

Neuronal Clusters in the Primate Motor Cortex during Interception of Moving Targets

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Abstract

■ Two rhesus monkeys were trained to intercept a moving target at a fixed location with a feedback cursor controlled by a 2-D manipulandum. The direction from which the target appeared, the time from the target onset to its arrival at the interception point, and the target acceleration were randomized for each trial, thus requiring the animal to adjust its movement according to the visual input on a trial-by-trial basis. The two animals adopted different strategies, similar to those identified previously in human subjects. Single-cell activity was recorded from the arm area of the primary motor cortex in these two animals, and the neurons were classified based on the temporal patterns in their activity, using a nonhierarchical cluster analysis. Results of this analysis revealed differences in

the complexity and diversity of motor cortical activity between the two animals that paralleled those of behavioral strategies. Most clusters displayed activity closely related to the kinematics of hand movements. In addition, some clusters displayed patterns of activation that conveyed additional information necessary for successful performance of the task, such as the initial target velocity and the interval between successive submovements, suggesting that such information is represented in selective subpopulations of neurons in the primary motor cortex. These results also suggest that conversion of information about target motion into movement-related signals takes place in a broad network of cortical areas including the primary motor cortex. ■

INTRODUCTION

Many of our actions are generated towards objects in motion relative to our body, whether the motion is due to the changes in position of the object, our body, or both. In such cases, different control strategies are needed, compared to when the movements are directed toward stationary objects. Psychophysical studies have shown that both the initiation and the kinematics of the movement are adjusted appropriately according to the visual information about the target motion (Lee, 1976; Lee, Port, & Georgopoulos, 1997; Port, Lee, Dassonville, & Georgopoulos, 1997; van Dankaar, Lee, & Gellman, 1992; Young & Zelaznik, 1992). Nevertheless, most previous neurophysiological investigations of the motor cortex have been focused on movements toward stationary targets, and neural mechanisms for interception of moving targets are largely unknown.

In primate brains, neural representation of visual motion (Albright, 1984; Maunsell & Van Essen, 1983) and its use in controlling eye movements (Eckmiller, 1987; Lisberger, Morris, & Tychsen, 1987) have been extensively studied. In addition, some of the anatomical substrates linking the cortical areas involved in analysis

of visual motion to the frontal areas underlying the control of arm movements have been elucidated (Wise, Boussaoud, Johnson, & Caminiti, 1997; Caminiti, Ferraina, & Johnson, 1996; Johnson, Ferraina, Bianchi, & Caminiti, 1996; Tanné, Boussaoud, Noëlle, & Rouiller, 1995), and these pathways are likely to be involved in controlling arm movements toward moving targets. In order to gain insights into the neural mechanisms underlying interception of moving targets, we examined the activity of the neurons in the primary motor cortex of the rhesus monkeys trained to produce arm movements toward moving targets.

Patterns of neural activity recorded in a single cortical area during performance of a particular behavioral task can be quite complex and heterogeneous (e.g., Chafee & Goldman-Rakic, 1998). In the present study, since activity in the motor cortex displayed complex waveforms and varied substantially across different neurons, it was difficult to develop a coherent framework of analysis based on visual inspection or comparison of activity across a set of arbitrary epochs. To overcome these problems, we applied a nonhierarchical cluster analysis to classify the neurons according to the temporal profiles of their activities. One of our goals was to determine if such methods could uncover any principles underlying the temporal patterning of the activity of motor cortical neurons

during interception of moving targets. Another goal was to determine whether signals related to the aspects of target motion that are important for successful interception are represented in selective subpopulations of neurons in the primary motor cortex. Preliminary results have been presented (Lee, Port, Kruse, & Georgopoulos, 1997).

RESULTS

Stability and Individual Differences in Performance

Two monkeys were trained in an interception task in which they were required to intercept a moving target in a predetermined location by controlling the position of a feedback cursor with their hand movements (Figure 1, Port, Lee, Kruse, & Georgopoulos, 2001). In both animals, the response times varied systematically across different target conditions. As described in the preceding paper (Port et al., 2001), movement initiation was delayed systematically for longer target motion time (TMT) in the first animal, whereas response time was relatively constant regardless of the

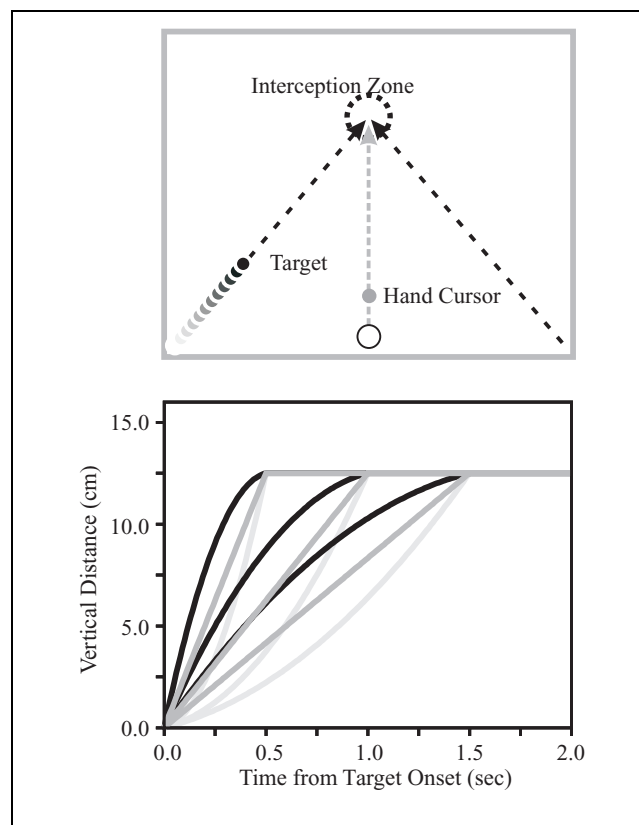


Figure 1. Top: Schematic illustration showing the spatial arrangements of the start circle, the hand cursor, the moving target, and the interception zone. Bottom: Vertical distance of the moving target from its starting position for the targets moving in constant acceleration (light gray), constant deceleration (black), and constant velocity (dark gray). All three TMT (0.5, 1.0, and 1.5 sec) are shown.

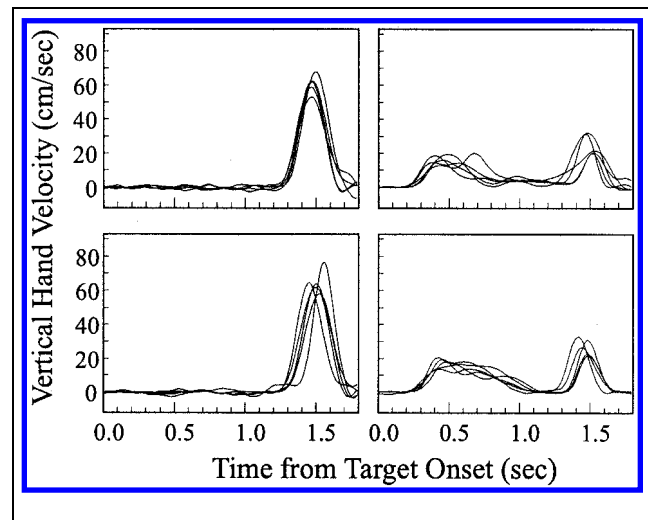


Figure 2. Vertical hand velocities in individual trials at two different phases during the course of the experiment (upper, early; lower, late), indicating that the behavior remained relatively stable in different animals over the course of the experiment. These examples are taken from the trials with 1.5-sec TMT and constant velocity.

