \(F\) and \(t\)-tests, respectively (Snedecor and Cochran 1989). Statistical significance of the regression coefficients were also tested using a permutation test in which the number of trials or the serial positions of targets were shuffled to estimate the probability that the observed practice or priming effects could arise by chance. The \(P\) values from this permutation test were obtained based on 1,000 shuffles.

RESULTS

Effects of practice on behavior

Behavioral and neural data described in this paper were obtained from a total of 27 daily sessions with a minimum of 200 trials/day. On average, the animal performed 311 trials/day, and this corresponds to 2,800 movements/day. To examine the time course of improvement in behavioral performance following experience with a particular movement sequence, the reaction times and acquisition times from all the sessions were obtained from a total of 27 daily sessions with a minimum of 200 trials/day. On average, the animal performed 311 trials/day, and this corresponds to 2,800 movements/day. To examine the time course of improvement in behavioral performance following experience with a particular movement sequence, the reaction times and acquisition times from all the sessions with
a minimum of 400 trials (n = 21 sessions) were averaged for each block, separately for the primary and random conditions. The results from the two animals were qualitatively similar and combined for simplicity. A total of 47,250 and 9,450 movements were analyzed for the primary and random conditions, respectively. The difference in the average reaction times for the primary and random conditions was 2 ms (242 vs. 244 ms, Fig. 2). Although this small difference was statistically significant (paired t-test, \( P < 0.05 \)) due to the large number of data points included in the analysis, this is unlikely to be the result of learning because the difference between the primary and random conditions did not change with the amount of training (Fig. 2A). This was quantified with the correlation coefficient between the number of blocks and the difference in the reaction times for the primary and random conditions. This was calculated for a variable number of blocks, beginning with the first 5 blocks and ending with all 50 blocks (Fig. 2C). The null hypothesis that this correlation coefficient was zero could not be rejected for any number of blocks. In contrast, the difference in the acquisition time for the primary and random condition was 24 ms (505 vs. 529 ms) and statistically significant (paired \( t \)-test, \( P < 10^{-23} \)). Unlike the reaction time data, this difference in the acquisition time gradually increased as the animal gained experience with repeated movement sequences in the primary condition (Fig. 2B). The correlation coefficients calculated for the number of blocks and the difference in the acquisition time were significantly different from zero (\( P < 0.05 \)) when more than 43 blocks of trials (344 trials) from the beginning of each session were included in the analysis (Fig. 2D).

Statistical structures of the target sequences presented in the primary and random conditions differed in several aspects. For example, the targets presented in the primary conditions appeared much more frequently compared with those in the random condition. In addition, only a small subset of possible target transitions (doublets, triplets, etc.) occurred in the primary conditions. Therefore the comparison between the primary and random conditions does not indicate whether the animals acquired any information other than the differences in the target frequency. However, the results from the switch trials provided some evidence that the animals acquired more information than just the target frequency (e.g., 2nd-order conditional probability). The transition from the sixth target to the seventh target in the switch trials was the same as in the primary trials. Nevertheless, both reaction time and acquisition time increased significantly following the switch from the secondary triplet to the primary triplet compared with those of the corresponding movement in the primary condition. This switch effect was 11.1 and 17.2 ms for the reaction time and acquisition time, respectively, and they were both statistically significant (\( P < 0.01 \)).

The pattern of eye movements during task performance was stereotyped. In most trials, the animal produced direct saccadic eye movements toward the next target location (Fig. 1). Saccadic reaction times were significantly shorter in the random condition than in the primary condition (\( P < 0.0001 \)), suggesting that generation of eye movements toward recently visited locations was suppressed (“inhibition of return”) (Bichot and Schall 2002; Maylor 1985; Posner and Cohen 1984; Tanaka and Shimojo 1996, 2000). The mean saccade reaction time in the primary condition was 204 ms, whereas it was 187 ms for the random condition. The mean saccade reaction times for the seventh target in the primary condition and switch condition were similar (187 vs. 185 ms), and this difference was not statistically significant.

**Neuronal database**

A total of 142 neurons were recorded in the left SMA of two monkeys. Of these, 108 neurons (69 and 39 neurons from the 2 animals, respectively) were examined for a minimum of 200 trials (=25 blocks) and included in the following analysis.

**FIG. 2.** Effects of practice on the reaction time and acquisition time. A and B: average reaction times (A) and acquisition times (B) of individual blocks for the primary (5 trials/block, filled circles) and random (1 trial/block, empty circles) conditions. Lines indicate the least-square fit to the data (thick line, primary condition; thin line, random condition). C and D: correlation coefficient between the block numbers and the difference in the reaction times (or acquisition times) between the primary and random conditions is shown for increasing numbers of 1st \( N \) consecutive blocks. Solid lines indicate the level of correlation coefficient at the significance level of 0.05 (1-tailed t-test).
Because the animal performed 9 movements in each trial, this corresponds to 1,800 movements, including 1,125 movements in the primary trials. The anatomical locations of the neurons included in the analysis are shown in Fig. 3. For the neurons included in the analysis, the mean number of trials was 441.8 ± 113.4 (SD) with a mean duration of recording of 88 ± 23 (SD) min. The average number of movements examined for each neuron was 3,976.

