Limited Functional Grouping of Neurons in the Motor Cortex Hand Area During Individuated Finger Movements: A Cluster Analysis

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Poliakov, Andrew V. and Marc H. Schieber. Limited functional grouping of neurons in the motor cortex hand area during individuated finger movements: a cluster analysis. J. Neurophysiol. 82: 3488–3505, 1999. Primary motor cortex (M1) hand area neurons show patterns of discharge across a set of individuated finger and wrist movements so diverse as to preclude classifying the neurons into functional groups on the basis of simple inspection. We therefore applied methods of cluster analysis to search M1 neuronal populations for groups of neurons with similar patterns of discharge across the set of movements. Populations from each of three monkeys showed a large group of neurons the discharge of which increased for many or all of the movements and a second small group the discharge of which decreased for many or all movements. Two to three other small groups of neurons that discharged more specifically for one or two movements also were found in each monkey, but these groups were less consistent than the groups with broad movement fields. The limited functional grouping of M1 hand area neurons suggests that M1 neurons act as a network of highly diverse elements in controlling individuated finger movements.

\textbf{Introduction}

Classification of neurons into groups based on similar patterns of discharge is a traditional approach to analysis of neuronal populations. Classification schemes may be suggested by externally observable features. Neurons of the primary motor cortex (M1) hand area, for example, might be classified into different groups apparently controlling different digits of the hand, as suggested by the iconic motor homunculus and simiusculus (Penfield and Rasmussen 1950; Woolsey et al. 1951). Alternatively, M1 neurons might be grouped according to which muscles or which movements they seem to control (Humphrey 1986; Phillips 1975). Recent evidence indicates, however, that single M1 neurons discharge during movements of several different fingers (Schieber and Hibbard 1993) and that the outputs of many single corticospinal neurons diverge to innervate multiple spinal motoneuron pools (Buys et al. 1986; Fetz and Cheney 1980; Kasser and Cheney 1985; Lemon et al. 1986; Mantel and Lemon 1987; Porter and Lemon 1993; Shinoda et al. 1979, 1981). These findings call into question whether or not groups of M1 neurons control particular digits, movements, muscles, or combinations thereof.

Other schemes for grouping neurons might be based on more abstract constructs. A number of recent theoretical consider-
could represent anything from concrete, externally observable features such as particular digits to more abstract constructs such as movement primitives. We also used clustering algorithms that made no a priori assumptions about the number of groups to be identified.

Cluster analysis revealed only two functional groups that were present consistently in the neuronal populations recorded from each of three monkeys: one large group of neurons that discharged for many or all finger movements and a second smaller group of neurons the tonic discharge of which paused during many or all movements. Although a few small groups of relatively movement-specific neurons were found in each monkey, these groups were less robust than the two groups with broad movement fields, differed from monkey to monkey, and failed to account for all the movements performed by each monkey. Many task-related neurons did not fall into any definable group. Our findings suggest that M1 neurons do not form large functional groups representing either concrete features or abstract constructs. Instead, we suggest that in controlling individuated finger movements M1 neurons act as a network of highly diverse elements. A preliminary report of this work has appeared in abstract form (Poliakov and Schieber 1998).

METHODS

All procedures for the care and use of these purpose-bred monkeys complied with the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals, followed the Public Health Service Guide for the Care and Use of Laboratory Animals, and were approved by the appropriate Institutional Animal Care and Use Committee.

Visually cued individuated finger movement task

Three juvenile (~4–6 yr old) male rhesus monkeys (Macaca mulatta; \( K \), 6 kg; \( A \), 5 kg; and \( C \), 7 kg) were trained to perform visually cued individuated finger movements. The behavioral paradigm, and the finger movements made by monkey \( K \), have been described in detail previously (Schieber 1991). The monkey’s elbow was restrained in a molded cast, and the right hand was placed in a pistol-grip manipulandum that separated each finger into a different slot. At the end of each slot, each fingertip lay between two microswitches. By flexing or extending a digit a few millimeters, the monkey closed the ventral or dorsal switch, respectively. This pistol-grip manipulandum was mounted, in turn, on an axis permitting flexion and extension wrist movements. A potentiometer coupled to the axis transduced wrist motion, and the output of this potentiometer was fed to level-crossing circuits that simulated flexion and extension switches for the wrist.

The monkey viewed a display on which each digit (and the wrist) was represented by a row of five light-emitting diodes (LEDs). The middle, yellow LED in a row was illuminated when neither the flexion or extension switch for that digit was closed. One of two green LEDs on either side of the middle yellow LED was lit whenever the flexion (leftward green LED) or extension (rightward green LED) switch was closed. When the monkey flexed or extended a digit, closing a microswitch, the middle yellow LED went out and the leftward or rightward green LED came on. The yellow and green LEDs thus informed the monkey which switches were open and which were closed. Red LEDs at either end of the row were illuminated as cues instructing the monkey to close either the flexion (leftward red LED) or extension (rightward red LED) switch.

The monkey initiated each trial by placing all digits and the wrist in their middle positions, so that no switches were closed and the middle yellow LED in each row was illuminated. After a pseudorandomly varied initial hold period of 500–750 ms, one red LED was illuminated under microprocessor control, instructing the monkey which switch to close (or to move the wrist). If the monkey closed the instructed switch within the 700-ms allowed response time after illumination of the red LED and held it closed for a final hold period (500 ms for monkeys \( K \) and \( C \); 300 ms for monkey \( A \)) without closing any other switches, then the trial had been performed correctly and the monkey received a water reward. After each rewarded trial, the finger movement to be instructed for the next trial was rotated in a pseudorandom order. Consecutive rewarded (correctly performed) trials of a given instructed movement therefore did not occur immediately after one another but instead were separated by trials of other instructed movements. In contrast, if the monkey failed to perform correctly—either by failing to close the instructed switch within the allowed 700-ms response time or by closing another switch before or after the instructed switch—no reward was delivered, and the same instruction was presented again for the next trial. (This protocol ensured that the monkey could not intentionally fail trials of difficult movements and earn rewards only on trials of easier movements.) After each trial, a minimum intertrial interval (1000 ms for monkey \( K \); 500 ms for monkeys \( A \) and \( C \)) was required before the monkey could initiate the next trial. Because the monkey had to initiate each trial by actively placing all digits and the wrist in their middle positions, the actual intertrial interval was variable, determined in part by the monkey.