TMT in the second animal. Accordingly, there were differences in the velocity profiles of hand movements between the two animals. In the first animal, the hand velocity profiles were symmetrical and bell-shaped in most target conditions (Figure 2, left). In the second animal, movements with symmetrical velocity profiles were found only in the trials with 0.5 sec TMT, whereas in all the other conditions the velocity profiles displayed multiple peaks. In most trials, the movement consisted of two submovements; the first submovement was generated immediately after the target onset and the second submovement was responsible for final interception of the target (Figure 2, right). These patterns were stable in both animals over the course of several weeks during which neurophysiological data were obtained (Figure 2).

Number of Neuronal Clusters

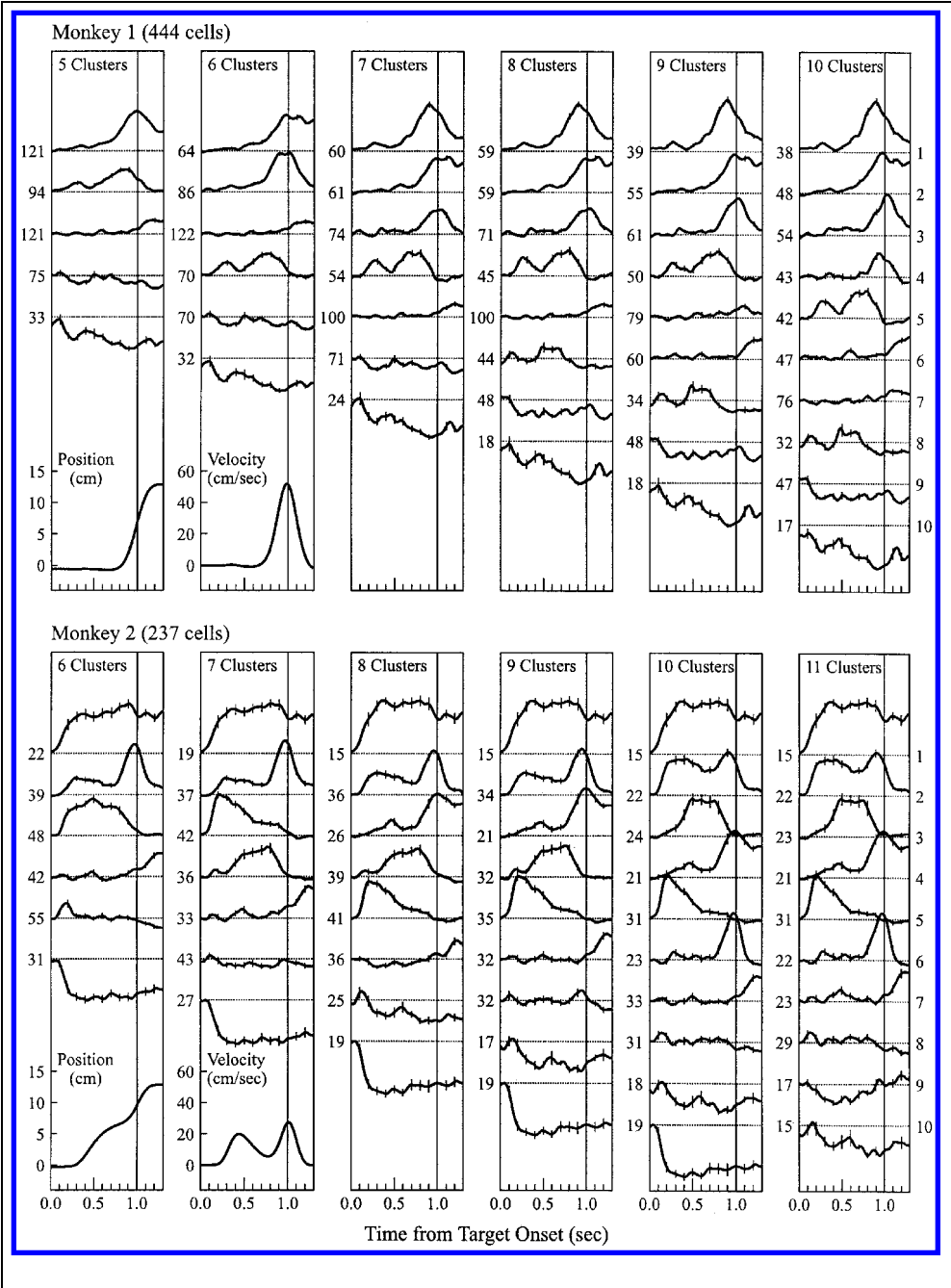
Consistent with the fact that the hand kinematics displayed a more complex pattern in the second animal, the activity of neurons recorded in the motor cortex also showed more diverse patterns in this animal. To quantify the diversity in the activity of motor cortical neurons, standardized spike density functions from all the neurons in each animal were averaged. Then, for each cell, the Euclidean distance was calculated between the average spike density function and that of a given neuron. The average of these Euclidean distances would indicate the amount of diversity present in the temporal patterns of activity among different neurons in each animal, independent of any differences in the level of overall activity. For example, if all the neurons displayed identical pattern of activity, the value of this average

distance would be zero. The value of this measure was 15% higher in the second animal (45.40) than in the first (39.49).

To examine the diverse pattern of neural activity present in the motor cortex, we applied a cluster analysis (see Methods). In this analysis, some qualitative judgments were required to decide the number of clusters. To this end, we inspected the average spike density functions (or “centroids”) of all the clusters for the number of clusters between 2 and 15. Some of these are shown in Figure 3 for the condition with 1.0-sec TMT and constant target velocity. Since there is no particular order among these different clusters, clusters are num-

bered according to the amount of activation (i.e., areas under the curves shown in Figure 3). In the present study, we focused on those clusters that increased their activity during target interception, and chose the smallest number of clusters that yielded stable patterns in these activated clusters. In the first animal, for example, the centroids of the first four clusters (1–4 in Figure 3, top) remained almost unchanged in their shape while the number of clusters was increased from 7 through 15 (shown only up to 10 clusters in Figure 3). With the number of clusters smaller than 7, some of these four clusters were unstable, therefore 7 was chosen as the number of clusters for the remaining analysis in the first

Figure 3. Average standardized spike density functions, or “centroids,” of individual clusters for different numbers of clusters. For Monkey 1 the number of clusters ranging from 5 to 10 are shown, and for Monkey 2 it ranges from 6 to 11. Only the conditions with constant velocity of the target with TMT = 1.0 sec are shown. Shown on the left of each centroid is the number of neurons assigned to the same cluster. The numbers of the far right indicate the indexes used to identify different clusters. The 11th cluster on the last column is omitted to avoid overcrowding. Also shown are the average vertical hand position (first column) and velocity (second column). Error bars represent SE.



animal. In the second animal, we selected 10 as the number of clusters, based on the same criteria (Figure 3, bottom).

