Skill Representation in the Primary Motor Cortex After Long-Term Practice

Yoshiya Matsuzaka, Nathalie Picard, and Peter L. Strick. Skilled representation in the primary motor cortex after long-term practice. J Neurophysiol 97: 1819–1832, 2007. First published December 20, 2006; doi:10.1152/jn.00784.2006. The acquisition of motor skills can lead to profound changes in the functional organization of the primary motor cortex (M1). For example, performance of movement sequences after prolonged practice is associated with an expansion of the effector representation in M1. Paradoxically, there is little evidence that the activity of M1 neurons reflects acquired skills, especially sequences of movements. We examined the activity of M1 neurons during skilled movement sequences in macaques trained to successively hit targets on a monitor. The targets appeared either pseudorandomly (Random mode) or in one of two repeating sequences (Repeating mode). With practice, response times for repeating sequences substantially declined and the monkeys performed the task predictively. Highly trained animals retained the acquired skill after long gaps in practice. After >2 yr of training, 40% of M1 neurons were differentially active during the two tasks modes. Variations in movement kinematics did not fully explain the task-dependent modulation of neuron activity. Differentially active neurons were more strongly influenced by task mode than by kinematics. Our results suggest that practice sculpts the response properties of M1 neurons. M1 may be a site of storage for the internal representation of skilled sequential movements.

INTRODUCTION

The ability to link elementary actions together to perform a meaningful sequence of movements is a key component of voluntary motor behavior. The classical view of the cortical control of sequential movements is that the premotor areas are critical for learning and storing the representations of movement sequences (Campbell 1905; Fulton 1935; Jacobsen 1934). In contrast, the primary motor cortex (M1) is thought to simply produce the patterns of muscle activity necessary to implement the plans generated by the premotor areas. However, results from a number of recent studies suggest that M1 plays a more active role in both the acquisition and retention of motor skills including sequential movements (Hund-Georgiadis and von Cramon 1999; Karni et al. 1995, 1998; Kleim et al. 1998; Pascual-Leone et al. 1994, 1995; Plautz et al. 2000; Remple et al. 2001; Sanes and Donoghue 2000; Tyč et al. 2005). For example, Karni et al. (1995, 1998) reported that practice on a sequence of finger movements resulted in large and lasting increases in the extent of M1 activation. Similar training also enlarged the map of cortical output that could be demonstrated using transcranial magnetic stimulation (Pascual-Leone et al. 1994, 1995). The map expansion was specific to the muscles used during training. These observations suggest that learning a sequence of movements alters the excitability and functional organization of M1. Furthermore, they imply that M1 is a site of storage for the long-term memory of motor skills acquired through practice (Karni et al. 1995).

Three additional lines of evidence provide further support for a direct role of M1 in the acquisition and maintenance of motor skills. First, correlates of motor learning are present in the patterns of activity of M1 neurons when animals perform arm movements in novel dynamic or kinematic environments (Li et al. 2001; Paz et al. 2003). Second, local changes in synaptic efficacy (e.g., long-term potentiation) occur locally within M1 and accompany motor learning (Asanuma 1991; Iriki et al. 1991; Monfils and Teskey 2004; Rioul-Pedotti et al. 1998, 2000; Stefan et al. 2006; Ziemann et al. 2004). Third, the activity of some M1 neurons encodes more than just patterns of muscle activity (e.g., Kakei et al. 1999). Indeed, a surprising number of M1 neurons (40–53%) encode abstract information such as the serial order of potential target stimuli (Carpenter et al. 1999) or display anticipatory activity related to upcoming movement sequences (Lu and Ashe 2005).

On the other hand, single-neuron recording studies in monkeys have found little evidence that M1 makes a special contribution to the actual performance of sequential movements, beyond its traditional role in movement execution (Ashe et al. 1993; Ben-Shaul et al. 2004; Lu and Ashe 2005; Mushiake et al. 1990, 1991). Thus, there is a disparity between the results of human studies that point to the involvement of M1 in the execution of sequential movements and monkey recording studies that do not. Given this disparity, we reexamined the activity of M1 neurons during the performance of sequential movements that were highly practiced.

We first characterized the behavior of four monkeys as they practiced two types of sequential movements: one type guided by randomly presented visual stimuli and the other guided by memory from practice with repeating sequences. We documented the time course of skill acquisition and the level of performance attained by these animals as they practiced for >1 year. Then, in two of the trained monkeys, we compared the activity of M1 neurons during performance of the two types of sequential movements. We observed that a substantial number of M1 neurons displayed different patterns of activity during the random and repeating sequences. Indeed, some of the neurons were exclusively active during the performance of the repeating sequences. Our results suggest that, with practice, aspects of learned sequences come to be represented in the activity of M1 neurons. Short reports of this work were previously presented in abstract form (Matsuzaka et al. 1998, 2000).

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METHODS

Subjects and task

Four monkeys (Macaca mulatta, 4–7 kg) were part of the behavioral study of sequence learning. Two of them were then used for single-neuron recording in M1. The remaining two animals were used for other experiments not described here. The care of the animals and the experimental protocols used adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the US Public Health Service Policy on Humane Care and Use of Laboratory Animals. All procedures used were approved by the relevant institutional committees.

The monkeys were trained to make sequential reaching movements to visual targets with their right hand. They faced a touch-sensitive monitor that displayed the outlines of five targets (Fig. 1A). The targets were arranged in a horizontal row and identified as numbers 1 to 5 from left to right. The targets measured 48 × 48 mm and were separated by 10 mm. On each trial, one of the targets was filled with yellow. We trained the monkeys to contact the filled target within 800 ms of its coloring. The yellow fill disappeared immediately on monitor contact. A new target was filled shortly after a correct response. Correct hits were signaled by a 1-kHz tone. Wrong hits were signaled by a 50-Hz tone. In the case of errors or no response, the trial was repeated. The task cycle of target display, contact, and new target display repeated continuously for 3,000 to 15,000 trials a day. Monkeys were initially rewarded with a drop of juice for each correct target hit. After the monkeys understood the task, the reward rate was gradually decreased to once every fourth correct hit.