Examination of the finger movements generated by monkeys performing this task showed that in each rewarded trial, the digit the monkey had been instructed to move underwent more movement than any other digit (Schieber 1991). Moreover, each digit had its greatest excursion when it was the instructed digit. In some movements, particularly when the monkey was instructed to flex the thumb or wrist, other digits remained stationary. In other movements, however, noninstructed digits moved to a greater or lesser degree. Each movement is therefore referred to as an instructed movement of a given digit in a given direction, recognizing that there was often some movement of noninstructed digits. For brevity, each instructed movement is denoted by the number of the instructed digit (1 for the thumb through 5 for the little finger, \( W \) for the wrist), and the first letter of the instructed direction (\( f \) for flexion, \( e \) for extension). Thus “2f” denotes instructed flexion of the index finger. Monkeys \( K \) and \( C \) were trained to perform 12 different finger and wrist movements. Monkey \( A \), in contrast, was trained to perform only six movements—\( 1f \), \( 2f \), \( 3f \), \( 4f \), \( 2e \) and \( 3e \)—all with the wrist axis fixed.

Neuron recording

Trained monkeys were prepared for single-unit recording by surgically implanting both a head-holding device and a rectangular Lucite recording chamber that permitted access to an area encompassing M1 contralateral to the trained hand. A few days after this procedure, daily 2- to 3-h recording sessions began. In each session, as the monkey performed the individuated finger movement task, a Trent-Wells hydraulic microdrive mounted on a custom XYZ micropositioner was used to advance a PtIr microelectrode (0.5–1.5 MΩ impedance) into the cortex. Signals from the microelectrode were filtered (300 Hz to 3 kHz), amplified 10,000 times, and monitored continuously on an oscilloscope and audiomonitor headphones. Single neuron action potentials were discriminated with a dual time/amplitude window and monitored by overlapping waveforms on a storage oscilloscope. Times of discriminated potentials were collected and stored to computer disk along with behavioral event marker codes.

In many microelectrode penetrations, intracortical microstimulation (ICMS) was used to confirm the location of the M1 hand area. The connections of the microelectrode were switched from the recording preamplifier to a stimulus isolator (BAK BSI-1) and trains of 12, biphasic, 200-μs, 5- to 40-μA constant current pulses at 330 Hz were delivered as the awake monkey performed the finger movement task.
ICMS was triggered under computer control as the monkey waited in the task’s initial hold period for an instruction cue or by the investigator as the monkey rested quietly between trials. Responses to ICMS were identified in monkey K by observing evoked movements of the fingers or wrist and by palpating contractions of forearm muscles. In monkeys A and C, ICMS responses also were identified in averages of rectified electromyograms (EMG) recorded through percutaneously implanted electrodes using both conventional trains and single-pulse ICMS (Cheney and Fetz 1985; Cheney et al. 1985). Placement of electrodes in each muscle was confirmed by observing that movement appropriate for the muscle was produced by tetanic electrical stimulation (trains of 12 biphasic pulses at 50–6,000 μA, pulses at 100 Hz) delivered between the bipolar electrode pair implanted in that muscle. In each of the three monkeys, the cortical territory from which ICMS evoked visible finger movements or EMG responses was coextensive with the region containing task-related neurons.

**Histology**

After the completion of all experiments on monkeys K and A, electrolytic lesions were made by passing DC current (40 μA for 40 s) through a microelectrode at selected locations. Several days later, the monkey was tranquilized with Ketamine (10 mg/kg), killed by lethal injection of thiopental (300 mg/kg iv), and perfused transcardially with phosphate-buffered saline followed by phosphate-buffered 4% paraformaldehyde. Before removing the brain from the cranium and photographing the cortical surfaces, marking ink tracks were placed at selected locations around the recording sites via a needle mounted on the same microrive. Frozen sections of both hemispheres were cut in the coronal plane at 30 μm, and every fourth section was mounted and stained for Nissl substance. The location of microelectrode penetrations was reconstructed based on examination of these sections. When particular penetration tracks could not be identified in the histological material, their locations were interpolated based on the locations of identified tracks, electrolytic lesions, and postmortem inked tracks. Histological confirmation of penetration locations in monkey C are unavailable at present because monkey C continues to be a subject in other studies. Nevertheless, the use of ICMS gives us a high degree of confidence that neurons recorded from monkey C were located in the M1 hand area (Widener and Cheney 1997).