To examine how unequivocally individual neurons were assigned to their clusters, we defined membership ambiguity as the distance between each neuron and its centroid divided by the distance between the same neuron and its next nearest centroid. Therefore, zero ambiguity indicates that a neuron is located precisely at the centroid of the current cluster, and the maximum ambiguity of one indicates that the neuron is located precisely halfway between the centroids of the two clusters. Distribution of membership ambiguity was quite different between the two animals (Figure 4). In the first animal, the percentage of neurons with membership ambiguity larger than .9 was 28.4% for 7 clusters, whereas it was 7.2% in the second animal for 10 clusters. This difference was not due to the difference in the number of clusters used, but rather due to the difference in the distribution of neurons in terms of their activity patterns, because similar differences were found when the comparison was made for the same number of clusters (Figure 4).

To check the reliability of clustering, we repeated the cluster analysis separately for two groups of neurons that were randomly divided, for the number of clusters for the remaining analyses. On average, 88.5% and 83.1% of the neurons were assigned to the same clusters as in the original analysis in the first and second animals, respectively. Therefore, our clustering results were rela-

tively stable despite the variability introduced in the sampling procedure.

Pattern of Activation in Different Clusters

In the first animal, five out of seven clusters were activated after the target onset, whereas in the other two clusters the activity was decreased. Out of five clusters that were activated, Clusters 1 and 4 displayed activation that began earlier than the other clusters (Figure 5, top). Activity of these two clusters diverged, however, near the movement onset. While activity of Cluster 4 began to decline near the movement onset, activity of Cluster 1 continued throughout the movement, decreasing with a time course more similar to that of the hand velocity. Activity of two additional clusters (2 and 3; Figure 5, top) lagged behind that of Clusters 1 and 4 by about 150 msec in most target conditions. Activity of Cluster 3 displayed a phasic pattern in that it declined with a time course similar to that of hand velocity, whereas activity of Cluster 2 showed a tonic pattern in which most of neuronal activity outlasted the movement. Activity of Cluster 5 showed a gradual increase throughout the movement (Figure 5, top).

Most of these activities began near the onset of the movement. However, the two clusters which showed earlier onset of activity (i.e., Clusters 1 and 4) displayed additional component in their activity immediately after the target onset. In the first animal, the movement onset was delayed substantially in most conditions with relatively long TMT (1.0 or 1.5 sec), thus making it possible to isolate the responses to the target from changes in the activity directly related to the upcoming movements. In some of these conditions, changes in activity were elicited 120 to 150 msec after the target onset in Clusters 1 and 4. The magnitude of such “early” activation was affected by the initial target velocity (Figure 6). Out of nine different target conditions, each defined by a unique combination of TMT and target acceleration, the onset of movement was substantially delayed from the target onset in five conditions (Figure 6, top panels). Of these five conditions, two conditions with the higher initial target velocities (i.e., constant velocity 1.0 sec TMT; constant deceleration 1.5 sec TMT) gave rise to “early” response in Cluster 1, whereas the other three conditions did not produce such response in the same cluster (Figure 6). In Cluster 4, the magnitude of such early response increased gradually with the initial target velocity (Figure 6). The same trend was found even when the trials where the trials with response times shorter than 0.5 sec were excluded in order to eliminate any contamination from the movement-related activation (Figure 6, bottom).

This early activation was further analyzed using more conventional methods based on the mean discharge rates of individual neurons (see Methods; Table 1). For

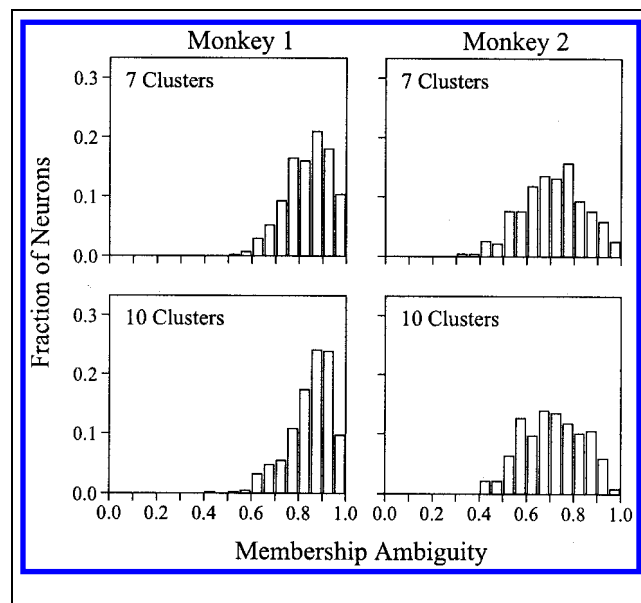
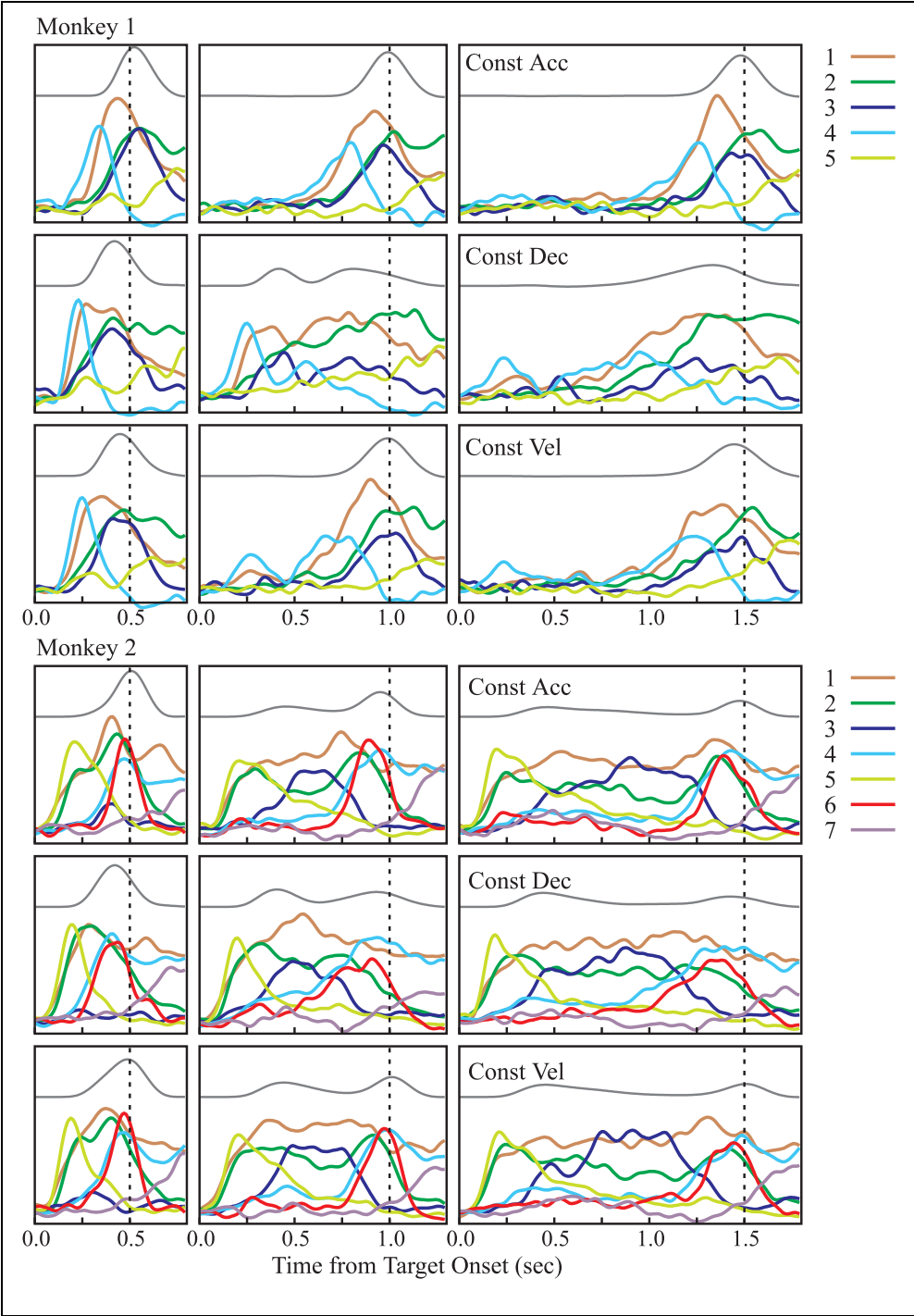