The monkeys performed the task in two modes: Random and Repeating. The same reward rate was used for the two modes. The monkeys were first trained only on the Random mode of the task. In this mode (Fig. 1B, top), new targets were filled according to a pseudorandom sequence with the constraint that the same target was not filled on successive trials. A 100-ms delay separated a response and the fill of the next target. With a delay of 100 ms, the animals had no time to initiate unguided responses and all responses were visually cued. The monkeys were not required to hold the target until the next target was presented in the Random (or the Repeating) mode trials. Response time (RT), defined as the time interval between successive target hits minus the delay, was the primary measure of performance. RT was measured with a 5-ms resolution.

When each animal’s performance on the Random mode reached an asymptote, we introduced the Repeating mode of the task. In this mode (Fig. 1B, bottom), targets were filled according to a predetermined repeating sequence. To promote the occurrence of predictive responses, defined as RT <150 ms, we delayed the fill of the next target after a correct response by 400 ms. Monkeys were permitted to make responses during the 400-ms delay, leading to negative RT values. When the monkeys made a correct response during this delay, the target was not filled and the task was incremented to the next target in the sequence. Thus a fully trained animal could (and did) perform the correct sequence in the Repeating mode without visual cues to guide their movements.

At the start of each daily training session, the monkeys performed roughly 500 trials of the Random mode. Then, they were switched to ≥500 trials of the Repeating mode. Blocks of 500–1,000 trials in the Random and Repeating modes continued to alternate for the duration of the training session. The first repeating sequence the monkeys learned consisted of targets 5–3–1. The monkeys were presented with the second repeating sequence (2–3–4) after 20–40 practice sessions on the first sequence. From then on, the two sequences alternated during a training session (e.g., Random, Repeating 5–3–1, Random, Repeating 2–3–4, Random, etc.). The monkeys were trained on both the Random and Repeating modes of the task for ≥1 to >2 yr. Thus the animals were clearly overtrained on both task modes.

To separate activity related to arm movements from that related to eye movements or attentional modulation, we trained the animals on a Watch task before neuron recording. A change in background color of the touch-sensitive monitor indicated the transition to this task. The task used the same target array as described above (Fig. 1A). However, during the Watch task targets were filled with yellow or red in a pseudorandom order at fixed intervals of 500 ms. The monkeys were required to keep their hand on a touch pad in front of them and to reach to contact only the yellow targets, not the red ones. On each trial, the probability of a
yellow target was 0.2 and a yellow target was displayed at least once every five target presentations.

**Electrophysiological recordings**

In two of the monkeys (GE and FN), we used conventional techniques to record the activity of single neurons. Recordings started when the monkeys were highly skilled on the two modes of the task (about 2 yr after the start of training). We implanted a head holder and a recording chamber over the left M1. All surgical procedures were performed using aseptic techniques. Anesthesia was induced with ketamine (30 mg/kg, administered intramuscularly) and maintained to surgical levels with 3% isoflurane and 1% N2O inhalation. After the monkeys recovered from the surgery, we used glass-coated Elgiloy electrodes to record the activity of single neurons in M1 (sampled at 1 kHz). We identified the arm area of M1 using intracortical microstimulation (ICMS; 12 pulses of 0.2-ms duration at 333 Hz, <50 μA). We also examined the somatosensory receptive fields of neurons using light tactile stimulation, tapping on muscle bellies, and joint manipulation.

The activity of neurons was usually sampled first during a block of trials (n > 200) in the Random mode, followed by blocks of trials in the Repeating mode for each sequence. Short blocks of the Watch task (roughly 100 trials) were inserted at the transitions between the other blocks of trials. For 82% of the neurons we recorded a second block of Random trials. For these neurons, we compared the activity in the two Random blocks for each move and, if similar (t-test, P < 0.01), trials from the two blocks in the Random mode were pooled for further analysis. On the other hand, data that differed during the two Random blocks were excluded from further analysis.

We recorded the electromyographic (EMG) activity of 22 axial, proximal, and distal forelimb muscles by inserting two single-stranded Teflon-coated, stainless wires percutaneously into a muscle. EMG was amplified, low-pass filtered (300 Hz), full-wave rectified, and transmitted (300 Hz, <50 μA).

**Analysis**

**Sampling.** We examined the activity of “task-related” neurons, which are single units that display phasic increases or decreases in discharge rate temporally coupled to the performance of one or more movements of the task. Most (234/256) of the single units we recorded in the arm representation of M1 fit this description. We excluded neurons that showed little modulation (<2 spikes on average in a 200-ms sampling period) or inconsistent modulation during the task.

We compared the activity of task-related neurons and arm muscles during the Random and Repeating modes of the task. For the Random mode, we examined only those trials with a move contained in a Repeating mode sequence (i.e., 5–3, 3–1, 1–5; and 2–3, 3–4, 4–2). Error trials (wrong hit or no response) were excluded from the analysis. Trials that immediately followed an error trial were also excluded because they were a repetition of the previous trial. Consequently, target location was always predictable on these trials.

For trials during the Random and Repeating modes, we measured the average activity in a 200-ms interval centered on a target hit or a target release. We selected hit or release based on which event gave the best timing and modulation in response averages. This event was target release for 69% of the task-related neurons (and 76% of the muscles) and target hit for 31% of task-related neurons (and 24% of the muscles).

**Measurement of task modulation.** To quantify task-dependent changes of activity, we used the mean activity in the analysis interval to calculate a modulation index (MI = [Repeating − Random]/[Random + Repeating]). Because the movements during a mode of the task differed in location, direction, and amplitude we calculated the MI separately for each move. Thus in principle six comparisons were possible for each neuron and muscle sampled. However, some comparisons were excluded from analysis for the following reasons: 1) low neural number (i.e., fewer than four neurons were recorded in either the Random or the Repeating mode); 2) low activity for both modes (i.e., mean discharge rate <10 Hz for neurons and <2% of the analog range for muscles, during both the Random and Repeating modes); and 3) large trial-to-trial variability during the analysis interval (i.e., coefficient of variation >80% for the greatest mean activity in Random or Repeating modes).