**Data analysis**

To determine whether each recorded M1 neuron was related to the individuated finger movements performed by the monkey, we tested the null hypothesis that the neuron’s firing rate modulation during the 1 s preceding the end of the movement (switch closure) could have occurred by chance alone. For each instructed movement, we compiled a histogram (20-ms binwidth) of the neuron’s spike discharge during all correctly performed trials, aligning the data at the time of switch closure in each trial (e.g., Fig. 2). We then averaged the neuron’s firing rate across the entire 1-s period preceding switch closure. If variation in the neuron’s firing rate from bin to bin had been unrelated to performance of the instructed movement, then deviations from the average rate for the entire 1 s would have resulted from chance alone. We therefore used a two-tailed Kolmogorov-Smirnov test to compare nonparametrically the neuron’s firing rate in the bins immediately before switch closure. To apply this test, the maximal deviation (D) between the cumulative sums for the neuron’s histogram and the hypothetical constant average firing rate was found. The null hypothesis for a given instructed movement was rejected at P = 0.05 when D exceeded the critical value of 1.36/√n where n is the number of spikes contributing to the neuron’s histogram during the 1 s preceding switch closure. Given that several movements were tested, however, a Bonferroni correction for multiple tests was applied. To test the hypothesis that a neuron was related to at least one of several instructed movements at the P = 0.05 confidence level, the P value for at least one of the m movements performed by the monkey should reach 0.05/m. For monkeys K and C that performed 12 movement, we therefore used a critical P value of 0.05/12 = 0.004167. For monkey A, which performed six movements, we used 0.05/6 = 0.008333. The corresponding critical value for the cumulative sum deviation, D, was interpolated from tabulated critical values for the Kolmogorov-Smirnov test. Only neurons found in this way to be related to at least one of the instructed movements performed by each monkey were included in the populations used for cluster analysis.

For purposes of cluster analysis, we characterized each neuron’s firing rate modulation during each instructed movement with a single numeric discharge measure. Initially, we used the change (CH) in firing for each movement, calculated by subtracting the average firing rate during a 200-ms baseline period (1,000–800 ms before switch closure) from the average firing rate during the 100 ms immediately before switch closure. Subsequently we repeated the cluster analyses with two additional discharge measures. For a second discharge measure, we used a baseline period from 1,000 to 700 ms before switch closure and thereafter computed the cumulative sum, using the sum at the time of switch closure as a discharge measure (CU), thus integrating the discharge over the preceding 700 ms. For a third measure, we computed the average firing rate during the 100 ms immediately before switch closure without subtracting a baseline value (FR). Note that unlike the first two methods, FR values can only be positive or zero; this would fail to reflect that neurons with tonic baseline discharge might have shown a decrease in firing rate during a particular movement. The cluster analyses described below thus were repeated on the neuronal population from each monkey using three different discharge measures: CH, CU, and FR.

Each neuron’s discharge across the instructed movements then could be characterized by a 12-dimensional vector consisting of the neuron’s discharge measures during each of the twelve movements. For monkey A, discharge measures for the six movements not performed were set to zero. To search for neurons with similar relative patterns of discharge across the movements rather than similar absolute discharge, we normalized each neuron’s vector to unit length by dividing each discharge measure by the sum of the root mean squares of the 12 values. The unit vectors of all neurons thus were normalized to lie on the surface of an imaginary 12-dimensional sphere.

To apply a cluster analysis procedure to our neuronal populations, a measure of similarity among any two neurons had to be established. Because a 12-dimensional unit vector was assigned to every neuron based on its relative discharge across the 12 movements, we defined the similarity of two neurons, Sij, as the Euclidean distance in 12-dimensional space between their unit vectors vi and vj.

\[
S_{ij} = \sqrt{(v_i - v_j)^2} = \sqrt{\sum_{k=1}^{12} (v_{ik} - v_{jk})^2}
\]

Thus defined, similarity ranged from 0 to 2 (0 ≤ Sij ≤ 2). The utility of normalization to unit vectors now can be appreciated by considering three hypothetical neurons: neuron A, which discharged 100 spikes/s during movement 1f only; neuron B, which discharged 10 spikes/s during movement 1f only; and neuron C, which discharged 10 spikes/s during movement 3e only. Without normalization, neuron B would be more similar (closer) to neuron C than to neuron A, because both have relatively low discharge. After normalization, however, neurons A and B are similar (close) because they both discharge only for movement 1f.

Agglomerative, hierarchical techniques were implemented for clustering (Johnson and Wichern 1992). To apply these techniques to N neurons, we started with N clusters, each containing a single neuron. An N × N symmetric matrix of similarities, S, then was calculated, and the pair of clusters closest to each other was identified. These were merged to form a new cluster. Then the similarity matrix was

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limited grouping of M1 neurons
FIG. 2. Selected examples of the variety of discharge patterns across instructed movements. Discharge patterns of 3 neurons selected from monkey K are shown in rasters and histograms of data recorded during correctly performed trials of the 12 instructed movements (1f to We; see text for explanation of number of instructed digits). Data from different trials have been aligned at the time of switch closure in the center of each 2-s display. Each histogram represents the average of 12–15 correctly performed trials, though only the 1st 5 trials are rastered below. First and 2nd dots beneath each raster line indicate the time of cue onset and then switch closure (aligned) in each of these 5 trials. Vertical calibration bars for the histograms indicate firing rates of 0–100 spikes/s.
Cluster analysis of EMG activity

For comparison with M1 neuronal activity, and to test our clustering methods, we performed a cluster analysis of EMG activity recorded from nine forearm muscles: flexor digitorum profundus, radial region (FDPr); flexor digitorum profundus, ulnar region (FDPu); flexor digitorum superficialis (FDS); palmaris longus (PL); extensor digiti secundi et tertii (ED23); extensor digitorum communis (EDC); extensor digiti quarti et quinti (ED45); extensor carpi radialis (ECR); and extensor carpi ulnaris (ECU). EMG recordings from these nine muscles were made simultaneously with the recordings of 10 neurons included in the population from monkey C as the monkey performed the individuated finger movement task. The raw activity from the intracortical microelectrode (ME) also was recorded in these 10 sessions, and we included these continuous waveform recordings in the cluster analysis with the EMG recordings. The change (CH) in activity for each of these 10 recordings (9 EMGs + 1 ME) from each of 10 sessions was computed from histograms of rectified waveforms averaged over all correctly performed trials of each instructed movement, parallel to the analysis of single neuron activity (see METHODS). The bottom display of Fig. 3 shows the 12-dimensional unit vectors for each of these recordings, again in the order in which they were recorded. Although at first glance these recordings may appear to be as diverse as the neuron recordings, because the monkey performed the finger movement task in a relatively stereotyped fashion each day, we anticipated that the EMG activity of a given muscle would show a similar pattern each day. Indeed, scanning the EMG unit vector display in Fig. 3 from left to right suggests a pattern repeating every 10 columns.