Figure 4. Top: Frequency histograms of membership ambiguity for seven clusters. Membership ambiguity was defined as the distance between the standardized spike density functions of a given neuron and the centroid of its current cluster divided by its distance to the next nearest centroid. Bottom: Frequency histograms of membership ambiguity for 10 clusters.

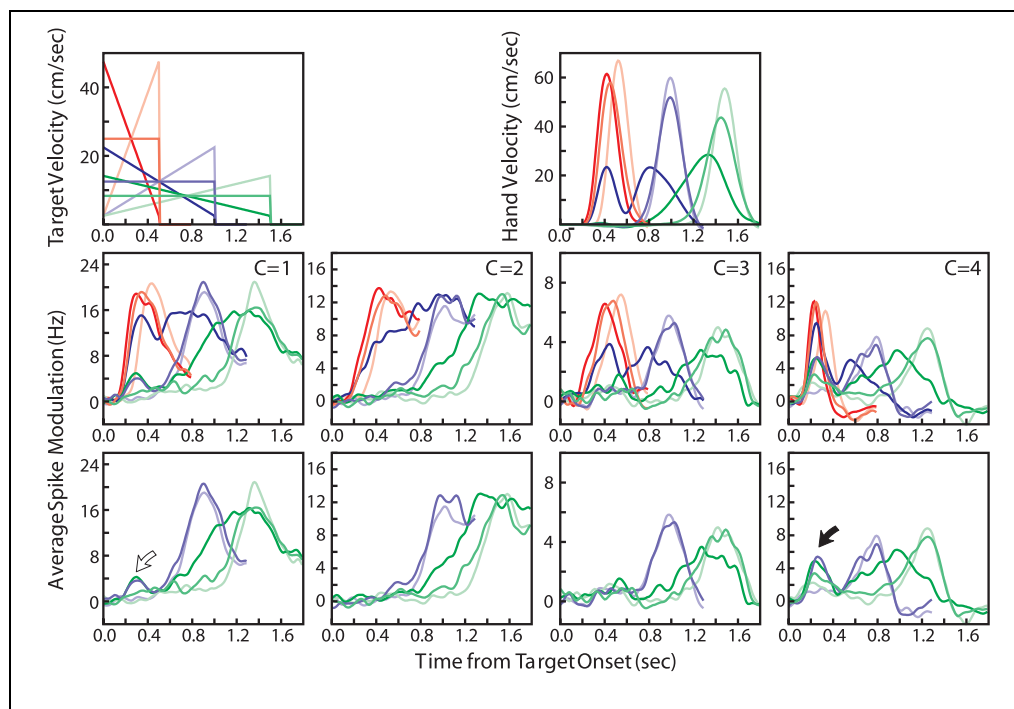
Figure 5. Average standardized spike density functions for the clusters with increased activity during target interception are shown for different target conditions. Different clusters are shown in different colors and indicated by the same numbers used in the text. In each animal, conditions with constant acceleration (Const Acc), constant deceleration (Const Dec), and constant velocity (Const Vel) are shown from the top to the bottom. Different columns correspond to different TMTs (0.5, 1.0, and 1.5 sec from the left to the right). In each panel normalized average hand velocity profiles are shown for comparison.



each neuron, we determined whether changes in the mean discharge rate during the 0.3-sec periods immediately following the target onset were statistically significant in the trials where the response time was larger than 0.5 sec. Overall, only 36 neurons (8.1%) showed significant changes in the activity during this period. On the other hand, some clusters included a substantially higher proportion of neurons with significant early activity, suggesting that this early activity was concentrated in these clusters. These results were not guar-

anteed, because our cluster analysis was applied blindly to the entire period of target motion and interception. In Clusters 1 and 4, 15% and 31% of the neurons, respectively, showed statistically significant increase during the first 0.3 sec after the target onset during the trials where the movement did not begin until 0.5 sec from the target onset, whereas on average only 3% of the neurons showed increased activity in the other clusters. Furthermore, neurons in these two clusters were more likely to change the magnitude of their early activity according to

Figure 6. Top: Target velocity profiles (left) and the average vertical hand velocity (right) for different target conditions in Monkey 1. A given target condition is indicated by the same color for all the panels in this and following figures. Middle: Average standardized spike density functions for different target conditions in the same animal. Four clusters that were activated during target interception are shown separately in different panels. Bottom: Average standardized spike density functions computed for the trials in which the response times were greater than 0.5 sec. Note that even in these trials, conditions with higher initial target velocity (constant velocity with TMT = 1.0 sec, medium blue; constant deceleration with TMT = 1.5 sec, dark green) were associated with the stronger early responses (0.2–0.4 sec from target onset) in some clusters (1, empty arrow; 4, filled arrow) compared to the other conditions.



the initial target velocity. In Clusters 1 and 4, 60.0% and 75.9% of the neurons showed positive correlation between the initial target velocity and the mean discharge rates during the initial 0.3 sec after the target onset, respectively, whereas in the other clusters the percentage of neurons with positive correlation was not statis-

tically different from the chance level as determined by the binomial probability (Table 1). In addition, in Clusters 1 and 4 the percentages of neurons with statistically significant correlation between the initial target velocity and the magnitude of the early activation (6.7% and 9.3%, respectively) were statistically different from the

Figure 7. Top: Target velocity profiles (left) and the average vertical hand velocity (right) in Monkey 2. Middle and bottom: Average normalized spike density functions in the same animal are shown for the eight clusters that were activated during target interception. Same format as in Figure 6.