**Determination of differential neuron activity.** To be designated as differentially active, we required a task-related neuron to meet three criteria:

1. MI greater than that of arm muscles.
2. We established the range of MIs for proximal arm and axial muscles from EMG recordings made during multiple sessions in both animals. Only neurons with MI outside of this range for one or more of the moves were considered differentially active. This criterion dissociated the effect of task mode on neuron activity from variations in motor output based on measures of EMG activity.
3. Activity in one mode of the task that was significantly different from that in the other mode. We compared the mean neuron activity during equivalent moves of Random and Repeating modes using a t-test. All neurons considered to be differentially active had statistically different activity during the Repeating and Random modes (P < 0.01, two-tail). Note that many neurons that also showed statistically different activity during the two modes were not considered differentially active because their MI was within the range of MIs for muscles.

**Mode-dependent activity that cannot be explained solely by modulation associated with kinematic variables.** We used an analysis of variance (ANOVA) to dissociate the effect of task mode from variations in motor output based on measures of movement kinematics during the task. For comparison, we performed a similar analysis on EMG activity. In both cases, we pooled all Random and Repeating trials for the six moves examined. For every trial, we measured four kinematic variables: movement direction, amplitude, speed, and target hold time. Movement direction had only two values: left or right. Movement amplitude was taken as the center-to-center distance between targets of a move. Movement speed was calculated as movement amplitude/movement time; the time used was the interval between the release of the touch screen and the next contact. Target hold time (THT) was the interval between the time of target hit and the time of target release. Although not a kinematic variable per se, THT was included in our analyses because it is the performance measure that showed the largest and most consistent variation between the two task modes (see RESULTS). THT is closely related to reaction time in our task. However, reaction time cannot be measured in the Repeating mode because the monkeys anticipated target presentation—THT can be viewed as an inverse correlate of preparatory processes.

We first determined the effect of task kinematics on the activity of all task-related neurons using a multiple linear regression analysis (General Linear Model procedure; Systat, Richmond, CA). The activity of M1 neurons can precede changes in kinematics and generate movement. The activity can also follow changes in kinematics and reflect somatosensory input during movement. As a consequence, we performed the multiple regression analysis using the kinematic variables of the move that followed the sampling interval of M1 activity and the kinematic variables of the move that was concurrent with the interval (THT was invariant). Next, we evaluated the effects of task
mode and mode × move interaction as factors in an ANCOVA with all seven kinematic variables measured as covariates (current direction, current amplitude, current speed, target hold time, next direction, next amplitude, and next speed). Because differences in activity between the Random and Repeating modes frequently occurred just for certain moves (see RESULTS), it was necessary to test for the significance of task mode as a main effect or as the interaction term with move. The ANCOVA tests for the significance of task mode on measures of activity adjusted for the influence of kinematics (Snedecor and Cochran 1980). The ANCOVA was computed only for cells that met the two preceding criteria for determination of differential activity. In these selected data, the significance of effects naturally split into two groups. All cases tested were either clearly nonsignificant (P > 0.01) or highly significant (P < 0.005). The same tendency was also seen in the results of the multiple regression analysis. For uniformity, we adopted a single significance threshold (P < 0.005) for all tests and variables. We performed the same analysis of the activity of arm muscles for comparison. We used the results of the ANCOVA to identify neurons that displayed differential activity that was not exclusively attributed to variations in movement kinematics between the two task modes.

RELATIVE CONTRIBUTIONS OF TASK MODE AND KINEMATICS. The majority (96%) of differentially active neurons also had a significant effect of at least one kinematic variable (ANCOVA). We compared the relative contributions of task mode and kinematics to the modulation of activity separately for each move showing differential activity using a multiple regression analysis (discharge rate = constant + mode + current speed + target hold time + next direction + next amplitude + next speed). Because this analysis was made separately for each move, direction and amplitude on the current trial did not vary between the Random and Repeating modes and these variables therefore could not be included in the model. The absolute standardized regression coefficients (beta weights) and the contribution of each variable to the coefficient of multiple determination (R²) were compared for neurons and for arm muscles analyzed in a similar fashion. Nonparametric tests were used for these comparisons because of the nonnormality of these data (Shapiro–Wilk test, P < 0.001).

Histology

After behavioral and recording experiments were completed, each animal was used in a terminal study using the 2-deoxy-glucose method (2DG) (for technical details see Picard and Strick 2003). At the end of the 2DG experiment each animal was deeply anesthetized (Nembutal, 40 mg/kg, administered intravenously) and perfused through the heart with buffered saline and fixatives. The brain was extracted and sectioned in a cryostat at 30 μm. Results of the 2DG experiments will be presented in a later report. Every tenth section was stained with cresyl violet for examination of cytoarchitecture. In one monkey (GE), small electrolytic lesions were made in the white matter of M1 5 days before sacrifice to verify the location of recording sites.

RESULTS

Sequence learning

Each monkey was initially trained on the Random mode of the task in which the targets for reaching movements were presented in a pseudorandom sequence (Fig. 1B, top; for details see METHODS). The monkeys’ response times (RTs) on this mode of the task improved with practice (Fig. 2). In the first six training sessions, RTs for the Random mode decreased to 77% of their maximum value, on average. Performance in the Random mode continued to improve gradually until it reached an asymptote after 25–40 training sessions. Clearly, at this time the animals knew the task and performed it at high speed.