**Coding of movement parameters in the SMA**

In this paper, short-term changes in neural activity related to the repetition of a particular movement sequence within a single trial is referred to as a priming effect, whereas more gradual changes in neural activity related to experience with a particular sequence across multiple trials is referred to as a practice effect. Visual inspection of raster plots and spike density functions suggested that both of these effects were present in many SMA neurons. However, several alternative causes must be excluded before such changes in activity could be attributed to learning. For example, some neurons might display systematic activity changes in a given trial according to the numerical order of the movements (Clower and Alexander 1998; Shima and Tanji 2000) or the temporal proximity of each movement to the reward delivery (Shidara and Richmond 2002; Shidara et al. 1998). These two types of within-trial nonstationarity should be separated from the priming effect. In addition, neurons recorded over an extended period of time often display a gradual drift in their overall excitability (Bach and Krüger 1986; Bair et al. 2001; Rose 1979). This cross-trial nonstationarity must be separated from the practice effect. To control for these alternative factors, the activity recorded in the random condition was examined. Because the movement sequence was random, neural activity specifically related to the learning of movement sequence was unlikely to occur in this condition. Because the movement sequence was random, however, it also increased the variability of neural activity. Therefore effects of movement parameters on neural activity were estimated and factored out before the level of nonstationarity was quantified.

The activity of many SMA neurons was often influenced by initial hand position and movement directions. For example, the activity of the neuron shown in Fig. 4 was more strongly related to the previous target position immediately before and after the onset of the next target, and movement direction became a more important factor beginning ~200 ms from target onset. The dip in the spike density function at the time of target onset was more pronounced when the data were sorted according to the current target position (Fig. 4A, black arrow), whereas the absence of movement-related activity for certain directions could be seen clearly only when the data were sorted by the movement direction (Fig. 4B, gray arrow). To determine the time course in which the neural activity was influenced by various movement-related variables, regression coefficients from a sliding regression model (Eq. 1, see METHODS) were calculated separately for each time step (Δ = 20 ms), and the relative contributions of different variables were expressed by the squares of standardized regression coefficients. For the neuron illustrated in Fig. 4, this analysis confirmed that the activity was influenced by the target position as well as movement direction. The influence of the target position reached its maximum at 100 ms from the onset of the next target, as it accounted for 34.4% of the variance in the spike density function (Fig. 4D, dashed line). The influence of movement direction reached its maximum at 280 ms from target onset, accounting for 35.3% of the variance in the spike density function (Fig. 4D, solid line). The relative influences of hand position and movement direction on the activity level varied across different SMA neurons. For example, the activity of the neuron shown in Fig. 5 was mostly related to the target position. The contribution of movement direction was negligible, and beginning from ~200 ms from target onset, the activity was strongly related to the position of the next target (Fig. 5D).

To evaluate the time course of the signals related to movement direction in the population of SMA neurons, the same sliding regression analysis was performed for the entire population of neurons examined in this study. The results show how signals about the initial hand position and movement direction evolve over time in the population activity of SMA neurons (Fig. 6). On average, ~12% of the variance in the spike density function was related to target position before the onset of a new target (Fig. 6A). At 140 ms from target onset, the fraction of variance related to movement direction exceeded 2 SD above the baseline level calculated during the 400-ms interval before target onset. This value reached its peak of 12% at 240 ms from target onset (Fig. 6A). The percentage of neurons that displayed statistically significant effects of hand position and movement direction was modulated similarly. During the 200-ms interval beginning from 100 ms before target onset, the effects of hand position were significant on average in 59.7% of the neurons. The percentage of neurons with significant effects of movement direction gradually increased after target onset and peaked at 50.9% at 200 ms from target onset (Fig. 6B).

**Patterns of nonstationarity in SMA activity**

Although the residuals from the sliding regression model for individual movements displayed large variability (e.g., Figs.
and $E$), examination of these residuals still revealed a substantial level of nonstationarity in the activity of many SMA neurons in the random condition. Nonstationarity was found across different targets in a given trial as well as across multiple trials during a given recording session, and individual neurons displayed diverse patterns in their combinations. For example, in the neuron illustrated in Fig. 4, there was no significant change in activity across trials ($F = 1.29, P = 0.2784$; Fig. 4E), suggesting that the activity of this neuron remained stable throughout the recording session. In contrast, a slight decrease in activity for the targets toward the end of each trial was statistically significant ($F = 3.17, P < 0.05$; Fig. 4F). Some neurons displayed substantial changes in their activity across different trials. For example, the neuron illustrated in Fig. 5 increased its activity across trials ($F = 54.64, P < 0.0001$; Fig. 5E). In this neuron, there was also a systematic change in the activity associated with different targets within a given trial ($F = 5.90, P < 0.005$; Fig. 5F).