Figure 4 shows the results of single linkage clustering applied to this population of 100 EMG and ME recordings. In
Fig. 4B, the order of the recordings shown in Fig. 3 EMGs, has been rearranged by the clustering process. The process began with each recording treated as a cluster. In reiterative steps, the most similar two clusters were identified and merged, creating progressively larger and larger clusters, until finally all the recordings were merged into a single cluster. As each step merged two clusters, the columns representing the recordings of one cluster were taken out of their position along the horizontal axis and placed next to the columns representing recordings of the other cluster. (Consequently, the position of groups at the end of the process depended in part on where similar members of the population were located before clustering, and in part on which members were most similar, i.e., clustered first.) The column representing each recording in Fig. 3 EMGs, therefore has been moved next to the columns representing other recordings with similar activity patterns across the 12 instructed movements (i.e., close on the 12-dimensional sphere) in Fig. 4B.

The clustering process successfully grouped all the 10 recordings made from each of seven muscles—FDPr, ED23, ECR, FDS, FDPu, EDC, and PL—which appear in Fig. 4B as bands with horizontal rows of similar color 10 columns wide. For example, the 10 recordings from FDPr (Fig. 4B, far left) all had greatest EMG activity during instructed movement Wf, which appears as a horizontal red band in the 6th row from the bottom (arrowhead); each FDPr recording also had substantial activity during 2f and 5e; this appears as yellow-orange bands in the 2nd and 11th rows, respectively. In contrast, the 10 recordings from ED23 all had greatest EMG activity during instructed movement 5f as well as substantial activity during...
1e, 2e and 3e. ED23 recordings therefore appear as a group 10 columns wide, with a red horizontal band in the fifth row from the bottom (5f), and yellow-orange horizontal bands in the seventh–ninth rows (1e, 2e, 3e). [Note that during individuated finger and wrist movements performed by monkey C, as described in detail previously for other monkeys (Schieber 1995), a given muscle was not necessarily most active as a prime mover of the digits it serves. FDPr was most active as a wrist flexor. ED23 was most active in limiting flexion of digits 2 and 3 during 5f. And similarly, FDPu was most active in limiting extension of digit 5 during 4e.] The EMG recordings from ED45 and ECU were so similar that the cluster analysis did not separate them, and recordings from these two muscles are intermingled in the 20 columns in Fig. 4B, far right. Of the 10 microelectrode recordings, 6 showed increased activity during most of the movements and were grouped together by the clustering process (ME*); the other 4 each showed a different pattern and were left as isolated columns (*).

Figure 4A shows the corresponding dendrogram. Here the recordings are represented by vertical lines rising from the abscissa in the same left-to-right order as in Fig. 4B. Horizontal lines join the vertical lines for two recordings at the ordinate value representing the distance between the two recordings in the 12-dimensional space. As additional recordings are agglomerated onto existing clusters of two or more, horizontal lines join the vertical line representing each newly added recording to a vertical line extending upwards from the existing cluster. The ordinate value of this horizontal line represents the distance from the newly added recording to the closest member of the existing cluster. To help identify major groups, the blue lines have been replaced with red lines when the two clusters being merged each already included ≥3% of the entire population. The dendrogram thus provides a more quantitative view of the groupings formed by the clustering process: the more the members of a group are similar to one another, the lower along the ordinate are the horizontal lines joining them; the more distinct the group’s members are from other members of the population, the longer the next vertical line segment above joining that group to other members of the population. The dendrogram of the clustered EMG recordings indicates that the recordings obtained from a given muscle in different sessions generally were very similar to one another—being joined at low ordinate values—and that the recordings from different muscles (except for ED45 and ECU) were relatively distinct—with long vertical lines joining the group of recordings from one muscle to the group from another.

Figure 4C shows the corresponding similarity (or distance) matrix. Here, each recording is represented in the same order as in B along both the abscissa and the ordinate. The distance in the normalized 12-dimensional space between each possible pair of recordings is displayed in the appropriate cell using a color scale to represent values from 0 (dark blue) to 2 (dark red). Note that this color scale for the similarity matrix (C) has an entirely different meaning than the color scale for the unit vector display (B). Similarity matrix cells representing the distance between two similar recordings are dark blue because the distance between the two recordings in the 12-dimensional space is close to the minimum possible distance of 0. Similarity matrix cells representing the distance between two dissimilar recordings are yellow, orange, or red because the distance between the two recordings is closer to the maximum possible distance of 2. Because the similarity matrix is symmetric about its main diagonal and because the values along the main diagonal all are 0, only the cells above the main diagonal are shown. In the similarity matrix, groups of similar recordings appear as blue triangles with hypotenuses along the main diagonal. The distances between the contiguous recordings in these groups are low (blue), indicating that the unit vectors of these recordings are close on the 12-dimensional sphere, i.e., the recordings are similar. The degree to which the edges of these blue triangles are sharply defined (vs. blending gradually into green, yellow, orange, and red) provides another indicator of the degree to which the clustered recordings are distinct from other somewhat similar recordings. The larger squares of warmer colors away from the main diagonal provide an indication of the distance between each grouping represented along the main diagonal. For example, the large yellow-orange square at the intersection of the FDPr columns and the ED23 rows indicates that the FDPr and ED23 recordings were relatively dissimilar, whereas the large light-blue square at the intersection of the EDC columns and PL rows indicate that the EDC and PL recordings were relatively similar.