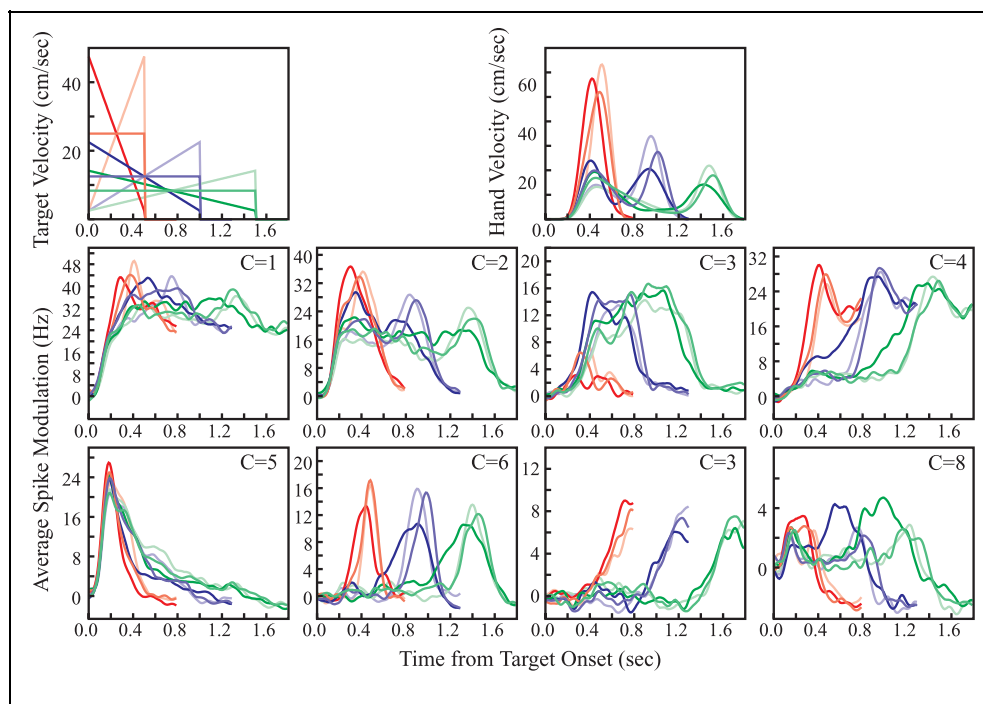


Table 1. Number of Cells With Early Activation

Cluster	1	2	3	4	5	6	7	Total
Number of neurons	60	61	74	54	100	71	24	444
Number of neurons with significant early activation	9	3	0	17	6	0	1	36
Number of neurons with positive correlation between the initial target velocity and the early activation	36	33	38	41	45	28	8	229
Number of neurons with significant effects of target velocity on the early activation	4	1	1	5	2	0	1	14

The number of clusters refer to the order of presentation in Figure 3. The clusters with early activation are indicated in **boldface**.

frequency of such neurons in the entire population (binomial test, $p < .05$).

In the second animal, activities of different clusters showed more dynamic patterns, consistent with the fact that their movements were more complex and included multiple submovements more frequently, compared to the first animal. In this animal, 7 out of 10 clusters were activated during target interception, whereas the remaining 3 were suppressed. Activation in three of the activated clusters began almost simultaneously, immediately after target onset (Figure 5, bottom). One of them (Cluster 1) sustained its activity throughout target interception and remained active even after the end of the movement, whereas another cluster (Cluster 2) decreased its activity near the end of the movement (Figure 5, bottom; Figure 7). The third cluster showed an even more phasic pattern of activity in that its activity peaked before the movement onset and declined rapidly (Cluster 5; Figure 5, bottom; Figure 7). There were two other clusters, one with tonic (Cluster 4) and the other with phasic pattern of activity (Cluster 6), in which the onset of activation was delayed consistently compared to the first group of three clusters (Figure 5, bottom; Figure 7). Activation in these two clusters began near the onset of the second submovement, but only one of them (Cluster 4) maintained its activity after the end of the movement. Another cluster (Cluster 3) was mainly activated during the period between the first and second submovements (Figure 5, bottom; Figure 7), and when the movements consisted of a single submovement in 0.5 sec TMT conditions, this cluster showed substantially attenuated activation. Finally, one cluster (Cluster 7) showed most of its activity after the end of the movement (Figure 5, bottom; Figure 7).

There are at least two possible explanations for the pattern of activation in Cluster 3 of the second animal, in which most activity was confined to the period between the two submovements (Figure 7). One possibility is that it is related to a temporary pause in the movement. Alternatively, it may be explained by a nonlinear relationship between the activity of the neurons in this cluster

and the vertical component of the hand position. To distinguish between these two possibilities, we analyzed the activity of these neurons during the center-out task. If this pattern were due to a nonlinear relationship between the neuron's activity and the vertical hand position, one would expect relatively high activity during the center hold period in the center-out task compared to the hold period after the movement in either 6 or 12 o'clock direction from the center. Our analysis showed

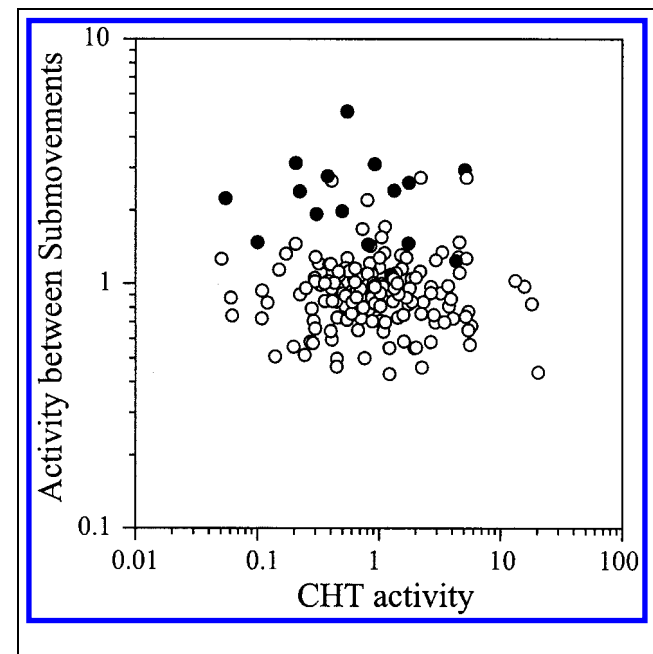


Figure 8. Relations between the activity between the submovements during target interception (ordinate) and the activity during the CHT in the center-out task (abscissa). For each neuron, the former is calculated as the mean activity during the TMTs = 1.0 and 1.5 sec divided by the average activity during the TMT = 0.5 sec, whereas the latter is calculated as the average activity during the CHT divided by the average activity during the target hold period after the movements in either 6 and 12 o'clock direction. The filled circles indicate the neurons that belong to the cluster (Cluster 3) that displayed most of its activity during the submovements. Notice that there is no relationship between these two measures either in this particular cluster or overall.