Next, we introduced the animals to the Repeating mode of the task in which the targets for reaching movements were presented in a fixed, three-element repeating sequence (Fig. 1B, bottom). After a period of training on the first sequence, we introduced a second repeating sequence. Examples of the learning curves from two of the monkeys are presented in Fig. 2. All four monkeys appeared to learn the task at nearly the same rate. Each monkey displayed a rapid improvement in performance on the Repeating mode within the first training session. The median RT for Repeating trials in the first session was on average 80% (range 60–90%) that of Random trials (Fig. 2). With additional practice, RTs in the Repeating mode continued to shorten. The decline of RT was accompanied by a rapid increase in the percentage of predictive responses (RT...
After a median of 22 wk (range: 6–27) of practice, the monkeys made >80% of their responses without visual cues (negative RTs). With further training over a prolonged period of time, RTs continued to decline gradually. After a median of 22 wk (range: 6–27) of practice, the monkeys made >90% of their responses without visual cues.

The animals appeared to learn the second sequence in the same manner as the first. One monkey appeared to learn the second sequence at a somewhat faster rate (Fig. 2, A and B), but not the others (e.g., Fig. 2, C and D). We saw no evidence of interference between the two sequences. Thus overall, the learning of the two sequences was similar.

We tested the retention of the learned sequences early in training (four monkeys, two sequences per monkey) and after extensive practice (two monkeys, one sequence per monkey). For the “early” test, training on the 5–3–1 sequence of the Repeating mode was interrupted for 3–4 wk (23–29 days), while the animals continued to practice the second sequence (2–3–4) and the Random mode of the task. The “interrupted” sequence is expected to occur by chance 15–23 times for 1,000–1,500 Random trials in a typical training session before a retention test. In contrast, the sequence occurs 333–500 times in the same amount of trials in the Repeating mode. Thus the amount of practice on a sequence arising from its chance occurrence in the Random mode is negligible compared with the normal amount of practice during the Repeating mode that is withheld for the retention test.

The early retention test occurred an average of 99 days after the first sequence (5–3–1) was introduced. On the first day after the interruption, the monkeys made significantly fewer predictive responses and more errors (Table 1). However, these RTs were still well below those of the Random mode (average 74% of Random RT). Except for one animal (monkey GI), the performance of the monkeys returned to near their previous level within the first session after the interruption (Fig. 3A). Thus although the interruption in practice resulted in a transient decline in performance, a modest amount of retraining enabled the animals to return to their prior skill level.

We also performed an “early” test of retention of the second sequence (2–3–4) while monkeys continued to practice the first sequence and the Random mode of the task. This test occurred an average of 123 days after the 2–3–4 sequence was first introduced. The interruption in practice on sequence 2–3–4 caused a decrement in its performance on the first day after the interruption. However, the decrement was somewhat less than that observed after the gap in practice on the first sequence (Table 1). For monkey GE, another interruption of sequence 2–3–4 (27 days in length) occurred after five additional practice sessions (Fig. 2B). The effect of the interruption was again seen as an increase in RT and an increase in the number of errors in the first block of trials during the session after the interruption (data not shown). Thus a small amount of additional practice had little effect on retention of the sequence at this stage of training. Results of these retention tests also indicate that the small amount of practice afforded by performance in the Random mode is insufficient to sustain the level of skill acquired by extensive practice in the Repeating mode.

Two animals were trained long enough to perform a second retention test (monkeys GE and FN). The “late” test occurred after nearly 200 additional sessions of practice (10–11 mo later). As with the early test, training on sequence 5–3–1 was interrupted while the animals continued to practice sequence 2–3–4 and the Random mode of the task. Although the interruption lasted 35 or 37 days, it had no detrimental effect.

<table>
<thead>
<tr>
<th>Sequence 5–3–1</th>
<th>Sequence 2–3–4</th>
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<tr>
<td><strong>Monkey</strong></td>
<td><strong>Random Post</strong></td>
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<tr>
<td><strong>Response time</strong></td>
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<tr>
<td>MA</td>
<td>−41.1 ± 99.1</td>
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<tr>
<td>GE</td>
<td>−1.6 ± 110.1</td>
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<tr>
<td>FN</td>
<td>−39.8 ± 119.8</td>
</tr>
<tr>
<td>GI</td>
<td>−153.0 ± 101.9</td>
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<td>Percentage predictive responses</td>
<td></td>
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<tr>
<td>Monkey</td>
<td></td>
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<tr>
<td>MA</td>
<td>95.0</td>
</tr>
<tr>
<td>GE</td>
<td>93.0</td>
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<tr>
<td>FN</td>
<td>93.5</td>
</tr>
<tr>
<td>GI</td>
<td>98.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>94.9 ± 2.2</td>
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<tr>
<td>Percentage errors</td>
<td></td>
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<tr>
<td>Monkey</td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>7.8</td>
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<tr>
<td>GE</td>
<td>4.1</td>
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<tr>
<td>FN</td>
<td>2.6</td>
</tr>
<tr>
<td>GI</td>
<td>7.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.6 ± 2.6</td>
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Response time values are mean ± SD (ms). Errors are responses where the monkey contacted the wrong target or the background of the touch screen outside of a target area. Pre measures are from the last block of trials before the interruption; Post measures are from the first block of trials after the interruption. *, Pre vs. Post P < 0.001 (t-test, two-tail). †, Pre vs. Post P < 0.01 (paired t-test, two-tail). ‡, Pre vs. Post P < 0.05 (paired t-test, one-tail).
on either animal's performance (Fig. 3B) (Table 2). Indeed, the average RT for the 5–3–1 sequence during the first block in the session immediately after the interruption was faster than that observed immediately before the interruption in one animal (Table 2). Thus prolonged practice on the learned sequences improved not only task performance, but also skill retention.

**Movement kinematics and EMG**

We quantified aspects of movement kinematics after extensive training in the two animals used for neuron recording. We pooled performance data from all recording sessions for this analysis. As a consequence of training, the monkeys’ RTs for the Repeating mode were substantially shorter than those for the Random mode (Fig. 2). Two factors contributed to the shorter RTs in the Repeating mode. The first and most important factor was that of differences in target hold time between the two modes (Table 3). Target hold time was substantially and consistently shorter in the Repeating mode (mean 151.6 ± 21.7 ms) than in the Random mode (337.3 ± 76.3 ms). In the Repeating mode, it was not necessary for the animals to wait for a cue to correctly perform the task. Thus the monkeys paused briefly on a target before moving to the next one. Conversely, in the Random mode the monkeys waited for the display of the next target and maintained contact with the screen until 237 ms (on average) after a new target was presented. Thus the ability to predict the location of the next target in the Repeating mode and, as a consequence, the absence of a reaction time account for the major portion of the RT difference between the two modes of the task.