**FIG. 4.** Clustering of EMG recordings. Results of clustering 90 EMG recordings and 10 raw microelectrode recordings (ME) using the CH discharge measure and single linkage clustering algorithm are shown in 3 complementary displays: dendrogram (A); unit vector display (B); and similarity matrix (C). In B, as in the unit vector displays of Fig. 3, each recording is represented by a column and each instructed movement by a row (from 1f at bottom to We at top), but here the columns representing different recordings have been reordered from left to right by the clustering process, such that the column representing each recording is close to the columns of other recordings with similar activity patterns. Groups of similar recordings therefore appear in B as contiguous columns with similar colors in each row, forming horizontal bands of relatively constant color. These horizontal bands indicate the shared features that make the recordings similar. At the left of the unit vector display, for example, a group of 10 contiguous columns each with a dark red cell in the 6th row from the bottom (arrowhead) represents a group of recordings that showed much more intense EMG activity during movement Wf than during any other movement. These recordings all were from flexor digitorum profundus, radial region (FDPr). Muscle name abbreviations indicate the groups of recordings from other muscles, all of which were clearly separated except for recordings from ED45 and ECU, which were so similar that they remain intermingled in the large group at the far right. Clustering process also separated the ME recordings from the EMGs, placing 6 of them in a group (ME*) and leaving 4 others as isolated members of the population (*). FDPr, flexor digitorum profundus, ulnar region; FDS, flexor digitorum superficiais; PL, palmaris longus; ED23, extensor digiti secundi et tertii; EDC, extensor digitorum communis; ED45, extensor digiti quarti et quinti; ECR, extensor carpi radialis; and ECU, extensor carpi ulnaris. In the corresponding dendrogram (A), the recordings are represented by vertical lines rising from the abscissa in the same left-to-right order as in B. Horizontal lines join the vertical lines for 2 recordings at the ordinate value representing the distance between the 2 recordings in the 12-dimensional space. Red lines replace blue lines when 2 merging clusters each already contained ≥3% of the entire population. In the corresponding similarity matrix (C), each recording is represented in the same order as in B along both the abscissa and the ordinate. Distance on the 12-dimensional sphere between each possible pair of recordings is displayed in the appropriate cell using a color scale (different from the color scale used in B to represent values from 0 (dark blue) to 2 (dark red). In the similarity matrix (C), groups of similar recordings therefore appear as blue triangles with hypotenuses along the main diagonal.
The dendrogram (A), unit vector display (B), and similarity matrix (C) thus provide complementary information on groups of recordings with similar activity patterns across the 12 instructed movements. The dendrogram provides a linear picture of the distance between members of a group and the distance between groups. The unit vector display provides a picture of the features of activity across the 12 instructed movement that render the members of a group similar. The similarity matrix provides a picture of the degree of similarity or dissimilarity between each group. Overall, the EMG recordings from each muscle were highly similar, clustering into a group, which in turn was relatively distinct from the groups of recordings from other muscles as well as being distinct from the ME recordings, which did not all cluster into a single group. These results indicate that the monkey used a stereotyped pattern of EMG activity to perform the task from session to session, while at the same time demonstrating that groups of similar recordings were formed by the clustering process. Such clear groupings were not produced, however, when the same clustering methods were applied to populations of single M1 neurons.

General results of cluster analysis of M1 neuronal populations

Because we could not classify M1 hand area neurons by simple inspection and because many neurons could not be treated as broadly tuned, we applied cluster analysis to examine the neuronal population in each monkey for groups of neurons the discharges of which varied similarly across instructed movements. The results of single linkage clustering of the M1 neuronal populations recorded from monkeys K, A, and C are shown in Figs. 5–7, respectively. The general results of clustering were consistent across all three monkeys. The largest group always consisted of neurons the discharges of which increased for many or all of the instructed movements performed by the monkey. This group appears in the neuronal unit vector displays (B in Figs. 5–7) as a broad band of columns containing predominantly yellow, orange, and red cells located toward the right of the display. In the similarity matrix (C in Figs. 5–7), this group appears as a large blue triangle toward the right of the matrix. The borders of the blue triangle are not necessarily sharply defined, indicating that this group of neurons was not necessarily distinctly isolated from other members of the population. The dendrograms (A in Figs. 5–7) show that members of this group were relatively close to one another, agglomerating at distances from ~0.2 to 0.5. The absence of long vertical lines leading upward from this group in the dendrogram again indicates that the group was not sharply isolated from other members of the population. Defining the movement field of a given neuron as the set of instructed movements in relation to which that neuron discharged, this large group can be described as consisting of neurons with broad field excitation (BFE).

A second, smaller group also appeared in each monkey. This group was composed of neurons the discharges of which decreased for most or all instructed movements, which we describe as broad field inhibition (BFI). The BFI group appears as several contiguous columns of light blue to dark blue cells toward the left end of each neuronal unit vector display (Figs. 5B–7B), with a corresponding blue triangle against the main diagonal of the similarity matrix below (Figs. 5C–7C). The column of red cells rising above the BFI group’s blue triangle in each similarity matrix indicates that most other neurons of the population were quite unlike neurons of the BFI group. Nevertheless, like the BFE group, the BFI group was not necessarily sharply isolated from other members of the population. Compared with the BFE group, members of the BFI group were less similar, agglomerating at larger distances of ~0.3–0.9 (Figs. 5A–7A).

