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Cortical cell types from spike trains

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Summary

The patterns of cell activity recorded extracellularly in the motor cortex of behaving monkeys were classified into the following three groups using a combination of cluster and discriminant analyses of 1925 spike trains: (a) cells with low discharge rate and low bursting (67.1%), (b) cells with low discharge rate but bursting (20.2%), and (c) cells with high discharge rate and low bursting (12.7%). The percentage of directionally tuned cells and of cells engaged during a memorized delay task were very similar in all three cell groups.

Introduction

Cells in the sensorimotor cortex are commonly classified into two major groups, namely output neurons and local interneurons (Ramón y Cajal, 1955; Porter, 1981; Steriade, 1978). The former are usually pyramidal and the latter stellate cells (Sloper et al., 1979; Houser et al., 1983), which in turn consist of excitatory and inhibitory cells (Sloper et al., 1979; Porter, 1981; Houser et al., 1983). The identification of the type of neuron is important for any operation performed by the cortical circuit. However, identification of the neuronal type in the behaving animal is almost impossible for it requires intracellular recording with subsequent injection of substances to stain the cell body and its processes; this has not yet been accomplished in the neocortex of the behaving monkey. Antidromic identification by electrical stimulation in different structures could identify a projection cell but not a local interneuron. A different approach relies on properties of the series of the action potentials (spike train) emitted by a cell. In intact animals, the activity of presumed local, presumably inhibitory, interneurons has been characterized by a large proportion of short interspike intervals (Steriade et al., 1974; Steriade, 1978). A significant advance in the correlation of discharge patterns, elicited by current injection, with specific cell types has been made using recordings from slice preparations of the sensorimotor cortex (McCormick et al., 1985) in which cells can be readily identified. Three basic types have been thus identified; namely, (a) cells with high frequency of discharge (presumed local inhibitory interneurons), (b) cells with low, regular frequency of discharge (presumed pyramidal cells) and (c) cells with low frequency but highly bursting discharge (presumed pyramidal cells or excitatory local interneurons) (Connors and Gutnick, 1991). Similar observations have been made in the motor cortex of the anesthetized rat (Pockberger, 1991). In the present analyses we sought to classify discharge patterns of 1925 cells recorded in the motor cortex of behaving monkeys based on properties of their spike trains.

Materials and methods

The analyses described below were performed on data recorded previously in our laboratory during the past 12 years. The activity of single cells was recorded extracellularly (Georgopoulos et al., 1982) in the arm area of the motor cortex contralateral to the perform-

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ing arm. The implantation of a recording chamber was performed aseptically under general pentobarbital (28 mg/kg body weight) anesthesia. The data used in the present analyses come from recordings in 15 monkeys. Although the behavioral tasks involved movement of the arm, the data analyzed were only those obtained while the animals held the arm in a stable posture ("control period") over a visual target before the target shifted to a peripheral location. One interspike interval distribution was analyzed for every cell ($n = 1925$ cells); it was derived from 47.3 ± 13.97 (mean $\pm$ SD) trials corresponding to 1.286 ± 0.0188 s per trial. Two analyses were performed in sequence: a cluster analysis and a discriminant analysis. In addition, standard analyses (Snedecor and Cochran, 1980) were used for statistical comparisons.

Selection of number of groups

The number of groups into which to classify cortical spike trains is somewhat arbitrary because a variety of morphological cell types exist (Porter, 1981). We chose to classify cells into three groups based on physiological observations in cortical recordings in behaving monkeys (Mountcastle et al., 1992), and on cell types described in slice (McCormick et al., 1985; Connors and Gutnick, 1991) and in vivo preparations (Pockberger, 1991) using functional and morphological techniques.

Initial cluster analysis

Our initial goal was to find out which measures of the spike train would be most effective in separating the cells into three distinguishable groups. For that purpose, we used cluster analysis based on variance-standardized Euclidean distance (Program KM, BMDP/386/Dynamic statistical package, Los Angeles, CA, 1992). Since we did not know which variables would be most effective, we used a large number of variables which were then rank-ordered with respect to their effectiveness (F test in the cluster analysis) in separating the groups. The variables tested came from three broad aspects of the spike train, namely (a) the frequency of discharge, (b) the interspike interval (ISI) distribution, and (c) the degree of bursting.