Overall, there was a significant change in the residual activity across trials in 71.3% of the SMA neurons examined in this study. The percentage of neurons showing significant change across different targets within a given trial was 37.0%. In this case,
analysis, the regression model for the trial number was a third-order polynomial, whereas a second-order polynomial was used for the target number to prevent overfitting of the data. Nevertheless, the difference in the percentage of neurons for cross- and within-trial nonstationarity was not due to the difference in the models used. The percentage of neurons with significant within-trial nonstationary changed only slightly to 38.9% when a third-order polynomial regression model was used.

**FIG. 5.** Example of another SMA neuron during the movements in the random condition. Same format as in Fig. 4.

**FIG. 6.** Coding of movement parameters in the entire population of SMA neurons sampled in this study. A: the population average for $R^2$ (gray solid line) and sums of squared standardized regression coefficients for starting target position (dashed line), movement direction (black solid line), and final target position (dotted line). B: the percentage of neurons in which the activity was significantly affected by the initial target position or movement direction. This was evaluated for each 20 ms time step in a sliding regression model (see METHODS).
Primed and practice-related activity in SMA neurons

The neuron illustrated in Fig. 7 displayed significant changes in its activity as the primary movement sequence was repeated within a given trial (Fig. 7A) and as the same sequence was repeated across successive trials within the recording session (Fig. 7B). Beginning ~200 ms prior to the onset of the second target in the primary triplet, the activity of this neuron increased as the primary triplet was repeated within each trial (Fig. 7A, left). The mean spike rate during the 400-ms interval starting from 200 ms before target onset was 20.0 spikes/s for the first triplet, whereas it was 27.1 and 26.4 spikes/s for the second and third triplet. The same neuron also displayed substantial changes in its activity as the same movement triplet was repeated throughout the recording session (Fig. 7B). This was particularly noticeable for the movement from the third target to the first target in the triplet (Fig. 7, B, right, and C). The mean spike rate during the 400 ms interval beginning from 200 ms before target onset was 12.9 spikes/s for the first 25 trials in the primary condition, whereas this increased to 20.0 spikes/s during the last 25 trials. This neuron did not display any significant cross-trial nonstationarity (Fig. 4E), and the level of within-trial nonstationarity was significant but small (Fig. 4F). For the same neuron, the regression analysis revealed that the priming effect was statistically significant for the first and the second movement in the primary triplet ($P < 0.0001$ for both movements), and the practice effect was significant for the second and the third movements ($P < 0.05$ and $P < 0.0001$, respectively). Although this neuron displayed a significant practice effect for the third movement ($C \rightarrow A$) in the primary triplet, a close examination of the raster plot (Fig. 7C) suggests that the practice effect became stronger as this movement was repeated withing a given trial, suggesting an interaction between the priming and practice effects. This was quantified with a modified regression model that incorporated an interaction term corresponding to the product of the trial number and target number (Eq. 8). As expected, the regression coefficient for interaction term was statistically significant for the third movement ($P < 0.01$).

For the same neuron, the pattern of activity during the switch trials provided additional evidence for learning-related activity. Because the activity of this neuron increased gradually as the primary triplet was repeated across many trials, one would expect a decrease in its activity when the primary triplet was introduced unexpectedly. The activity of this neuron in the switch trials was consistent with this prediction. The mean spike rate during the 400-ms interval beginning from 200 ms

![FIG. 7. Activity of an example SMA neuron (same neuron shown in Fig. 4) in the primary trials. A: spike density functions averaged according to target numbers. The 3 movements in the primary triplet ($A \rightarrow B \rightarrow C$) are shown in different columns, and line colors indicate the 1st (green), 2nd (blue), and 3rd (black) repetition of each movement. Right: the red line corresponds to the target that switched from the secondary triplet to the primary triplet. B: spike density functions averaged for successive groups of 25 primary trials, and the line colors indicate the trial numbers for the 1st trials in each group. C: the raster plots for the 3rd movement ($C \rightarrow A$) in the primary triplet. Dots denote individual action potentials, and circles reaction times for individual trials. Left, middle, and right: the 1st, 2nd, and 3rd repetition, respectively, of the 3rd movement of the primary triplet within a given trial. Bottom: the activity after the switch during the same movements.]

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before the switch was 11.3 spikes/s (Fig. 7A, right, red line), whereas it was 15.4 spikes/s for the corresponding movements generated in the primary condition.