Table 2. Number of Muscles Whose Activity Patterns Were Most Closely Associated With Each Neuronal Cluster

<i>Cluster</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>	<i>10</i>
Monkey 1	1	5	2	0	0	0	1			
Monkey 2	0	3	2	4	0	3	0	0	1	1

The number of muscles associated with neuronal clusters with late activation is indicated in **boldface**.

that this was not the case (Figure 8), suggesting that the activity in Cluster 3 between the submovement is related to the pause of the movement, not merely to the intermediate vertical hand position.

Comparison of Electromyographic (EMG) Activity and Neuronal Clusters

Compared to the neurons in the motor cortex, muscle activation patterns resembled the clusters with late activation, i.e., Clusters 2 and 3 in the first animal and Clusters 4 and 6 in the second (Table 2). The percentage of muscles resembling these clusters was 77.8% (7/9 muscles) and 46.7% (7/15 muscles) in the first and second animal, respectively, whereas the corresponding percentage for the motor cortical neurons were 30.4% and 18.6%, respectively. In addition, there were some neuronal clusters that were not associated with any muscles. Of particular interest among these are Cluster 4 in the first animal, and Cluster 5 in the second animal: both of these clusters displayed early activation and their activity was phasic.

DISCUSSION

Significance of Neuronal Clusters and Individual Differences

A natural question arising in any application of cluster analysis is the nature of the boundaries among different clusters. To address this issue, we examined how unequivocally each neuron was assigned to its own cluster by comparing the distance between each neuron and the centroid of its current cluster and the distance between the same neuron and the next nearest centroid. The results were quite different between the two animals examined in the present study. In the first animal, there were many neurons with comparable distances to the centroids of multiple clusters, regardless of the total number of clusters used in the analysis, whereas in the second animal most neurons were assigned to their clusters rather unequivocally (Figure 4). We think this is ultimately related to the differences in the complexity of the kinematics between these two animals. In the first animal, the movements consisted of a single bell-shaped velocity profile in most conditions, whereas in the second animal multiple submovements were observed much more frequently. Multiple submovements are likely to engage more complex

control mechanisms, and this is reflected in more diverse and complex patterns of neural activity at the motor cortical level.

Another interesting difference in the strategy adopted by the two animals was the way in which the movement was initiated. In one animal (Monkey 1), the response time increased in most cases with the TMT, whereas in the other animal (Monkey 2) the response time remained almost constant. These results suggest that Monkey 1 relied more on information about the target motion for its decision regarding the initiation of movement than Monkey 2. Thus, in the first animal one might expect to find an aspect in the neural activity related to target motion (e.g., position or velocity) independently from that related to the movement onset. We found that in two clusters (1 and 4) identified in Monkey 1, activation began shortly after the target onset in most conditions, irrespective of whether the movement was initiated immediately or not. When movement initiation was delayed, this activity subsided and increased again before the movement onset. Stimulus-related activity in the primary motor cortex has been reported in previous studies (Riehle, 1991; Alexander & Crutcher, 1990; Wannier, Maier, & Hepp-Reymond, 1989; Kwan, MacKay, Murphy, & Wong, 1981; for a review, see Georgopoulos, 1994). On the other hand, the magnitude of the earlier activity found in this study was graded according to the initial target velocity, suggesting that this early activation reflects processing of target motion rather than simple detection of target appearance. Furthermore, the results of our cluster analysis suggest that stimulus parameters (e.g., velocity) may be represented in the activity of subpopulation of neurons in the primary motor cortex when these parameters play an important role in controlling behavioral responses.

In the second animal, all three clusters (Clusters 1, 2, and 5) that began their activation immediately after the target onset initially followed the same time course regardless of the initial target velocity, consistent with the assumption that in this animal the decision regarding the movement initiation was not so much based on the target motion, but rather on the detection of target onset. Once the movement was initiated, these clusters then revealed different activation patterns according to the movement kinematics (Figure 6), and therefore we cannot exclude the possibility that these differences are entirely due to the differences in the movement kinematics among different target conditions.

Common Features of the Neural Clusters in the Motor Cortex

Despite the above differences in the behavioral strategies and the pattern of activity in different neuronal clusters, some features were common to both animals. As mentioned in Methods section, the particular algorithm we adopted for our cluster analysis tends to generate clusters that are distributed along the axis of maximum variance. Therefore, if stable features are found in the clusters from different animals, it suggests that differences revealed by those clusters are related to dimensions that are consistently responsible for large variance in the patterns of neural activity in those animals. There were two such common features.

First, phasic and tonic patterns of activity were found in both animals. In the first animal, for example, Clusters 2 and 3 displayed similar patterns of activity in most target conditions during most of the TMT, but clear differences were found in terms of the activity remaining after the movement offset in that Cluster 2 displayed much more tonic activity. In the second animal, the same tonic–phasic pairs were found in Clusters 1 and 2 as well as in Clusters 4 and 6. The fact that motor cortical neurons display both phasic and tonic patterns of activity in relation to the generation of force ramp or actual limb movements has been known from previous studies (Ashe & Georgopoulos, 1994; Fetz, Cheney, Mewes, & Palmer, 1989; Georgopoulos & Massey, 1985; Georgopoulos, Caminiti, & Kalaska, 1984), and our results are consistent with these earlier reports. Second, some clusters were distinguished based on the onset of activation relative to either the target onset or the movement onset. This is also consistent with the previous findings that the neurons in the motor cortex display a wide range of latencies relative to the movement onset (Schwartz, Kettner, & Georgopoulos, 1988; Georgopoulos, Kalaska, Caminiti, & Massey, 1982). Also consistent with these earlier study was the fact that the EMG activities were mostly associated with the clusters that had relatively late onset of activation.

Role of the Motor Cortex in Interception of Moving Targets

Activity of neurons in the primary motor cortex has been linked to various aspects of the movements (for review, see Georgopoulos, 1994), ranging from rotation around single joints (Evarts, 1968) and generation of isometric forces (Evarts, 1969) to the multijoint drawing movements (Schwartz, 1994). Therefore, it is not surprising that activity in most of the clusters was related to the movement while displaying different characteristics in terms of amount of tonic activation, or onset of activation relative to the movement. On the other hand, some features of the clusters identified in the current study may be specifically related to the interception task itself,

which required the end of the movement to coincide with the arrival of the moving target at a particular location in space.