(a) One measure was used from the frequency of discharge, namely the mean frequency of discharge (spikes/s).

(b) The following eight measures were derived from the ISI distribution: (i) the mean ISI; (ii) the mode ISI; (iii) the trimean ISI (Tukey, 1977, p. 46); and (iv–viii) the percentage of ISI's below 5, 10, 15, 25, and 30 ms.

(c) The following five measures of bursting (Legéndry and Salcman, 1985) were calculated as described in Aldridge and Gilman (1991): (i) the total number of bursts; (ii) the Poisson surprise; (iii) the number of bursts per 1000 spikes; (iv) the burst rate (number of bursts/s); and (v) the burst index.

Second cluster analysis

The above 14 factors were entered in the initial cluster analysis above. The contribution of each factor in cluster separation was assessed by the value of the F test. The results obtained (see below) identified three factors as most significant: the frequency of discharge, the percentage of ISI below 20 ms, and the burst index. The cluster analysis was then repeated using these three factors to classify each cell into one of three groups.

Linear discriminant analysis

In this analysis the three factors above were used to discriminate among the three cell groups identified by the cluster analysis. There were two objectives of the discriminant analysis: first, to validate the classification obtained by the cluster analysis by calculating the percentage of cells that were classified into the same group(s) by both techniques; and second, to derive classification functions, one for each cell group, so that other cells, not contained in this sample, could be classified in one of the three groups. The BMDP statistical package (program 7M) was used for this analysis.

Other analyses

It is of interest to know whether functional or other properties of the cells studied were distributed differentially among the groups identified. For that purpose we used the χ^2 test (Snedecor and Cochran, 1980). With regard to functional properties, we compared the distribution of directionally tuned cells (Georgopoulos et al., 1982; Schwartz et al., 1988) in the three cell groups, and of cells engaged in a memorized delay task (Smyrnis et al., 1992). We also tested whether the relative proportions of cells in the three groups differed in the upper (II and III) and lower (V and VI) cortical layers. Finally, we wanted to assess the processing of directional information by the neuronal population of each cell type. For that purpose we calculated the neuronal population vector (Georgopoulos et al., 1983) within each cell group and evaluated the goodness of the prediction by calculating the angle between the population vector and the direction of the movement.

Results

Cluster analysis

The initial cluster analysis identified the mean frequency of discharge, the percentage of interspike intervals < 20 ms, and the burst index as the most important variables for separating the cells into three groups ($P < 10^{-5}$ for each variable, F test). These variables had the highest F score from those tested within each of the three broad aspects of the ISI distribution (see Methods). This analysis was then repeated using the three measures above to classify each of the, 1925 cells into one of three groups.

Discriminant analysis

This analysis validated the results of the clustering analysis: its concordance with the clustering analysis was 97.6% which means that a cell was assigned to the same group by both analyses in 97.6% of cases. The following classification functions were obtained:

$$\text{Type A: } C = -1.8187 + 0.1346X + 0.0424Y + 0.0870Z \quad (1)$$

$$\text{Type B: } C = -10.6262 + 0.1060X + 0.0776Y + 1.6321Z \quad (2)$$

$$\text{Type C: } C = -13.1596 + 0.4255X + 0.3238Y - 0.0684Z \quad (3)$$

where C is the classification function, X is the mean frequency of discharge, Y is the percentage of interspike intervals < 20 ms, and Z is the burst index. Given X, Y and Z for a specific cell, C can be calculated for each group and the cell classified to the group that yields the largest C.

The group means $\pm$ SD are given in Table 1. The pairwise separation of the three groups was statistically highly significant ($P < 10^{-5}$ for each comparison, multivariate Hoteling's T^2 test). It can be seen in Table 1 that type A cells (67.1%) had low discharge rate and low bursting; type B cells (20.2%) had low but bursting discharge rate; and type C cells (12.7% of cells) had high discharge rate with low bursting. Examples are shown in Figs. 1 and 2.