For the neuron illustrated in Fig. 8, activity in the random condition displayed significant nonstationarity for both target number ($F = 24.9075, P < 0.0001$) and trial number ($F = 6.3622, P < 0.0005$). As a result, similar changes in the activity found in the primary trials could be attributed to such nonstationarity. The spike density functions displayed a systematic increase as the primary triplet was repeated within a given trial (Fig. 9A), but the regression analysis did not find a significant priming effect for any movement in the primary triplet because such an activity increase was attributed to the within-trial nonstationarity. Although the activity of this neuron included a significant cross-trial nonstationarity (Fig. 8C), a significant practice effect was found for the third movement in the primary triplet ($P < 0.0001$) because the pattern of changes in the primary trials was different from the cross-trial nonstationarity. The activity of this neuron before the onset of the third movement in the primary triplet increased gradually throughout the recording session (Figs. 9B, right, and 10). The same changes were observed even when the activity was aligned to movement onset (Fig. 9C), suggesting that they were not entirely due to the systematic changes in the reaction times throughout the recording session. In contrast, the cross-trial nonstationarity found in the random condition displayed a U-shaped pattern (Fig. 8C). This neuron also displayed a significant reduction in its activity following the switch from the secondary to the primary triplets (Figs. 9A, right, red line, and 10, switch trials), suggesting that the gradual activity change in the primary condition was indeed related to the learning of the primary triplet.

![Figure 8](https://example.com/fig8.png)

**Fig. 8.** Effects of movement parameters and nonstationarity on the activity of an SMA neuron. The formats of A–D are the same as those of C–F in Fig. 4.

![Figure 9](https://example.com/fig9.png)

**Fig. 9.** Spike density functions of the same neuron shown in Fig. 8 during the primary trials. The formats of A and B are the same as those in Fig. 7. C: same as B except that the activity is aligned to movement onset.
Although many SMA neurons displayed gradual changes in their activity during the course of the recording session, such changes did not always reflect the learning of a movement sequence. For example, for the neuron illustrated in Fig. 11, the spike density functions for all three movements in the primary triplet displayed a systematic increase with trial number. However, the same change was also found in the random condition (Fig. 5E), suggesting that such change in activity was not related to learning. The results of the regression analysis indicated that this neuron did not display significant practice effect. The priming effect was significant for the first movement in the primary triplet (Fig. 11A).

Population analysis

The above regression analyses were applied to the entire population of neurons recorded in the SMA. For each neuron, the activity associated with each of the three movements in the primary triplet was analyzed separately. From a total of 324 cases (108 neurons × 3 movements), 1 case was excluded because it did not include any spikes during the 400-ms window used in the analysis. The percentages of neurons that showed statistically significant effects for each of the variables included in the sliding regression analysis (Eq. 6) are shown in Fig. 12, along with the proportion of variance in neural activity accounted for by the same variables. For hand position and movement direction, results for the horizontal and vertical components were combined after correcting for multiple comparisons according to the Bonferroni equation. For the 400-ms window used for the analysis of learning-related activity that begins 200 ms before target onset, the average percentage of neurons with significant nonstationarity in the random condition was 34.7 and 49.3% for the within-trial and cross-trial nonstationarity, respectively (Fig. 12, left). For the same temporal window, hand position and movement direction exerted significant changes in neural activity in 50.7 and 23.2% of the neurons, respectively. Finally, reaction time, movement time, and saccadic reaction time affected the activity in 33.2, 35.6, and 23.1% of the neurons, respectively.

Priming and practice effects were evaluated after all the variables related to nonstationarity and behavioral performance were factored out. Nevertheless, a substantial number of neurons displayed significant learning-related effects. The percentage of cases with statistically significant priming and practice effects was 31.9 and 23.2%, respectively (t-test, P < 0.05;
Table 1), and these results were similar to those obtained with a permutation test (29.0 and 20.1%, respectively). For both priming and practice effects, the proportions of neurons that significantly increased their activity with training and those with decreasing activity were statistically indistinguishable (binomial test, \( P > 0.05 \); Table 1). In addition, the magnitude of a priming effect was not related to that of practice effect for the same movement. The correlation coefficient between the standardized regression coefficients for the priming and practice effects was slightly negative (\( r = -0.086 \)) and not significantly different from zero, suggesting that these two types of experience-related changes in activity did not originate from a single factor. To test the possibility that the practice and priming effects might interact, as described for the neuron shown in Fig. 7, a regression model with an interaction term (Eq. 8) was applied to the entire population of SMA neurons. Overall, significant interaction was found in 17% of the SMA neurons (Table 2), suggesting that these two effects were combined in a multiplicative fashion in some SMA neurons.

For the neurons with statistically significant practice effects or priming effects, the corresponding regression coefficients were relatively unaffected by excluding the performance-related variables (RT, MT, and SRT) from the regression model (Fig. 13). Therefore it is not likely that much larger learning-related activity in the SMA neurons was washed away by the performance-related variables. The percentage of cases in which the regression coefficients changed by more than a factor of 2 with the introduction of the performance-related variables in the regression model was 18.4 and 2.7% for those with significant practice and priming effects, respectively. For the priming effects, the regression coefficients were similar even when the entire population was considered (\( r = 0.96 \)). For the practice effects, however, there were some cases in which potentially learning-related effects might have been absorbed by the performance-related variables (Fig. 13, O), and the corresponding regression coefficients in the two regression models were less strongly correlated (\( r = 0.65 \)).