Other small groups in each monkey were characterized by more specific movement fields. The nature of these small groups varied from monkey to monkey, however. For example, whereas monkey K had one group of neurons that discharged almost exclusively for movement 1f, monkey C had no 1f group but rather had a 5f group that was not found in monkey K. The members of these other small groups generally were separated by distances intermediate between the BFE and BFI groups. Like the BFE and BFI groups, these other small groups were not necessarily sharply isolated from other members of the neuronal population in each monkey. Many other neurons did not fall into any sizable group. Such neurons can be viewed as lying in the interstices between groups. As the clustering process proceeded from closest neurons to most distant (from lowest to highest horizontal lines of the dendrograms in Figs. 5A–7A), the BFE group formed a core onto which the smaller groups and the interstitial neurons gradually were agglomerated.

Nonrandom features of the M1 population

The diversity of the neuronal population in each monkey, the limited number of groups apparent in the cluster analysis, and the fact that the groups were not sharply demarcated from other members of the population, all raised the possibility that the observed groupings could have arisen by chance alone. To examine this possibility, we performed cluster analyses on two imaginary neuronal populations.

First, we created a population of 177 neurons (equal to the number of neurons in the population from monkey C) in which the discharge measure for each neuron during each instructed movement was chosen randomly with an even distribution from −1 to +1. These values were normalized to unit vectors for each neuron and then clustered using the single-linkage algorithm (Fig. 8). In comparison with the real neuronal populations, this randomized population lacked neurons that discharged much more intensely for one instructed movement than for any others (Fig. 8B). Such movement-specific neurons appeared as columns with one dark red cell in the unit vector displays of the real populations (Figs. 5B–7B).

Clustering of this randomized population showed that its most similar neurons were separated by distances >0.4, whereas in each of the real populations several small groups started to form at distances <0.4. Furthermore clustering of this random population produced no BFE, BFI, or other groupings like those seen in the real populations. The dendrogram revealed no clusters of short vertical lines; the unit vector display showed no columns of similar neurons; and the similarity matrix showed no dark blue triangles against the main diagonal. This randomized population thus was much more evenly distributed in the 12-dimensional clustering space than any of the three real neuronal populations. These observations suggest that the real neuronal populations did contain groups of
neurons the similarity of which was not the result of random variation in neuronal discharge from one instructed movement to the next.

An important difference between this random population and the real neuronal populations, however, lies in the distributions of their discharge measures. Whereas discharge in the randomized population varied evenly from negative to positive and was symmetric about zero, the discharge distribution of a real population of cortical neurons is neither even from maximum to minimum nor symmetric about zero discharge. We therefore generated a second imaginary population in which the discharge measures from the real population of monkey C were reshuffled randomly. This process maintained the same distribution of discharge measures for the imaginary population as a whole (Fig. 9D), while eliminating any association of particular discharge values during particular instructed movements in single neurons.

Figure 9 shows the results of clustering this randomly reshuffled population. Most striking again was the absence of groups characterized by BFE and BFI. In place of these BFE and BFI neurons, however, the randomly reshuffled population had many neurons that discharged much more intensely for one instructed movement than for others, appearing as columns with single dark red cells in the unit vector display (Fig. 9B). Indeed, whereas only a few groups of neurons so selective for a particular instructed movement appeared in the real population from each monkey, such groups appeared for many instructed movements in the randomly reshuffled population.
The occurrence of these groups reflects the fact that the distribution of real discharge measures included many small positive values and only a few large positive values (Fig. 9D). One large value thus frequently was associated with 11 much smaller values by chance alone. Unlike these groups of neurons that discharged selectively for a particular movement in the real neuronal populations, however, the minimum distance separating these similar neurons in the randomly reshuffled population was >0.4.

The occurrence of a large number of BFE neurons in each monkey therefore did not result simply from chance association of increased discharge during multiple instructed movements in a single neurons. Likewise, the groups of BFI neurons did not result from chance association of decreased discharge during multiple movements. Such results suggest that neurons in the BFE and BFI groups are likely to have functional importance in cortical control of individuated finger movements.

Reproducibility of functional groups

That most of the functional groups identified by the present cluster analysis were not sharply isolated from other members of the neuronal population, that the movement fields characterizing the more movement specific groups varied from monkey to monkey, and that such movement specific groups could arise by chance association of one large discharge value with eleven smaller values for a given neuron, all raised the possibility that the presence of these groups might have resulted from the particular method we chose to quantify neuronal
discharge or from the algorithm we used for clustering. We therefore repeated the cluster analysis of each monkey’s neuronal population using two other measures of neuronal discharge (CU and FR, see METHODS) and one other clustering algorithm (average linkage, see METHODS). This produced a total of six different cluster analyses of each monkey’s neuronal population (3 discharge measures × 2 clustering algorithms). We used these six cluster analyses to examine the neuronal population in each monkey for groups that were stable across the discharge measures and clustering algorithms.

The large BFE group appeared in all six cluster analyses performed on the neuronal populations from each of the three monkeys. The smaller BFI group never appeared in analyses using the FR discharge measure because the spike frequency during the 100 ms preceding switch closure could never be negative. The BFI group did appear consistently, however, in the other four cluster analyses using either CH or CU with either single- or average-linkage clustering performed on the neuronal populations from each of the three monkeys. The large BFE and smaller BFI groups thus appeared consistently across discharge measures and clustering algorithms.