In the first animal, successful performance was achieved by delaying initiation of the entire movement for the longer TMT. Thus, for the shortest TMT of 0.5 sec, the movements were initiated immediately after the target onset. Such an immediate response could be triggered by a simple threshold mechanism combined with the activity of neurons that increases monotonically with the initial target velocity. Activity in some clusters in the first animal displayed systematic relationship between the initial target velocity and neural activity even in the absence of movement, and this could be a part of the mechanisms responsible for triggering the immediate initiation of the movement after the onset of high-velocity targets. The finding that signals related to initial target velocity and those related to overt behavioral responses coexist together at the level of single neurons in the primary motor cortex suggests that such decisions take place through a broad network of cortical areas including the primary motor cortex. Similar conversion of sensory signals into movement-related activity has been found in the dorsolateral prefrontal cortex during a motion discrimination task (Kim & Shadlen, 1999), suggesting that such a mechanism may be commonly employed in a variety of behavioral task.

In the second animal, it was the interval between the two submovements that was adjusted according to the TMT, whereas the first submovement was initiated immediately after the target onset in all cases. One cluster (Cluster 3) became activated mostly during the period between the two major submovements (Figure 5, bottom; Figure 7), and therefore displayed little activation in the trials with the shortest TMT of 0.5 sec where the movements followed bell-shaped velocity profiles. This pattern of activity was not due to some nonlinear effects of vertical hand position (Figure 8), and therefore, such activity may be directly related to initiation of the second submovement. At present, we do not know how the duration of this activation is controlled, or whether the primary motor cortex plays any role in controlling its duration. Since we did not find any clusters in the second animal that included a pattern of activation that could be uniquely related to some aspects of the target motion, it is possible that such a control mechanism is located outside the primary motor cortex.

Finally, in both animals, successful target interception required precise timing of a critical event, which was different for the two animals; namely, the onset of the entire movement in the first animal, and the onset of the second submovement in the second animal. The primary motor cortex may be involved with the decision as to whether the movement should be initiated immediately following the target onset, since some clusters displayed changes in their activity modulated by the initial target velocity. When the entire movement or

the submovement is not initiated immediately after the target onset, a separate “GO” signal would be needed for the successful interception. We do not have any evidence for or against the involvement of the primary motor cortex in the timing of such signal, although such decision is likely the outcome of interaction among multiple cortical areas, such as the premotor cortex and the supplementary motor area.

MATERIALS AND METHODS

Animals

Two adult rhesus monkeys (male, BW = 8–11 kg) were used in the present study. Animal care and surgical procedures have been described previously (Lurito, Georgakopoulos, & Georgakopoulos, 1991; Georgopoulos et al., 1982). They conformed to the principles outlined in “Guide for the care and use of laboratory animals” (NIH publication no. 85-23, revised 1985).

Apparatus

The monkey was seated in a primate chair and grasped the handle of a 2-D articulated manipulandum (Georgopoulos, Kalaska, & Massey, 1981). By moving the manipulandum, the animal controlled the location of a feedback cursor on a 14-in. computer monitor (Gateway 1020NI), located 57 cm from the animal’s eyes. The position of the manipulandum in *x-y* coordinates was digitally sampled at a rate of 100 Hz and a spatial resolution of 0.125 mm. The gain of the feedback cursor was set to 1, so that a centimeter displacement in the manipulandum corresponded to a degree in the visual angle at the distance of 57 cm. The eye position was monitored using the scleral search coil technique (Robinson, 1963; CNC Engineering, Seattle WA) in the first animal, and an infrared oculometer (Dr. Bouis, Karlsruhe, Germany) in the second animal.

Behavioral Paradigm

The behavioral paradigms used in the present study were described in detail in the preceding paper (Port et al., 2001). Briefly, the animal was trained to make reaching movements towards either stationary targets (the center-out task) or moving targets (interception task). In the center-out task, the monkey initiated a trial by moving the feedback cursor (0.3 cm radius disk) into a circular window (1 and 0.5 cm radius for the first and second animals, respectively) presented in the center of the screen. After a pseudorandom delay of 1–3 sec, a second target was presented 8 cm away from the initial target in one of eight directions. The animal was required to move the cursor into a positional window around the second target within 3 sec. In the interception task, a trial began when the cursor was placed in the initial target near the bottom of the screen along the

midline, and after a random delay (1–3 sec), a second target appeared at either the left or right lower corner of the screen and immediately began traversing a 45° trajectory towards an interception zone (1.2- and 1.0-cm radius for the first and the second animals, respectively) located directly above the initial target (Figure 1). The animal was required to intercept the second target with the feedback cursor within 130 msec of the target’s arrival at the interception zone. For a given trial, the acceleration type (constant acceleration, constant deceleration, or constant velocity; Figure 1) and the TMT (time from the onset of the second target to its arrival at the center of the interception zone; 0.5, 1.0, or 1.5 sec) were randomized.

Neural Recordings

After the animal had been trained to the criterion (85%), extracellular recording of single-unit activity was carried out in the motor cortex during task performance using a seven-microelectrode system (Lee, Port, Kruse, & Georgopoulos, 1998; Mountcastle, Reitboeck, Poggio, & Steinmetz, 1991). The techniques used for recording neural activity have been described previously (Port et al., 2001; Lee et al., 1998; Lurito et al., 1991).

EMG Recordings

The EMG activity was recorded during task performance using intramuscular, Teflon-coated, multistranded, stainless steel wires. The EMG signals were recorded differentially, amplified through a Grass amplifier system with an amplification of 10,000–20,000, bandpass filtered at 30–300 Hz, sampled at a rate of 1 kHz, and rectified. The sampled muscles were as follows: in the first animal, trapezius (cervical, upper), pectoralis, paraspinus, triceps (middle and lateral), posterior deltoid, forearm extensor, and forearm flexor; in the second animal, trapezius (cervical, upper, middle), rhomboid, triceps, biceps, paraspinus, supraspinatus, infraspinatus, latissimus dorsi, anterior deltoid, posterior deltoid, forearm extensor, forearm flexor, and pectoralis.

Statistical Analysis of the Effects of Target Motion on Discharge Rates

In some neurons, there were changes in activity immediately after the target onset in the interception task even when the initiation of the movement was delayed substantially. To appraise these changes statistically, we excluded all the trials in which the response time was shorter than 0.5 sec, and the mean discharge rate was calculated on a trial-by-trial basis during the two 0.3-sec periods immediately preceding and following the target onset. A paired *t* test was performed to determine whether the mean discharge rate was significantly different between these two periods. In addition, to de-

termine whether the discharge rate during the same period was systematically influenced by the initial target velocity, we calculated the correlation coefficient between the initial target velocity and the mean discharge rate during this period for each neuron. Whereas these analyses were performed only on the data from the trials with response times longer than 0.5 sec in order to exclude activity directly related to the upcoming movement, the following cluster analysis was performed on the data from all trials.

Cluster Analysis

Cluster analysis can be divided into two major categories; hierarchical and nonhierarchical. In biology, hierarchical cluster analysis has been used extensively to analyze developmental or evolutionary relationship within a data set. We used a nonhierarchical cluster analysis in our study because hierarchical methods do not guarantee an optimal solution in terms of the clustering criterion (Anderberg, 1973). Furthermore, we did not have any theoretical motive to postulate any hierarchical relationship among different neurons based on the temporal patterns of their activities.