**Table 1.** Percentage of samples with significant priming effect and practice effect (2-tailed t-test, \( P < 0.05 \)), evaluated with the regression model without the interaction term (Eq. 7).

<table>
<thead>
<tr>
<th>Interval</th>
<th>Priming</th>
<th>Practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 ms before target onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>10.2</td>
<td>13.4</td>
</tr>
<tr>
<td>Decrease</td>
<td>9.0</td>
<td>16.1</td>
</tr>
<tr>
<td>Either</td>
<td>19.3</td>
<td>29.5</td>
</tr>
<tr>
<td>200 ms after target onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>9.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Decrease</td>
<td>9.6</td>
<td>15.2</td>
</tr>
<tr>
<td>Either</td>
<td>18.6</td>
<td>29.2</td>
</tr>
<tr>
<td>Combined*</td>
<td>12.1</td>
<td>13.3</td>
</tr>
<tr>
<td>Increase</td>
<td>11.2</td>
<td>18.6</td>
</tr>
<tr>
<td>Decrease</td>
<td>23.2</td>
<td>31.9</td>
</tr>
</tbody>
</table>

Number of samples was 323. *Combined refers to the 400-ms interval beginning 200 ms before target onset.

**Table 2.** Percentage of samples with significant priming effect, practice effect, and interaction between the two (2-tailed t-test, \( P < 0.05 \)).

<table>
<thead>
<tr>
<th>Priming</th>
<th>Practice</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase</td>
<td>9.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Decrease</td>
<td>10.2</td>
<td>11.8</td>
</tr>
<tr>
<td>Either</td>
<td>19.8</td>
<td>23.2</td>
</tr>
</tbody>
</table>

Number of samples was 323. Samples were evaluated with the regression model with the integration term (Eq. 8).
We also examined whether SMA neurons coding the movement-related variables, such as target position and movement direction, tended to display stronger or weaker learning-related activity. To answer this question, a correlation coefficient was calculated between the absolute value of the standardized regression coefficients for the practice (or priming) effect and the maximum value obtained by the sum of the squared standardized regression coefficients for the horizontal and vertical components of the starting target position (or movement direction) in the sliding regression model (Eq. 1). The values of these correlation coefficients were relatively small (Table 3).

The practice effect was not significantly correlated with the coding of initial hand position or movement direction, suggesting that many SMA neurons are involved in the control of individual movements as well as the learning of movement sequences. The only correlation coefficient significantly different from zero was between the movement direction and the priming effect ($r = -0.144$; 2-tailed $t$-test, $P = 0.0386$, corrected for multiple comparisons according to Bonferroni equation).

Although the activity of many SMA neurons was significantly affected by experience with repeated movement sequences, the magnitude of such effects was relatively small (Fig. 13). The median absolute value for the significant practice effect was 0.004 spikes/s/trial, which corresponds to an increase or decrease of 1.6 spikes/s after 400 trials of practice. The median absolute value for the significant priming effect was 0.2 spikes/s/target. This corresponds to a change of 1.8 spikes/s following three repetitions of a primary triplet.

**Effects of unpredicted switching in SMA activity**

The effect of unexpected switch from the secondary to the primary triplet was examined by comparing the mean spike rate during the 400-ms interval starting from 200 ms before the switch to the level of the activity for the same movement in the primary condition. As in the analysis of practice and priming effects, potential confounding effects of variable behavioral performance were factored out using a regression model. In 45.8% of the neurons, the difference in the mean activity during this interval was statistically significant ($P < 0.05$). If this switch effect was related to the learning of the primary triplet, one must be able to predict its size based on the practice effect and priming effect estimated from the primary trials, because both of these effects must be reduced or absent in the switch trials. Using the regression coefficient $h_2$ (Eq. 7) as an estimate of the practice effect, the expected switch effect related to the practice effect would be $h_2 N_{max}/2$, where $N_{max}$ is the number of trials in a given recording session. Similarly, using the regression coefficient $h_1$ as an estimate of the priming effect, the expected switch effect related to the priming effect would be $h_1 N_{max}/2$.

**TABLE 3. Relationship between the coding of kinematic variables and the size of learning-related activity changes**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Position</th>
<th>Movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming</td>
<td>−0.0312</td>
<td>−0.1438*</td>
</tr>
<tr>
<td>Practice</td>
<td>0.0291</td>
<td>0.0720</td>
</tr>
</tbody>
</table>

Sample size was 323. The relationship presented is as indicated by the correlation coefficient between the absolute value of the standardized regression coefficient for the practice (or priming) effect (Eq. 7) and the maximum values obtained by the sum of the standardized regression coefficients for the horizontal and vertical components of the target position (or movement direction; Eq. 1). * $P < 0.05$ (corrected for multiple comparisons).
effect, the expected switch effect related to the priming effect would be $6h_1$ because the switch effect would correspond to the difference in the priming effect expected for the first and seventh target in the primary condition. The correlation coefficient between the sum of these two terms ($6h_1 + h_2N_{\text{max}}/2$) and the actual switch effect was 0.287 (Fig. 14), which was significantly different from zero ($P < 0.005$). The correlation coefficient between the actual switch effect and the predictions based on either the priming effect ($r = 0.180$) or the practice effect ($r = 0.248$) was both positive and significantly different from zero.