The remaining small groups of movement selective neurons were more difficult to identify across cluster analyses. To employ a consistent criterion, we therefore identified neuronal groups in which a minimum percentage of the total population had been clustered together before being joined to the remainder of the population. Because the single linkage clustering algorithm tends to form smaller groups than the average linkage algorithm (see METHODS), we used 3% of the population as the criterion for cluster analyses performed with the single linkage algorithm, and 5% for average linkage. In Figs. 5–7, these groups are identified in the dendrogram by red lines...
joining them to the rest of the population. Note that one or two of the small groups identified in this way typically were part of the larger BFE group. Table 1 shows the number of small groups identified using each of the six cluster analyses in each of the three monkeys.

We then examined the small groups identified by the six cluster analyses to determine the degree to which the groups varied or remained stable. First we examined the movement field of each small group in one analysis and searched the other analyses for groups with the same movement field. Occasionally a group identified in several analyses appeared to have been subdivided in one analysis. The 4e,5e group in monkey C, for example, appeared subdivided into two groups in the analysis using the CH discharge measure and single-linkage algorithm (Fig. 7), though it appeared as a single group in the other five analyses. Table 2 lists the small groups in each monkey identified in at least four of the six cluster analyses along with the number of analyses in which each group was identified. These groups are denoted by black bars above the neuronal unit vector display (B) in Figs. 5–7.

Besides appearing consistently across analyses, a stable group should include a core of neurons that remain part of the group whenever that group is identified. Each of the relatively stable small groups listed in Table 2 therefore was examined to determine how many neurons were present consistently across the different cluster analyses in which that group was identified. Table 3 illustrates this consistency for the 5f group in monkey C. The 5f group was identified in all six cluster analyses of monkey C’s neuronal population. In the six different cluster analyses, from 6 to 22 neurons were included in the 5f group (counts at bottom). Of the 177 neurons in the population from monkey C, 29 were clustered in the 5f group by at least one of the six cluster analyses. Five of these 29 neurons were clustered in the 5f group by all six cluster analyses.
Although the 5f group thus was quite variable across the six cluster analyses, it contained a stable core of five neurons.

For each relatively stable small group in each monkey, Table 2 lists the number of core neurons consistently in the group whenever that group was identified. For two of the relatively stable groups—4e,5e in monkey C and 3e,5e in monkey K—no such core neurons were found. If we then count only those groups identified in at least four of the six analyses containing a core of at least one neuron consistently present whenever the group is identified, we find only two to four such groups in each monkey, one being the BFI group in each monkey.

**Spatial location of functional groups**

We found previously that M1 neurons discharging in relation to any given finger movement are distributed throughout the M1 hand area (Schieber and Hibbard 1993). Nevertheless members of the functional groups identified by the present cluster analyses might be located close together in the M1 cortex, and different groups might be spatially segregated. To examine this possibility, we plotted the spatial location of the

---

**TABLE 1. Number of small groups identified with different cluster analyses**

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Single Linkage</th>
<th>Average Linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measure</td>
<td>CH</td>
<td>CU</td>
</tr>
<tr>
<td>Monkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

CH, change; CU, cumulative sum; FR, firing rate.
TABLE 2. Small groups identified in at least four of the six cluster analyses

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Movement Field*</th>
<th>Analyses Identified/Number Possible</th>
<th>No. of Core Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>BFI</td>
<td>4/4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1f</td>
<td>4/6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1e</td>
<td>4/6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3e, 5e</td>
<td>5/6</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>BFI</td>
<td>4/4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2f</td>
<td>6/6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2e</td>
<td>5/6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3e</td>
<td>6/6</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>BFI</td>
<td>4/4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5f</td>
<td>6/6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4e, 5e</td>
<td>6/6</td>
<td>0</td>
</tr>
</tbody>
</table>

BFI, broad field inhibition. * Each instructed movement is denoted by the number if the instructed digit (1 for thumb to 5 for little finger, w for wrist) and the first letter of the instructed direction (f, flexion; e, extension).

Functional groups in M1 identified by cluster analysis

In the M1 neuronal populations recorded from each of three monkeys, cluster analysis revealed only a limited number of groups of neurons with similar discharge patterns across the finger and wrist movements performed by each monkey. In all three monkeys, the largest group consisted of neurons the firing of which increased during many or all of the individuated movements performed by the monkey. Although this BFE group was not sharply isolated from other members of the neuronal population, the BFE group appeared consistently in the neuronal population from each of the three monkeys, no matter which measure of neuronal discharge or clustering algorithm was used. Furthermore the BFE group consistently formed a sizable fraction (roughly 20–25%) of the population.

A second group identified reliably in all three monkeys consisted of neurons with tonic discharge at rest whose firing decreased during many or all of the finger movements, a pattern to which we refer as BFI. Although much smaller than the BFE group, the BFI group was identified in each monkey using two measures of neuronal discharge (our FR measure could not identify inhibition) with either clustering algorithm. Neither a BFE nor a BFI group appeared on clustering two imaginary neuronal populations: one in which discharge measures were randomly distributed from −1 to +1, another in which measures were drawn randomly from a real distribution. Although BFI and BFE neurons may appear relatively nonspecific for control of individuated finger movements, their robust presence in all three monkeys and their absence in the randomized populations suggest that the BFE and BFI groups both represent types of neurons fundamentally important for M1’s contribution to control of individuated finger movements. The physiology of these neurons was organized such that their discharge either increased (BFE) or decreased (BFI) to a similar extent during many or all individuated finger and wrist movements.