TABLE 2

Distribution of cell types in upper and lower cortical layers

	Type A	Type B	Type C	Total
Upper layers (II–III)	36 (64.3)	18 (32.1)	2 (3.6)	56 (100.0%)
Lower layers (V-VI)	61 (66.3)	23 (25.0)	8 (8.7)	92 (100.0%)
Total	97	41	10	148

Laminar distribution

In a subset of 148 cells the location within the cortical layers was histologically identified (Georgopoulos et al., 1984). The laminar distribution of the cell types identified above is given in Table 2. This distribution did not differ among the upper (II–III) and lower (V–VI) layers ($P < 0.36$, χ^2 test).

Directional tuning

The prevalence of directional tuning was similar in the three cell groups identified (Table 3), although the percentage of tuned cells was slightly lower in the type B group than in the other two groups. This difference was statistically significant ($P < 0.02$, χ^2 test).

Memory signal

The prevalence of a directional memory signal (Smyrnis et al., 1992) changes in cell activity during a memorized delay period were observed in all three cell groups (Table 4) ($P < 0.11$, χ^2 test).

Neuronal population vector analysis

The neuronal population vector (Georgopoulos et al., 1983, 1986) predicted equally well the direction of

TABLE 1

GROUP MEANS ($\pm$SEM) OF THE VARIABLES USED FOR CLASSIFICATION

	Type A	Type B	Type C
Mean frequency of discharge (spikes/s)	8.565 $\pm$ 0.242	4.349 $\pm$ 0.199	27.760 $\pm$ 0.908
Percentage of interspike intervals < 20 ms	6.192 $\pm$ 0.192	19.445 $\pm$ 0.633	38.282 $\pm$ 1.125
Burst index	0.589 $\pm$ 0.039	10.457 $\pm$ 0.233	1.070 $\pm$ 0.111
n = 1925 (100%)	1 292 (67.1%)	388 (20.2%)	245 (12.7%)