Spatial and temporal properties of learning-related activity

The results described so far were based on the activity during the 400-ms interval spanning two successive 200-ms periods before and after target onset. However, it is possible that the pattern of learning-related activity might change during this 400-ms interval. To examine this possibility, the same regression model (Eq. 7) was applied separately for the 200-ms intervals before and after target onset. Overall, the pattern of practice and priming effects remained similar across these two intervals (Fig. 15). The cases in which statistically significant practice (or priming) effects changed its direction across these two intervals were rare (1.6 and 0.9% for the practice and priming effect, respectively). Thus both the practice and priming effects were manifested on a relatively slow time scale.

In some neurons, the directions of learning-related activity changes were different for different movements. For example, in the neuron illustrated in Fig. 7, the practice effect led to a significant increase in the activity before the third movement (C→A) but a decrease before the second movement (B→C). To determine how the practice effect varied across different movements for a given neuron, we counted, for each neuron, the number of movements with significant positive practice effect and those with significant negative practice effect. By assuming that the practice effect for different movements in a given primary triplet is independent, the expected distribution of neurons with different numbers of positive and negative practice effects was calculated, as shown by the numbers in the parentheses in Table 4. For example, if the overall frequency of the positive practice effect is $x$, the expected percentage of neurons showing positive practice effects for all three movements would be $x^3$ when the effects for different movements are independent. The same analysis was performed separately for the priming effect. In both cases, the actual distribution differed significantly from the expected distribution ($x^2$ test, $P < 0.01$). This was mostly due to the fact that there were more neurons with the same sign of significant practice effect and fewer neurons with opposite signs, compared with what was expected under the independence assumption. These results suggest that there is a tendency for SMA neurons to display similar practice (or priming) effect for multiple movements in a given sequence.

**DISCUSSION**

Activity of SMA neurons during sequence learning

Learning of complex skillful movements proceeds in multiple stages, from the initial stage in which individual movements are separately controlled to a later stage in which the entire sequence of movements can be automatically executed (Adams 1971; Fitts and Posner 1967). Similarly, it has been proposed that new and familiar movement sequences are controlled by separate populations of neurons or even by distinct cortical areas (Hikosaka et al. 1999; Nakamura et al. 1998; Sakai et al. 1998; van Mier et al. 1998). In evaluating the results of empirical studies in light of this proposal, it is important to distinguish alternative causes of differential activation of various brain areas associated with the performance of new and familiar movement sequences. One possibility is that apparent specialization for new versus familiar movement sequences is related to additional processing requirements involved in the learning of new movement sequences, such as working memory and error correction. Localization of these various functions in different cortical areas or network of neurons would produce differential activation for new and familiar sequences. Another possibility is that different groups of neurons or cortical regions might be recruited for the control of familiar movement sequences due to the storage of information about movement sequences in specific neuronal populations.

In a previous single-cell recording study performed in the SMA and the pre-SMA (Nakamura et al. 1998), activity patterns of neurons in these cortical regions were compared for new and familiar sequences of movements, and it was found that some neurons in both cortical areas change their activity according to the amount of practice with a particular movement sequence. In that study, however, the animal was required to discover the correct movement sequence by trial and error and memorize it. In addition, the changes in neural activity were accompanied by a substantial performance improvement. As a result, it was not clear whether the observed changes in neural activity were related to the storage and retrieval of information about the sequence itself or whether they reflected working memory processes necessary for error correction during the learning of a new sequence or other factors related to performance changes. In the present study, the animals performed a modified version of the serial reaction time task (Nissen and Bullemer 1987). During this task, information about the next target location was continuously available until the corresponding movement was completed, and therefore the working mem-

**FIG. 14.** Comparison of the observed switch effect (ordinate) and the predicted switch effect (abscissa) estimated from the priming effect and the practice effect in the primary trials.
ory requirement was minimized. The fact that the reaction time difference for repeated and random movement sequences was relatively small suggests that the animals indeed relied on the incoming visual input to initiate hand movements in both conditions. Furthermore, the effects of changes in behavioral performance were factored out in the quantitative analysis of learning-related activity. Changes in the SMA activity found in the present study are, therefore likely to reflect the changes in neural circuitry involved in the processing of information about familiar movement sequences. These changes might in turn contribute to the behavioral improvement manifested in the relative shortening of acquisition time in the primary condition relative to that in the random condition (Fig. 2).