The second factor that contributed, in some instances, to the RT differences was movement speed during the two modes. Movements in the Repeating mode were generally performed faster than those in the Random mode, but not always (Table 4). Monkey GE moved 3–23% faster in the Repeating mode than in the Random mode (Table 4). In this monkey, movement times were 7.3 to 48.7 ms shorter in the Repeating mode. However, monkey FN performed four of six movements at a slower speed in the Repeating mode than in the Random mode (Table 4). Movement times for moves 5–3, 3–1, 1–5, and 4–2 were between 6.9 and 46.5 ms longer in the Repeating mode than in the Random mode. On the other hand, movement times for moves 2–3 and 3–4 were 5.3 and 18.2 ms longer in the Random mode than in the Repeating mode. Thus differences in movement speed made a modest but variable contribution to the shorter RTs during the Repeating mode.

We calculated the MI of EMG activity separately for each move, for each arm muscle and recording session, and for each monkey (see METHODS for details). This resulted in 179 MIs from proximal and axial muscles taken in 35 separate recordins (Fig. 4, bottom). The average MI in this sample was 0.04 ± 0.13 (mean ± SD). This indicates that muscle activity was essentially similar for the two modes of the task. The distribution of muscle MIs had a relatively low kurtosis.
proximal arm representation of M1 in an area 6 mm² apart in the anterior–posterior axis and 1 mm apart in the mediolateral axis. Most penetrations were located on the pre-central gyrus. We recorded 234 task-related neurons from the proximal arm representation of M1 in an area 6 × 8 or 8 × 6 mm². We defined the proximal arm representation as the region where ICMS evoked elbow or shoulder movements at threshold intensity (<50 μA) and neurons responded to manipulation of muscles and/or joints associated with the shoulder, upper arm, or elbow (e.g., Kwan et al. 1978; Park et al. 2001). The proximal arm representation defined in this way was essentially separate from the distal hand representation. ICMS in some penetrations (14%) within the proximal arm representation evoked response from distal muscles. However, these effects were evoked at different depths along a penetration. Mixed effects were observed at sites near the border of the distal arm representation.

As a control, we examined the activity of task-related neurons during the Watch task (see METHODS). None of these neurons showed activity consistently related to target presentation alone. Because the results from the two monkeys were similar in all respects, they were pooled for simplicity of presentation.

We calculated the MIs for each neuron and for each move separately. This resulted in a total sample of 945 MIs from all task-related cells (Fig. 4). The average MI in this sample was −0.014 ± 0.30. The population of MIs displayed a wide range and SD and a significant kurtosis (1.94, SE = 0.195). Values of these variables exceeded those of the MIs for muscles. These results suggest that many neurons in M1 display a larger task modulation than that of muscles.

Surprisingly, 40% (94/234) of the task-related neurons were differentially active during the Repeating and Random modes (Table 5). We considered a neuron to be differentially active and Repeating enhanced, if it had MI > 0.24 (i.e., more than that of any arm muscle) or differentially active and Repeating depressed if it had an MI < −0.43 (i.e., less than that of any arm muscle). To be considered differentially active a neuron also had to display a highly significant (P < 0.005) effect of mode or mode × move interaction determined by the ANCOVA (see METHODS and below). Differentially active neurons were distributed uniformly throughout the proximal arm representation.

The largest group of differentially active neurons (roughly 27% of total sample) had enhanced activity for the Repeating mode of the task. For example, the neuron illustrated in Fig. 5 displayed phasic activity during both the Random and the Repeating modes, although this activity was considerably enhanced for five of the six movements when made in the Repeating mode (MI 0.5–0.78). Another group of neurons (12% of the total sample) had depressed activity during the Repeating mode of the task. For example, the neuron illustrated in Fig. 6 was active during all moves but showed task-dependent modulation of its activity for only one move of sequence 2–3–4 (MI −0.48). A small fraction of the sample (roughly 2%) had mixed properties. The activity of these neurons was enhanced during the Repeating mode for certain moves, but was depressed for others. Neurons of all types were intermingled within the arm representation of M1.

### TABLE 4. Mean movement speed

<table>
<thead>
<tr>
<th>Move</th>
<th>Monkey GE</th>
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<th>Monkey FN</th>
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<tbody>
<tr>
<td></td>
<td>Repeating mode</td>
<td>Random mode</td>
<td>% Change</td>
<td>Repeating mode</td>
</tr>
<tr>
<td>5–3</td>
<td>56.6 ± 4.6</td>
<td>51.1 ± 8.1*</td>
<td>10.8</td>
<td>42.6 ± 3.6</td>
</tr>
<tr>
<td>3–1</td>
<td>53.5 ± 4.1</td>
<td>51.9 ± 4.6*</td>
<td>3.1</td>
<td>44.5 ± 4.2</td>
</tr>
<tr>
<td>1–5</td>
<td>62.0 ± 7.9</td>
<td>54.7 ± 5.9*</td>
<td>13.3</td>
<td>57.8 ± 4.9</td>
</tr>
<tr>
<td>2–3</td>
<td>41.6 ± 5.4</td>
<td>35.2 ± 4.4*</td>
<td>18.2</td>
<td>28.1 ± 2.9</td>
</tr>
<tr>
<td>3–4</td>
<td>39.4 ± 4.0</td>
<td>35.9 ± 4.8*</td>
<td>9.7</td>
<td>29.5 ± 3.6</td>
</tr>
<tr>
<td>4–2</td>
<td>63.4 ± 5.1</td>
<td>51.4 ± 6.1*</td>
<td>23.3</td>
<td>41.3 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± SD, obtained by first averaging the values from all trials of the type indicated for each task-related neuron and then averaging across all neurons. Units are cm/s % Change = 100 × (Repeating − Random)/Random. *, Repeating vs. Random P < 0.001 (paired t-test, two-tail); †Repeating vs. Random P < 0.01 (paired t-test, two-tail).