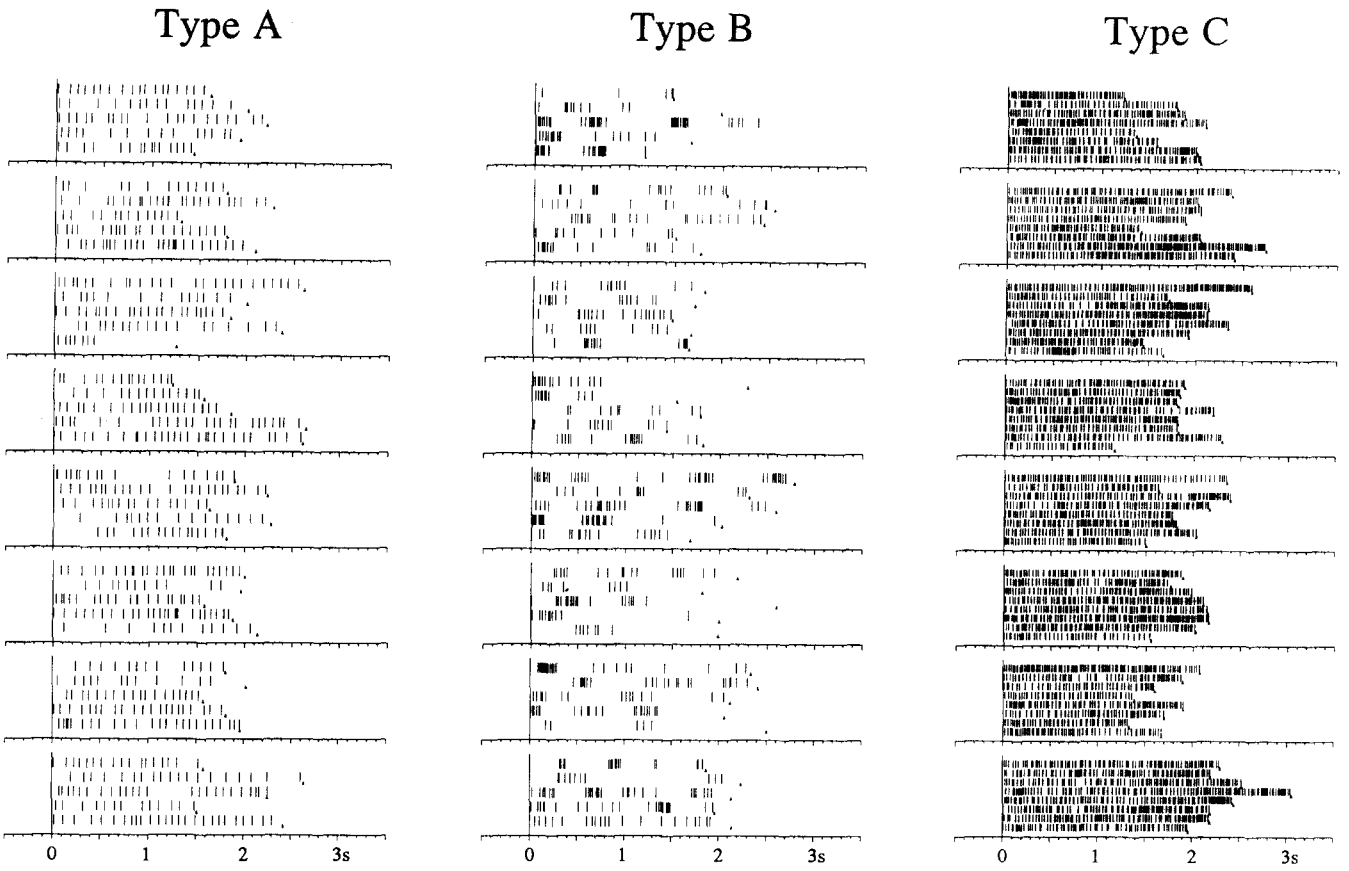

Fig. 1. Typical examples of the three types of cells identified. Each raster represents spike activity in one trial; 40 trials are shown for types A and B, and 64 for type C. Rasters start (time 0) at the onset of the period used for analyses ("control period", see Methods) and end at the end of that period.

movement within each group. For example, the average absolute angle between the direction of the population vector and the direction of movement in two-dimensional space was (mean $\pm$ SD, $n = 8$ directions) $4.90 \pm 3.47°$, $7.11 \pm 3.94°$ and $5.14 \pm 2.47°$ for type A, B, and C, respectively.

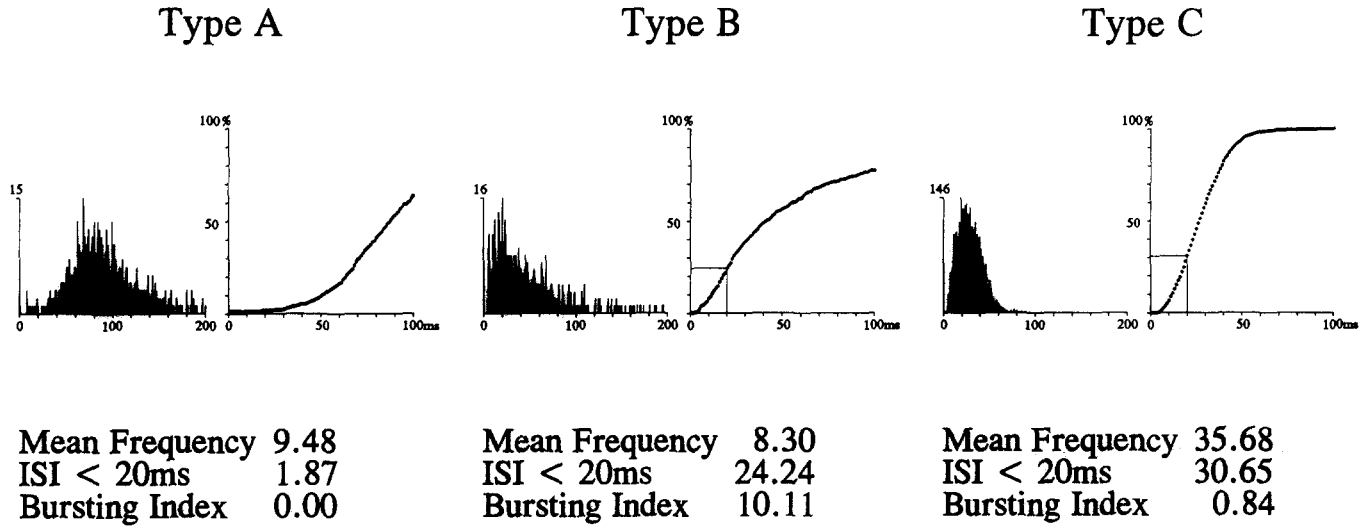

Fig. 2. Interspike intervals and cumulative functions are shown for the data illustrated in Fig. 1. ISI, interspike interval.