In this study, we have demonstrated that about a third of the neurons in the SMA displayed gradual changes in their activity across different trials when a particular movement sequence was repeatedly performed. This is referred to as a practice effect to distinguish it from the second type of learning-related activity changes occurring within a single trial, which is referred to as a priming effect. Accordingly, the practice and priming effects reflect the activity changes occurring over two different time scales. The extent to which these two different types of learning-related activity change were manifested in individual neurons varied within the population of SMA neurons. We found that there was no systematic relationship between the practice and priming effects across different SMA neurons. In addition, in some neurons, there was a significant statistical interaction between the two, indicating that the magnitude of the priming effect might also depend on the level of practice. These results suggest that the activity of neurons in the SMA was influenced by at least two distinct learning-related factors: short-term priming and long-term storage of information about movement sequence.

Neurophysiological studies aimed at understanding the neural basis of skill learning are often made difficult because the spike trains of individual neurons often display a substantial amount of nonstationarity over time (Bach and Krüger 1986; Bair et al. 2001; Li et al. 1993; Rose 1979). To distinguish learning-related changes in neural activity from other time-dependent variables, such as ongoing fluctuations in the neuronal excitability or disturbances caused by the electrode advancement, appropriate control conditions must be included. In the present study, the neural activity during movements toward randomly selected target locations was examined concurrently, and the nonstationarity found in this condition was factored out from the estimates of learning-related effects. This approach is conservative, since some of the activity changes seen in the random condition might also reflect genuine learning-related phenomenon.

Performance- versus learning-related activity in the SMA

When new motor skills are acquired, learning can be quantified by the improvement in performance. Performance improvement is not, however, necessary for learning. For example, certain aspects of movement sequences can be learned merely by observation (Howard et al. 1992), and perceptual training with a specific temporal interval can later contribute to better performance in timing behavior (Meegan et al. 2000). Learning of movement sequences can also occur without performance improvement if the subjects are distracted by a concurrent task (Seidler et al. 2002). Because learning and performance improvement are closely related but separate processes, it is important to distinguish them in studying the neural basis of sequence learning. In the present study, changes in neural activity related to performance-related parameters, such as the reaction time and movement time, were removed from the estimates of learning-related activity. Therefore it is possible that some of the learning-related activity was disregarded as changes in activity related to behavioral performance. To examine this possibility, the analysis of learning-related activ-

TABLE 4. Percentage of neurons (N = 107) classified according to the number of movements for which practice (priming) effects were significantly positive (Increase) and the number of movements for which these effects were significantly negative (Decrease)

<table>
<thead>
<tr>
<th>Increase</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Practice effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38.3 (31.3)</td>
<td>14.0 (18.5)</td>
<td>7.5 (3.7)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>1</td>
<td>18.7 (25.9)</td>
<td>3.7 (10.2)</td>
<td>1.9 (1.0)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>12.7 (7.1)</td>
<td>0.9 (1.4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>1.9 (0.7)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Priming effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>52.3 (45.0)</td>
<td>9.4 (21.4)</td>
<td>9.4 (3.3)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>1</td>
<td>17.8 (19.8)</td>
<td>4.7 (6.3)</td>
<td>0.0 (0.5)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>4.7 (2.9)</td>
<td>0.9 (0.5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>0.0 (0.1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The numbers in the parentheses indicate the percentage expected when the practice (or priming) effects for different movements are independent.
ity was repeated without factoring out the effects of performance variability. This altered the estimates of learning-related activity in some cases. However, for many SMA neurons examined in this study, the estimates of learning-related activity were relatively unaffected, suggesting that they play a role in sequence learning independent of their relationship with performance changes.

The results from the present study also suggest that many SMA neurons are involved in the control of individual movements as well as the learning of movement sequences. If separate populations of neurons exist in the SMA for random (new) and repeated (familiar) movement sequences, the neurons that are strongly related to movement-related parameters in the random condition would display little or no learning-related activity. This would lead to negative correlation between the magnitude of learning-related activity and the coding of movement parameters, such as initial hand position and movement direction. We found no significant relationship between the coding of movement parameters and the practice effect. There was a weak but significant negative correlation between the coding of movement direction and the priming effect, indicating that neurons with a robust tuning for movement direction were less likely to display the priming effect.

**Comparison to previous studies**

Previous lesion and imaging studies have suggested that the SMA plays a role in the retrieval and execution of previously learned movement sequences (Doyon et al. 2002; Goldberg 1985; Grafton et al. 1995, 1998; Hazeltine et al. 1997; Jenkins et al. 1994; van Mier et al. 1998). However, lesion studies tend to focus disproportionately on the functional deficit resulting from a particular lesion rather than on functions that might be normally supported by a given cortical area but spared by the lesion due to redundancy or recovery. Similarly, imaging methods tend to focus on the difference in the level of activation and, due to its limited resolution, cannot detect the functional reorganization occurring within a particular brain area. For example, the results from a previous study (Nakamura et al. 1998) as well as those from the present study showed that the number of neurons increasing their activity with practice was similar to that of neurons with decreasing activity. Therefore experience might alter the pattern of neural activity in the SMA without altering the overall level of activity within the population of SMA neurons. These results raise the possibility that the role of the SMA in learning of movement sequences might have been underestimated in some imaging studies (Rauch et al. 1995, 1997; Sakai et al. 1998, 2002; Willingham et al. 2002) because metabolic measures utilized in such studies reflect the aggregate activity within each cortical region.