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In a small number of instances M1 neurons were exclusively active during one mode of the task (MI \(>0.95\) or \(<-0.43\), Repeating enhanced; \(<-0.43\), Repeating depressed). Additional constraints were applied for neurons to be considered differentially active (see METHODS). Labels of the abscissa indicate the maximum value included in the corresponding bin, except for the first bin \((-1\)), which indicates the minimum value included in that bin.

In a small number of instances M1 neurons were exclusively active during one mode of the task (MI \(>0.95\) or \(<-0.95\) for at least one move). For example, the neuron illustrated in Fig. 7 displayed a burst of activity for movements from target 3 to target 4 during the 2–3–4 sequence of the Repeating mode. The same neuron was entirely inactive in the sampling period for the comparable movements in the Random mode. Similarly, this neuron displayed bursts of activity for movements from target 5 to target 3 and movements from target 1 to target 5 during the 5–3–1 sequence of the Repeating mode. In contrast, the neuron was inactive when comparable movements were performed during the Random mode. In total, two neurons were exclusively active during the Repeating mode and five were exclusively active during the Random mode.

Most neurons that were differentially active displayed modulation of their activity during the performance of only one of the two sequences (Table 5). For example, 74% of the Repeating enhanced neurons displayed their differential activity either for a single move in one of the two sequences (41/62) (Fig. 8) or for multiple moves in one of the two sequences (5/62). Similarly, 61% of the Repeating depressed neurons displayed their differential activity either for a single move (14/28) (Fig. 6) or for multiple moves in one of the two sequences (3/28). Thus although we observed a variety of activity patterns, the training associated with our task appeared to exert some specific influences on the response properties of M1 neurons not only at the level of task mode, but also at the level of a specific move that is part of a sequence.

**RELATION OF NEURON ACTIVITY TO KINEMATICS.** An obvious question is whether the differential response properties of the M1 neurons during the Random and Repeating modes arise from or are strongly influenced by differences in movement kinematics. Therefore we used a combination of tests to assess the effect of movement kinematics on the activity of M1 neurons. For comparison, we used the same tests to evaluate relationships between the activity of arm muscles and movement kinematics. We first quantified the influence of movement kinematics on neuron activity using a regression analysis (see METHODS for details). Almost all task-related neurons (98%) and muscles (97%) we recorded displayed a significant fit to some aspects of the task kinematics. In general, the activity of neurons and muscles alike was more closely related to the kinematics of the movement that the monkey was preparing to do than to the one he was just completing. The mean multiple \(R^2\) of the regression model was 0.38 \(\pm\) 0.2 (range: 0.02–0.90) for neurons and 0.47 \(\pm\) 0.2 (range: 0.13–0.83) for muscles \((t\)-test, \(P<0.02\)). It is noteworthy that the mean multiple \(R^2\) for differentially active neurons was not significantly different from the mean for those that were not differentially active \((t\)-test, \(P=0.24\)). This result suggests that the differential activity of Repeating enhanced or Repeating depressed neurons cannot be explained solely by kinematics. This suggestion was confirmed by the ANCOVA analysis in which the effect of task mode was evaluated with all movement kinematics as covariates (see METHODS). As with the global neuron or muscle sample, differentially active neurons tended to have more frequent significant effects for the kinematic

![Graph](image.png)

**FIG. 4.** Distribution of modulation indices (MIs) for neurons in the arm representation of primary motor cortex (M1) and proximal arm/axial muscles. Data from all task-related neurons and EMG recordings are included (both monkeys combined). Based on these data, differentially active neurons were defined as those with a MI outside of the range of MI for EMG activity (\(>0.24\), Repeating enhanced; \(<-0.43\), Repeating depressed). Additional constraints were applied for neurons to be considered differentially active (see METHODS). Labels of the abscissa indicate the maximum value included in the corresponding bin, except for the first bin \((-1\)), which indicates the minimum value included in that bin.

**TABLE 5.** Classification of task-related neurons in the arm area of M1

<table>
<thead>
<tr>
<th>Neuron Classification</th>
<th>Number, %</th>
<th>One Sequence</th>
<th>Two Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>One move only</td>
<td>Two to three moves</td>
</tr>
<tr>
<td>Differential Repeating +</td>
<td>63 (26.9%)</td>
<td>38/63 (60%)</td>
<td>6/63 (10%)</td>
</tr>
<tr>
<td>Repeating -</td>
<td>25 (10.7%)</td>
<td>10/25 (40%)</td>
<td>3/25 (12%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>4 (1.7%)</td>
<td>0/4 (0%)</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>Nondifferential</td>
<td>142 (60.7%)</td>
<td>0/142 (0%)</td>
<td>0/142 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>234 (100%)</td>
<td>0/234 (0%)</td>
<td>0/234 (0%)</td>
</tr>
</tbody>
</table>

Repeating +, Repeating enhanced; Repeating −, Repeating depressed.
variables of the upcoming movement than the current one (Fig. 9). Likewise, direction and amplitude were the dominant factors. However, differentially active neurons were less frequently influenced by kinematics than were arm muscles. Conversely, muscles were less frequently influenced by task mode. The $R^2$ increment with the inclusion of mode terms in the ANCOVA relative to the multiple regression on kinematics was 0.1 ± 0.004 on average (range: 0.02–0.40) for differentially active neurons. The corresponding increment for muscle activity was significantly lower (0.06 ± 0.002; range: 0.003–0.20; $t$-test, $P < 0.002$). These results confirm that differentially active neurons are influenced by task mode independently of other variables and that this influence is greater for neurons than for arm muscles.