Discussion

The results of this study provide a way to classify motor cortical cells into three well differentiated groups. The value of this analysis comes from the fact that the classification functions above were derived from a large database ($n = 1925$ cells) and therefore the resulting classification of specific cells should be quite reliable. The characteristics of the three cell types distinguished by the present analyses are essentially those observed in the spontaneous activity of cortical neurons in waking monkeys (Mountcastle et al., 1992).

Methodological considerations

An important methodological consideration of this study concerns the behavioral conditions under which the cells analyzed were recorded. These conditions were motorically stable; i.e., the impulse activity of cells was recorded while monkeys held a steady posture with the contralateral arm and exerted a constant force during a waiting period preceding an aimed movement to a target. Under these conditions the impulse activity of a cell reflects intrinsic membrane properties as well as a stable synaptic input. Therefore, the results of this study relate to both of these factors and, through them, are distinguished from the results of other studies in which the classification of cortical cells was addressed. For example, in studies of patterns of firing in slice preparations (McCormick et al., 1985; Connors and Gutnick, 1991) or in anesthetized animals (Pockberger, 1991), injections of current were used, whereas in studies in awake animals the behavioral conditions of "quietness" (Evarts, 1965) or "wakefulness" (Steriade, 1978) are not sufficient to define a stable motor output.

Another methodological consideration pertains to the analyses used for classification. First, the methods applied are established statistical techniques. Second, the choice of measures of the spike train on which the analyses were based are reasonable, for they cover the basic aspects of impulse activity, namely frequency of discharge, interval distribution, and degree of bursting. These are characteristics on which qualitative classifications have been based (Mountcastle et al., 1992). Finally, the decision to classify cells into a minimum of three types was based on physiological observations in cortical recordings in behaving monkeys (Mountcastle et al., 1992), on cell types described in slice (McCormick et al., 1985; Connors and Gutnick, 1991) and in in vivo preparations (Pockberger, 1991), and on the application of "Occam's razor" which states that "Entities are not to be multiplied without necessity" (see Russell, 1945, p. 472). Although cells could be classified in a large number of categories, we believe that there is merit in the approach we followed in this study, namely to keep this number small: it is interesting to know whether cells can be separated in *at least* three groups. This is a needed validation for possibly additional subclassification. Our analyses indeed provided a clear distinction among three cell groups: one with low frequency of discharge and low bursting (type A), one with low frequency of discharge but bursting (type B), and one with high frequency of discharge and low bursting (type C).

TABLE 3

NUMBERS OF DIRECTIONALLY TUNED AND NON-TUNED CELLS IN THE THREE GROUPS IDENTIFIED

	Type A	Type B	Type C	Total
Tuned	978 (75.7)	266 (68.6)	179 (73.1)	1423
Not-tuned	314 (24.3)	122 (31.4)	66 (26.9)	502
Total	1292 (100%)	388 (100%)	245 (100%)	1925

TABLE 4

NUMBERS OF CELLS IN THE THREE GROUPS WITH AND WITHOUT CHANGES IN ACTIVITY DURING A MEMORIZED DELAY TASK

	Type A	Type B	Type C	Total
Cells that changed activity	49 (42.2)	14 (56.0)	9 (69.2)	72
Cells that did not change activity	67 (57.8)	11 (44.0)	4 (30.8)	82
Total	116 (100%)	25 (100%)	13 (100%)	154