A number of single-unit recording studies have examined the pattern of neuronal activity in the SMA. These studies have found that some neurons display a dramatic increase in their activity only for a particular movement sequence (Nakamura et al. 1998; Shima and Tanji 2000; Tanji and Shima 1994). In contrast, learning-related activity changes found in the present study were more modest in their magnitude and did not alter the main characteristics of the activity patterns associated with the production of individual movements. This is likely due to the differences in the behavioral paradigms. In previous studies of sequence learning in the SMA, the animals were required to memorize a given movement sequence explicitly, whereas in the present study, the individual movements were always instructed by visual stimuli. In addition, the present study focused mostly on the initial changes in neural activity after the introduction of a new movement sequence. The results from the present study suggest that repeated performance of a given movement sequence brings incremental changes to the cortical network involved in the control of complex movement patterns. However, it should be also noted that the amount of practice in the present study was limited by the duration of a daily recording session. It is possible and remains to be determined whether learning-related activity changes described here ultimately lead to strong sequence-specific activity found in earlier studies (Shima and Tanji 2000; Tanji and Shima 1994).

**Neural network involved in the learning of a motor sequence**

When human subjects produce a particular sequence of movements repeatedly, the metabolic activity changes in various brain regions. Although the present study focused on the activity of individual neurons in the SMA, previous imaging studies have reported experience-dependent changes in the level of activation in various cortical and subcortical areas, including the prefrontal cortex (Grafton et al. 1995; Hazeltine et al. 1997; Jenkins et al. 1994; Sakai et al. 1998, 2002; Willingham et al. 2002), the premotor cortex (Grafton et al. 1995; Sakai et al. 1998; Willingham et al. 2002), the basal ganglia (Grafton et al. 1995; Hazeltine et al. 1997; Jueptner et al. 1997a, 1997b; Rauch et al. 1995; van Mier et al. 1998), the primary motor cortex (Grafton et al. 1995; Hazeltine et al. 1997; Karni et al. 1995), the posterior parietal cortex (Jenkins et al. 1994; Sakai et al. 1998; Willingham et al. 2002), and the cerebellum (Doyon et al. 2002; Jenkins et al. 1994; Jueptner et al. 1997a, 1997b; Rauch et al. 1997; Seitz and Roland 1992; Willingham et al. 2002), and the cerebellum (Doyon et al. 2002; Jenkins et al. 1994; Jueptner et al. 1997a; Seidler et al. 2002; van Mier et al. 1998; Willingham et al. 2002). These results suggest that both the cortical and subcortical processes involved in the preparation and execution of movement sequences are concurrently influenced by experience. For example, it has been proposed that the interactions between the cortex (e.g., SMA) and the basal ganglia may be important for the late stage of sequence learning in which the successive individual movements are prepared in parallel (Hikosaka et al. 1999). The cerebellum may be involved in this process through its role in coordinating the timing of multiple movements (Braitenberg 1967; Ivry et al. 1988). However, there is also a substantial variability across different imaging studies as to whether and how the activation level of a particular brain area changes with experience. For example, the activation in the SMA has been found in some (Doyon et al. 2002; Grafton et al. 1998; Jenkins et al. 1994), but not all (Rauch et al. 1997; Sakai et al. 1998, 2002; Willingham et al. 2002), imaging studies. Although some of this variability might be related to the differences in the behavioral paradigms, the results were not consistent even among the studies that used the serial reaction time task (Doyon et al. 2002; Grafton et al. 1998; Rauch et al. 1997; Sakai et al. 2002; Willingham et al. 2002). Similar variability was also found for the pattern of activation in the dorsolateral prefrontal cortex. Activation of the dorsolateral prefrontal cortex might be related to explicit knowledge of the movement sequence, and therefore the time course of its activation might...
be influenced by when such knowledge becomes available during the course of training (Sakai et al. 2002). Understanding the factors responsible for the pattern of activation in each of these different areas remains an important task for the future imaging studies of sequence learning. Similar to the results of imaging studies, single-unit recording studies have found the neural activity associated with specific movement sequences in various cortical and subcortical areas (Barone and Joseph 1989; Kermadi and Joseph 1995; Mushiake and Strick 1995; Shima and Tanji 2000; Tanji and Shima 1994). All of these studies were performed with the behavioral paradigms in which the animals were required to memorize various movement sequences explicitly, and therefore the functions of various brain areas at different stages of sequence learning remain poorly understood. A serial reaction time task used in this study might provide an advantage because it focuses on the incremental changes in behavioral performance without evoking additional processes, such as working memory. Further studies will be also needed to understand how information flow among various cortical and subcortical areas is modified by experience.

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