Finally, we examined the relative contribution of task mode and kinematics to differential activity (Fig. 10). For differentially active neurons. The corresponding increment for muscle activity was significantly lower (0.06 ± 0.002; range: 0.003–0.20; $t$-test, $P < 0.002$). These results confirm that differentially active neurons are influenced by task mode independently of other variables and that this influence is greater for neurons than for arm muscles.

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FIG. 5. Repeating enhanced neuron. This neuron was active in both the Random and the Repeating modes but its activity was appreciably enhanced for certain moves in the Repeating mode. Boxes highlight the moves for which the neuron showed differential activity. Diagrams above the rasters illustrate the target array and the moves shown in each panel (black arrow). For the Repeating mode, the gray arrows represent the additional series of moves that constitute the repeating sequence. Each panel in the 1st column shows the neuronal activity during movements from targets 4 to 2, 2 to 3, and 3 to 4 in the 2–3–4 repeating sequence. Panels in the 2nd column show the neural activity during the same movements in the Random mode. Panels in the 3rd and 4th columns show the activity of the same neuron during the Repeating and Random modes for the 5–3–1 sequence. Trials are aligned at the time of target hit for the move displayed (e.g., hit target 2 in moves 4 to 2). Triangles in the rasters represent the time of target hits. Some of these events occurred outside the time window illustrated. Bin width of the histograms is 15 ms. s/s, spikes per second.

FIG. 6. Repeating depressed neuron. This neuron was active in both the Random and the Repeating modes but its activity was depressed for moves 3 to 4 in the Repeating mode. Trials are aligned at the time of target release for the move displayed (e.g., release target 2 in moves 4 to 2). Inverted triangles in the rasters indicate target release. Other conventions as in Fig. 5.
tially active neurons, the absolute value of the regression coefficient for task mode was significantly greater (mean = 0.68) than the comparable coefficient for any of the kinematic variables (mean = 0.14–0.36) (Wilcoxon signed-rank test, \( P < 0.001 \)). The absolute coefficient for task mode was greater than the coefficients of all kinematic variables in 64% of the cases of differential activity. The mean value for task mode for neurons also was greater than that for arm muscles (mean = 0.56) (Mann–Whitney \( U \) test, \( P < 0.001 \)). On the other hand, the mean values of the regression coefficients for direction, amplitude, and movement speed on the next trial were significantly greater for muscles (mean = 0.32–0.38) than for neurons (mean = 0.19–0.20) (Mann–Whitney \( U \) test, \( P < 0.001 \)). Similar results were obtained when comparing the total contribution of task mode and kinematics to the variance of activity (\( R^2 \)) (Fig. 11). The \( R^2 \) for task mode was greater than the sum of \( R^2 \) values for kinematic variables in 65% of the cases of differential activity. Thus our results show that 1)
task-related differences in activity were greater in neurons than in arm muscles, 2) task mode made a relatively greater contribution to the modulation of differentially active neurons than kinematic variables, and 3) task mode made a relatively greater contribution to the modulation of differentially active neurons than to the modulation of arm muscles.

DISCUSSION

Our observations reveal that motor cortex neurons display some unique patterns of activity in animals trained to perform highly skilled sequential movements. The task the monkeys performed contained an element that is central to many motor skills—extensive practice. Practice normally results in the gradual acquisition of a skill and a sustained high level of execution. In our experiment, practice led to improvements in multiple aspects of the animals’ performance. Our monkeys were able to produce repeating sequences of movements entirely without external cues after nearly 22 wk of practice. With more prolonged practice the monkeys’ skill on the task continued to improve as evidenced by the gradual reduction in response time. The monkeys’ nearly flawless retention of the learned sequences after an interruption of >4 wk suggests that once acquired, the central representation of a motor sequence is robust. Although extended practice had a progressively smaller effect on measures such as response time, it had a dramatic impact on the retention of the learned sequence. Thus extended practice served to enhance the consolidation and retention of the acquired skill. We believe that the unique patterns of activity are a consequence of the extended practice and reflect the acquisition of a high level of skill on the task. In the following paragraphs we present other possible interpretations of our results and discuss the basis for our perspective.

Changes in motor performance constitute an inevitable result of the acquisition of a motor skill. Thus these performance changes are a potential confound for any study of motor skills. In this study, one could argue that the task-dependent modulation of M1 activity is explained by kinematic differences between the two task modes. It is well known that the activity of many M1 neurons can be related to kinematic variables, such as movement direction, amplitude, and velocity (e.g., Ashe and Georgopoulos 1994; Fu et al. 1993, 1995; Kakei et al. 1999; Moran and Schwartz 1999; Sergio et al. 2005). The kinematics of movements during the Random and Repeating modes of the task differed and the activity of almost all neurons in our sample was related to some aspect of movement kinematics (see the regression analysis in RESULTS). However, we found that the kinematic variables explained a relatively small portion of the variance in the activity of M1 neurons that were differentially active. In addition, all of the differentially active neurons...
neurons showed a significant effect of task mode (ANCOVA). Furthermore, for the majority of cases, the contribution of task mode to the total $R^2$ was greater than that of all kinematic variables summed. Other aspects of movement that we did not measure such as movement trajectory, velocity profile, and force may also have influenced neuron activity. However, these influences are likely to be relatively small in a simple and basically one-dimensional task involving unconstrained, unperturbed, and overtrained arm movements (Georgopoulos et al. 1981; Prablanc and Martin 1992). Any differences in these variables would at least be partly reflected by the output measures that we did consider (EMG activity, movement time, target hold time). Our analysis suggests that the task-dependent activity in M1 that we observed was not simply a reflection of the performance differences in the two modes of the task. Instead, our observations support the growing evidence that M1 is involved in operations beyond simply controlling movement kinematics and dynamics (see also Ben-Shaul et al. 2004; Carpenter et al. 1999; Kakei et al. 1999; Li et al. 2001; Lu and Ashe 2005; Sergio et al. 2005).