Three important choices are made in each application of cluster analysis. First, a measure of similarity is selected to yield a numerical value reflecting how similar two elements are; second, these values are combined into a single value in the form of clustering criterion; and finally, a search method is selected to find an optimal clustering according to the clustering criterion. For the measure of similarity, we used the Euclidean distance between the standardized spike density functions of a given pair of neurons. For each trial, a spike density function (MacPherson & Aldridge, 1979; Levick & Zacks, 1970) was calculated at 10 msec resolution by applying a Gaussian kernel ($SD = 30$ msec) to the original spike train. These were then averaged separately for each combination of target acceleration type and TMT, and the baseline activity, calculated from the last 500 msec before the target onset, was subtracted. These spike density functions were then divided by their standard deviations. A Euclidean distance between neurons i and j , $d^{i,j}$, was calculated as

$$d^{i,j} = \sqrt{\sum \sum_{t \in T} (f^i(t) - f^j(t))^2},$$

where $f^i(t)$ indicates the value of the standardized spike density function of neuron i at time t , and T represents the time interval included in the analysis. This interval covers the period from target onset through 300 msec after the target's arrival at the center of the interception zone. For a given pair of neurons, this distance was calculated across all target conditions, and therefore the total duration of the time interval T above was 13.5 sec. Since the spike density function was sampled at 100 Hz,

each neuron contributed a total of 1350 data points to the cluster analysis.

Given a measure of similarity, one can still consider a variety of clustering criteria to choose from. The criterion we used was the sum of the distances between individual neurons and the average spike density functions (or, "centroid") of all the neurons in the same cluster. Traditionally, simple gradient-descending techniques have been used to search for the optimal solution according to a clustering criterion (Anderberg, 1973; Wishart, 1969). These techniques all have the same problem; namely, they cannot escape from a local minimum. To avoid this problem, we used simulated annealing (Lee & Malpeli, 1994; Kirkpatrick, Gelatt, & Vecchi, 1983). The idea behind simulated annealing is to allow changes accompanied by an increase in the criterion function (or "energy") with a certain probability. Initially, each neuron was randomly assigned to a given cluster, and its assignment was updated iteratively. In

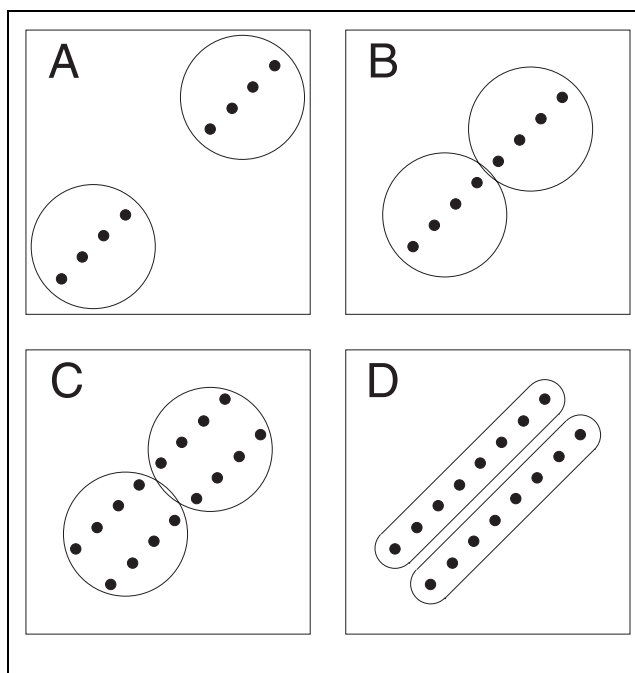


Figure 9. Results of different cluster algorithms applied to several hypothetical data sets. In these diagrams, small dots represent individual data points that are distributed in two-dimensional space. (A) Clusters can be identified relatively easily when the data points are clustered into distinct groups that are separated by a large gap. (B) When there are no natural borders among potential clusters, nonhierarchical cluster analysis based on minimum distance criterion will form clusters mostly along the axis of maximum variance (45° line in this example). (C,D) Sometimes, these two tendencies can be in conflict, for example, if a group of data points form a nonellipsoidal cluster, or "chain." Such cluster can be outlined by a method (e.g., single-linkage method) that requires every member to be more similar to some other member of the same cluster than to any other entity not in the cluster. In this case, algorithms based on minimum sum of distance (C) and single-linkage principle (D) produce different groupings.

each iteration the distance between a given neuron and its nearest centroid other than the centroid of its current cluster was calculated (d_{new}). If this distance was smaller than the distance between the same neuron and the centroid of its current cluster (d_{old}), it was assigned to the new cluster. Otherwise, the probability of assignment to the new cluster was given by $\exp(-dE/T_N)$, where dE is ($d_{\text{new}} - d_{\text{old}}$), and T_N is the "temperature" for the N -th iteration. This was repeated for all the neurons in each iteration. T_N was then lowered gradually according to a geometric schedule ($T_{N+1} = 0.97 T_N$), making a change with an increase in the criterion function increasingly more difficult. For the present analysis, the initial temperature was 10.0, which allowed more than 90% of the neurons to change their clusters.

Although cluster analysis has been used in analyzing neurophysiological data (e.g., Gallant, Connor, Rakshit, Lewis, & Van Essen, 1996; Erickson, Rodgers, & Sarle, 1993), it may be useful to discuss briefly the goals of different cluster analysis algorithms because they may produce quite different results, and the choice should be made based on the characteristics of the data set and the nature of the questions. Here, we use a series of artificial data set to illustrate several different circumstances (Figure 9). In a situation where there is obvious clustering in the data set (e.g., Figure 9A), the clustering algorithm usually provides the same result as our perceptual grouping. In fact, one benefits by relying on computerized algorithms only when the number of dimensions in the data set is greater than 2. When there are no natural borders across different clusters (e.g., Figure 9B), any clustering algorithm will still provide some clusters in such a way that is optimal for the clustering criterion that is being applied. If one tries to minimize the sum of distances, as in our current analysis, the clusters will be formed mainly along the axis that accounts for the maximum variance in the data set (Figure 9B,C). In this aspect, the cluster analysis achieves a goal similar to that of principal component analysis (PCA). However, cluster analysis has an advantage over PCA in that the average behavior of different clusters can be examined more directly. In some cases, however, such criterion could give rise to an outcome that is contradictory to what one may see as more natural grouping (e.g., C vs. D in Figure 9). Some clustering algorithm (e.g., single-linkage hierarchical clustering) requires that every member be closer to some other member in the same cluster than to any other entity not in the cluster, and can give rise to different results (Figure 9D). In this study, we adopted the minimum sum of distance criterion because this minimizes the variability within each cluster while maximizing the intercluster variability (Figure 9C).

In order to compare the patterns of EMG activity to those of the neural activity, we filtered and standardized the EMG activity in the same way as was the spike density function, and assigned each muscle to the cluster that

minimized the Euclidean distance between the centroid of the neuronal cluster and the EMG activity.

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