Relation to histologically or antidromically identified cell types

Ideally, one would like to establish a firm relation between the cell types above and those types identified in other studies. However, comparisons can only be tentative for various reasons. First, we did not stain the cells we studied and therefore we do not know their histological type. Second, we did not record intracellularly and therefore we have no data on cell responses to injection of current, as done in slice (McCormick et al., 1985; Connors and Gutnick, 1991) or in in vivo preparations (Pockberger, 1991). Third, we did not attempt to activate cells antidromically, and therefore we do not know which might have been pyramidal tract neurons. Finally, we analyzed cell activity during a

stable motor posture which was better defined behaviorally than "quietness" or "wakefulness" mentioned in other studies (Evarts, 1965; Steriade, 1978). Since motor cortical activity does relate to the status of the motor system and since this status is ill defined under the last two conditions, spike train properties of cells recorded during simply quietness or wakefulness may not be very comparable to our data which were recorded during a repeatable, stable motor posture.

Nevertheless, some statements can be made concerning our cells. First, given the bias of metal microelectrodes for larger cells (Humphrey and Corrie, 1978), it is probable that a large percentage of our sample consists of pyramidal cells. Second, the fact that the distribution of cells types we identified did not differ between upper and lower layers indicates that none of our three cell types belongs specifically to pyramidal tract neurons, since these are located in layer V. Third, a common feature of the discharge of putative inhibitory interneurons in the neocortex has been the high percentage of short interspike intervals (Steriade, 1978); conversely, such neurons do not seem to have a low frequency, non-bursting discharge. Therefore, it is unlikely that this latter type (type A) in our classification would correspond to inhibitory interneurons. Given the considerations above, it is reasonable to suppose, although by no means proven, that type A probably corresponds to pyramidal cells, whereas types B and C would comprise both interneurons and pyramidal cells.

Functional properties of cell groups identified using spike train analysis

The cell classification achieved in this study opens the possibility of testing specific hypotheses concerning intracortical processing. Indeed, we used the results of this analysis to answer three questions concerning the processing of directional information in the motor cortex. We wanted to know, first, whether directional tuning (Georgopoulos et al., 1982) is more prevalent in some of the cell types; second, whether the neuronal population vector (Georgopoulos et al., 1983, 1986) can provide accurate information about the direction of movement when calculated within each one of the three cell types; and third, whether a directional memory signal (Smyrnis et al., 1992) is present in some cell types only. We found that the proportions of tuned and non-tuned cells were similar in the three cell types (Table 3), although the percentage of tuned cells was slightly lower in Type B than in the other two types. The neuronal population vector predicted equally well the direction of movement within each type. Finally, changes in cell activity during a memorized delay period were observed in all three cell types (Table 4). These results show that directional and memory processing is truly distributed among the different cell types. This remains to be seen for other cases of neuronal population operations (Steinmetz et al., 1987; Young and Yamane, 1992).

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References

Aldridge, J.W. and Gilman, S. (1991) The temporal structure of spike trains in the primate basal ganglia: afferent regulation of bursting demonstrated with precentral cerebral cortical ablation. Brain Res., 543: 123–138.

Caminiti, R., Johnson, P.B., Galli, C., Ferraina, S. and Burnod, Y. (1991) Making arm movements within different parts of space: The premotor and motor cortical representation of a coordinate system for reaching to visual targets. J. Neurosci., 11: 1182–1197.

Connors, B.W. and Gutnick, M.J. (1990) Intrinsic firing patterns of diverse neocortical neurons. Trends Neurol. Sci., 13: 99–104.

Evarts, E.V. (1965) Relation of discharge frequency to conduction velocity in pyramidal tract neurons. J. Neurophysiol., 28: 216–228.

Georgopoulos, A.P., Kalaska, J.F., Caminiti, R. and Massey, J.T. (1982) On the relations between the direction of two-dimensional arm movements and cell discharge in primate motor cortex. J. Neurosci., 2: 1527–1537.

Georgopoulos, A.P., Caminiti, R., Kalaska, J.F. and Massey, J.T. (1983) Spatial coding of movement: a hypothesis concerning the coding of movement direction by motor cortical populations. Exp. Brain Res., Suppl., 7: 327–336.

Georgopoulos, A.P., Kalaska, J.F., Crutcher, M.D., Caminiti, R. and Massey, J.T. (1984) The representation of movement direction in the motor cortex: single cell and population studies. In: G.M. Edelman, W.E. Gall and W.M. Cowan (Eds.), Dynamic Aspects of Neocortical Function, John Wiley and Sons, New York, pp. 501–524.