Two other aspects of the task—movement guidance and movement preparation—varied between the Random and Repeating modes. The movements made in the Repeating mode were, for the most part, internally generated and self-initiated, whereas movements in the Random mode were externally cued and triggered. One could argue that the differential activity during the two task modes is a consequence of the manner of movement generation. However, previous work showed that M1 neurons generally do not discriminate between similar movements that are internally and externally guided (Ashe et al. 1993; Halsband et al. 1994; Mushiake et al. 1990, 1991). Similarly, functional imaging studies report that M1 displays comparable activation during internally and externally triggered movements (Cunnington et al. 2002; Debaere et al. 2003; Jahanshahi et al. 1995; Jenkins et al. 2000). In neuron recording studies, Mushiake et al. (1991) found that, at most, 7% of M1 neurons were differentially active during performance of memorized and externally cued movements. In contrast, we found that roughly 40% of the neurons we examined were differentially active during the Random and Repeating modes of the task. These observations suggest that internal versus external generation does not in and of itself explain the task-dependent differences in M1 activity we observed.

The potential also exists for differences in motor preparation during epochs of the two task modes to contribute to task-dependent activity. In the Repeating mode the location of the next target was always predictable and therefore motor preparation for the upcoming movement could occur around the time of target hit or target release. In contrast, in the Random mode, target location was unpredictable and therefore no preparation was possible around the time of target hit. However, once the new target was displayed, motor preparation was possible. In the Random mode, target release occurred on average 237 ms after the presentation of the next target. Thus a period of motor preparation could occur around target release in the Random and the Repeating modes because in both instances the monkey knew the location of the next target. As noted in METHODS, we examined activity aligned on target release for 69% of the task-related neurons because this event gave the best timing and modulation in response averages. Thus in most cases we compared equivalent periods of the task when the monkeys had knowledge of the forthcoming movement. Despite having the same motor preparation, 60% of the differentially active neurons displayed their task-dependent changes around the time of target release. This result indicates that differences in motor preparation cannot explain the task-dependent changes we observed.

**Sequence representation in M1**

As noted earlier, our interpretation is that the task-dependent changes in M1 are the result of extensive practice on a repeating sequence of movements. Thus the differential activity of M1 neurons reflects the specific associations that are
formed through practice between the linked movements. This explanation applies to the 27% of the differentially active neurons that were Repeating enhanced and the 11% of the neurons that were Repeating depressed. Both types of activity in M1 are qualitatively similar to the sequence-related activity observed by others in the medial motor areas—that is, a change in discharge rate for conditions requiring the use of sequential information (e.g., Lu et al. 2002; Nakamura et al. 1998; Tanji and Shima 1994). Repeating enhanced and Repeating depressed neurons together contribute to sequence representation and/or skilled performance by shaping the patterns of motor outputs across a population of neurons. We do not consider Repeating depressed neurons as reflecting some learned process associated with the Random mode because extended practice on the Random mode did not result in an observable change in the animals’ motor performance on this mode of the task.

Two prior studies also reported that M1 activity during movement can be specifically related to memorized sequences (Ashe et al. 1993; Mushiake et al. 1991). Our results differ from these previous studies with respect to the surprisingly large number of neurons we found with sequence-related activity. Methodological aspects of these studies guided us to hypothesize that the prolonged training and high level of motor skill attained by our animals explain the different results. For example, Ashe et al. (1993) observed “a few cells” with sequence-related activity during memorized sequences of movements, although their animals performed correctly on only 56% of the trials. Mushiake et al. (1991) trained their monkeys for 3–5 mo and found that roughly 7% of M1 neurons were preferentially active during the memorized sequences. In contrast, after >2 yr of training on simple sequences of movements, we found that 40% of the neurons in M1 had sequence-related properties. The relation between training duration, stages of motor skill acquisition, and the emergence of M1 neurons with sequence-related properties remains to be examined. However, our hypothesis is that with accrued practice the representation of the motor skill or some aspect of it becomes embedded in the activity of M1 neurons. This suggestion is compatible with the observation that a functional reorganization occurs in M1 after sufficient training (e.g., Karni et al. 1995; Kleim et al. 2004). Our hypothesis can be tested in future experiments by comparing M1 activity (neuron or metabolic) and the effects of functional disruption of M1 at early and late stages of training on the Repeating and Random modes of our task.

Our results raise the possibility that M1 may be a site of storage for internal representations of sequential movements as first suggested by Karni et al. (1995). The ability of monkeys to learn two sequences with no clear evidence of interference suggests that they can develop at least two independent internal models of the task. The large proportion of neurons (70%) whose activity was task dependent for certain moves in a single sequence may provide the basis for independent representations of the two learned sequences within M1. The fact that sequence-related activity was frequently seen for a single move may indicate that sequence representation exists in M1 as linked elements of a local network (e.g., Hatsopoulos et al. 2003).

Our findings are compatible with the occurrence of large-scale changes in the functional organization of M1 as a result of sequence or skill learning in humans (Hund-Georgiadis and von Cramon 1999; Karni et al. 1995, 1998; Pascual-Leone et al. 1994, 1995; Tycˇe et al. 2005) and other mammals (Kleim et al. 1998; Plautz et al. 2000; Remple et al. 2001). Our results are also consistent with recent studies showing marked changes in the properties of a sizable population of M1 neurons as monkeys learn to adapt to new dynamic or kinematic environments (Gandolfo et al. 2000; Li et al. 2001; Paz et al. 2003). Thus our work adds to the ever-widening view that M1 is a plastic structure capable of remarkable dynamic and long-term changes under a variety of conditions (Kleim et al. 2003, 2004; Nudo et al. 1996, 1997; Qi et al. 2000; Sanes and Donoghue 2000). In conclusion, we suggest that, with long-term practice on a sequence of movements and the acquisition of a high level of motor skill, aspects of the sequence come to be represented in the activity of individual M1 neurons.

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REFERENCES


Fulton JF. Definition of the “motor” and “premotor” areas. Brain 58: 311–316, 1935.


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