In each monkey we also found a few small groups of neurons characterized by discharge patterns that showed much more intense firing during one or two particular finger movements than during others. Using a criterion that the group be identified using four of our six combinations of discharge measure and clustering algorithm, we identified three such groups in monkey K, three in monkey A, and two in monkey C. Of these, only two groups in monkey K, three in monkey A, and one in monkey C, had core neurons that were included in the group across all the combinations of discharge measure and clustering algorithm with which the group was identified. These small, movement-specific groups differed from the BFE and BFI groups, however, in that the same groups were not found from monkey to monkey. Furthermore we found that such small, movement-specific groups could appear in an imaginary neuronal population the discharge measures of which were drawn randomly from a real distribution. Although we cannot exclude the possibility that the movement-specific groups in each monkey represented some important function unique to each monkey—such as assisting in movements the
monkey found particularly difficult—the functional importance of these small, movement-specific groups thus remains less certain than that of the BFE and BFI groups.

Of course, the population of recorded neurons constituted only a small sampling of the total number of neurons in each monkey’s M1 hand area. Groups consisting of large absolute numbers of neurons therefore might have gone undetected. Nevertheless, because we sampled relatively evenly through the M1 hand area and because our analysis would have identified groups constituting $\geq 5\%$ of the population, groups of this size are unlikely to have gone undetected. Studies comparing the postspike effects of single cortical neurons with the effects of intracortical microstimulation at the same site have provided evidence of local groups of M1 neurons with similar patterns of output connections to spinal motoneuron pools (Cheney and Fetz 1985; Cheney et al. 1985). Our findings suggest that these local groups each constitute $<5\%$ of the population or else that in spite of their similar output connections the different members of such local groups discharge differently across a set of individuated movements.

**Comparison with other means of analyzing the neuronal population**

Our application of cluster analysis to explore M1 neuronal populations for functional groups of similar neurons differs from traditional approaches, which we felt might not incorporate the diverse features displayed by different neurons during individuated finger movements. One traditional approach would have been to classify M1 neurons into a number of predefined groups based on features of the behavioral tasks studied. For example, ventral premotor cortex neurons have been classified as discharging either during precision pinch or else during power grasp (Rizzolatti et al. 1988). In the present context, M1 neurons could have been classified as best-related to a particular movement (e.g., 1f or 2f or 3f or . . . 5e or We) based on the neuron’s maximal discharge. Alternatively, M1 neurons could have been classified as best-related to a particular digit (1, 2, 3, 4, 5, or W) depending on the movements for which the neuron showed the greatest flexion/extension discharge differential; a neuron discharging most intensely during movement 3f and pausing entirely during movement 3e then would be classified as a digit 3 neuron. With any such classification, however, many of the present M1 neurons would have been difficult to assign to one category or another.

A second traditional approach would have been to assume that each M1 neuron was tuned broadly for individuated finger movements with discharge varying continuously in relation to the spatial geometry of the hand (Georgopoulos et al. 1982). A population vector then could be used to extract information about which finger movement was performed from the discharge of the M1 neuronal population. The population vector approach has been applied successfully to neuronal populations carrying information on movement direction, movement force, complex movement trajectories, and even facial features (Georgopoulos et al. 1986, 1989, 1992, 1993; Schwartz 1993; Young and Yamane 1992). Indeed, many of the present M1 neurons show a component of broad tuning in relation to individuated finger movements, and the population vector computed from their discharge during different finger movements can specify the instructed movement (Georgopoulos et al. 1999). The population vector approach assumes, however, that the discharge of a given neuron varies systematically across a finger movement space. This assumption characterizes

**FIG. 10.** Spatial location of functional groups in the M1 cortex of monkey A. Members of each group shown in Fig. 6—BFE, BFI, 2f, 2e, and 3e—are plotted as spheres of constant size in a separate 3-dimensional lollipop diagram for each group. An additional plot shows the location of all neurons in the population. Each sphere is centered at the location of the neuron it represents in a constant reference frame, oriented as if viewing the cortex in the anterior bank of the central sulcus from the frontal pole of the hemisphere, with the motor face representation to the viewer’s far right, leg representation to the far left. a, anterior; p, posterior; m, medial; l, lateral; s, superficial (toward the hemispheric surface); d, deep.
the discharge of many M1 neurons incompletely, especially neurons that discharged intensely for movements of nonadjacent fingers while not discharging for movements of the fingers in between (e.g., $K_{2505}$ in Fig. 2).

Implications for the control of individuated finger movements

Whether M1 controls body parts, movements, or muscles has been a topic of long-standing discussion and debate (Humphrey 1986; Lemon 1988; Walshe 1948). If different groups of M1 neurons exerted control on particular fingers, particular movements, or particular muscles, we would have expected to find different groups of M1 neurons that discharged in relation to particular sets of fingers, movements, or muscles. Enough groups should have been present to account for all the movements performed by each monkey, but such was not the case. Nor did the groups we identified in each monkey appear to represent a set of more abstract features—principle components, movement primitives, or virtual fingers—the different combinations of which could represent the movements performed. Our findings thus provide little evidence that different groups of M1 neurons act to control different movement features. An alternative hypothesis would be that M1 neurons are broadly tuned such that each neuron’s discharge varies systematically in relation to the spatial geometry of the hand. Although this might be an accurate description of many neurons in the present populations, it fails to characterize many others.

How might M1 neurons control individuated finger movements using neither groups of neurons to control particular digits, movements, or muscles nor a population of broadly tuned neurons? We suggest that the different combinations of muscle activity needed to produce many different movements could be controlled by the output of a network composed of diverse neuronal elements. The elements of such a network would not necessarily be specific for any given digit or movement nor would their activity necessarily resemble that of any particular muscle. Furthermore the discharge of single neuronal elements would not need to be related systematically to the spatial geometry of the hand nor would the population of neurons necessarily contain groups with similar behavior. Rather, behavioral diversity of different neurons would increase the variety of different outputs the network could achieve, providing an extensive and flexible movement repertoire.

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REFERENCES


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