Georgopoulos, A.P., Schwartz, A.B. and Kettner, R.E. (1986) Neuronal population coding of movement direction. Science, 233: 1416–1419.

Houser, C.R., Hendry, S.H.C., Jones, E.G. and Vaughn, J.E. (1983) Morphological diversity of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J. Neurocytol., 12: 617–638.

Humphrey, D.R. and Corrie (1978) Properties of the pyramidal tract neuron system within a functionally defined subregion of primate motor cortex. J. Neurophysiol., 41: 216–243.

Jones, E.G. and Wise, S.P. (1977) Size, laminar and columnar distribution of efferent cells in the sensory-motor cortex of monkeys. J. Comp. Neurol., 175: 391–438.

Kalaska, J.F., Caminiti, R. and Georgopoulos, A.P. (1983) Cortical mechanisms related to the direction of two-dimensional arm

movements: relations in parietal area 5 and comparison with motor cortex. Exp. Brain Res., 51: 247–260.

Legéndry, C.R. and Salcman, M. (1985) Bursts and recurrences of bursts in the spike trains of spontaneously active striate cortex neurons. J. Neurophysiol., 53: 926–939.

McCormick, D.A., Connors, B.W., Lighthall, J.W. and Prince, A.D. (1985) Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. J. Neurophysiol., 54: 782–806.

Mountcastle, V.B., Atluri, P.P. and Romo, R. (1992) Selective output-discriminative signals in the motor cortex of waking monkeys. Cereb. Cortex, 2: 277–297.

Pockberger, H. (1991) Electrophysiological and morphological properties of rat motor cortex neurons in vivo. Brain Res., 539: 181–190.

Porter, R. (1981) Internal organization of the motor cortex for input-output arrangements. In: J.M. Brookhart, V.B. Mountcastle, V.B. Brooks and S.R. Geiger (Eds.), Handbook of Physiology, Section 1: The Nervous System, Volume II: Motor Control, Part 2, American Physiological Society, Bethesda, MD, pp. 1063–1081.

Ramón y Cajal, S. (1955) Histologie du Système Nerveux, Tome II, Instituto Ramón y Cajal, Madrid.

Russell, B. (1945) A History of Western Philosophy. Simon and Shuster, New York.

Schwartz, A.B., Kettner, R.E. and Georgopoulos, A.P. (1988) Primate motor cortex and free arm movements to visual targets in three-dimensional space. I. Relations between single cell discharge and direction of movement. J. Neurosci., 8: 2913–2927.

Sloper, J.J., Hiorns, R.W. and Powell, T.P.S. (1979) A qualitative and quantitative electron microscopic study of the neurons in the primate motor and somatic sensory cortices. Phil. Trans. R. Soc. Lond. B, 285: 141–171.

Smyrnis, N., Taira, M., Ashe, J. and Georgopouilos, A.P. (1992) Motor cortical activity in a memorized delay task. Exp. Brain Res., 92: 139–151.

Snedecor, G.W. and Cochran, W.G. (1980) Statistical Methods, 7th edn., Iowa State University Press, Ames, IA.

Steinmetz, M.A., Motter, B.C., Duffy, C.J. and Mountcastle, V.B. (1987) Functional properties of parietal visual neurons: radial organisation of directionalities within the visual field. J. Neurosci., 7: 177–191.

Steriade, M. (1978) Cortical long-axoned cells and putative interneurons during the sleep-waking cycle. Behav. Brain Sci., 3: 465–514.

Steriade, M., Deschênes, M. and Oakson, G. (1974) Inhibitory processes and interneuronal apparatus in motor cortex during sleep and waking. I. Background firing and responsiveness of pyramidal tract neurons and interneurons. J. Neurophysiol., 37: 1065–1092.

Tukey, J.W. (1977) Exploratory Data Analysis. Addison-Wesley, Reading, MA.

Young, M.P. and Yamane, S. (1992) Sparse population coding of faces in the inferotemporal cortex. Science, 256: 1327–1331.