transmitting junctions that have been investigated (*1*). The slow EPSP in frog sympathetic ganglion cells thus appears to be the first example of a postsynaptic potential generated by an inactivation of membrane conductance. It has recently been reported that acetylcholine acting on muscarinic receptors causes a depolarization of cortical neurons that is not associated with an increased membrane conductance (*27*), and norepinephrine causes a hyperpolarization of cerebellar Purkinje cells that is associated with an increase in membrane resistance (*28*). Although the mechanism of such phenomena needs further elucidation, the similarity of those responses to the slow EPSP in sympathetic ganglion cells suggests that synaptic inactivation of membrane conductance may be a mechanism of general significance in the regulation of neuronal activity.

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11. In Fig. 1B, part 2, the bridge had been previously balanced in such a way that the hyperpolarizing current pulses did not produce a voltage deflection. This method indicates any change in membrane resistance (ΔR). During the slow EPSP, the —0.5 na hyperpolarizing current pulses produced a hyperpolarizing voltage deflection of 1 mv, indicating that the input membrane resistance had increased by 2 megohms. This increase in resistance was consistently observed and lasted for the duration of the slow EPSP. In this cell, the slow EPSP lasted for 50 seconds beyond the end of the oscilloscope sweep shown; during this time the resistance returned to the level before stimulation. In other experiments, without nicotine blockade, we have found [F. Weight and J. Votava, *Pharmacologist* **12**, 225 (1970)] that after repetitive stimulation of B fibers the mechanism of increased conductance of the fast EPSP lasts many seconds. In this cell, the increased resistance was not maximum during the early part of the slow EPSP, presumably because the fast EPSP (with increased conductance) was not totally blocked by nicotine. In other cells, however, the peak of the increased resistance occurred at the peak of the slow EPSP.
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13. With hyperpolarizing current the current-voltage relationship of the ganglion cell membrane is approximately linear (*8*); however, with depolarizing current we have found that most bullfrog ganglion cells have an increased membrane conductance (delayed rectification). Figure 1E shows that the size of the slow EPSP was approximately linearly related to hyperpolarizing current. Presumably, the falloff in size of the slow EPSP from a linear slope with increasing depolarizing current is due to delayed rectification.
14. In Fig. 1E, the slow EPSP reversed with a hyperpolarizing current of —0.55 na. From the current-voltage relationship of Fig. 1D it can be seen that a current of —0.55 na produced a voltage deflection of approximately —23 mv below resting membrane potential. Since the resting potential of this cell was —65 mv, the reversal potential of the slow EPSP was approximately —88 mv.
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17. In Fig. 1D, the after-hyperpolarization reversed with hyperpolarizing currents between —0.5 na and —0.6 na. From the current-voltage relationship in Fig. 1D, the reversal potential of the after-hyperpolarization was near —88 mv (*14*).
18. The composition of the Ringer solution was: Na_2SO_4, 78 mmole/liter; K_2SO_4, 1.6 mmole/liter; $NaHCO_3$, 2 mmole/liter; $CaSO_4$, saturated; and glucose, 1 g/liter.
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21. In most nerve cells the predominant resting ion conductances are K^+ and Na^+; however, there may also be some leakage conductance ions such as Cl^- (*15*, *20*).
22. The equilibrium potential for the fast EPSP is approximately —10 mv (*6*). There is some evidence that increased Na^+ conductance predominates, but there may also be some increase in K^+ conductance associated with the fast EPSP (*7*).
23. In most membrane circuit diagrams, E_m represents the electromotive force of all resting conductances including E_K. In Fig. 2C, however, E_K is represented separately, and E_m represents the electromotive force for the other resting conductances. Since this would be g_{Na} and leak conductances, E_m is indicated with positive inside and negative outside.
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Predicting Measures of Motor Performance from Multiple Cortical Spike Trains

Abstract. *Recordings have been obtained simultaneously from several, individually selected neurons in the motor cortex of unanesthetized monkey as the animal performed simple arm movements. With the use of comparatively simple quantitative procedures, the activity of small sets of cells was found to be adequate for rather accurate real-time prediction of the time course of various response measurements. In addition, the results suggest that hypotheses concerning the response variables "controlled" by cortical motor systems may well depend upon whether or not the temporal relations between simultaneously active neurons are taken into account.*

The firing patterns of single neurons in the cerebral or cerebellar cortices of unanesthetized animals during the performance of conditioned motor responses have been described in several reports (*1*, *2*). In each of these studies, the basic experimental approach has been the same; recordings have typically been obtained from one cell at a time, and attention has been focused on the extent to which the temporal discharge pattern of a given single unit, or class of sequentially observed units, might convey information concerning the intensity and time course of the animal's movements. Evarts' studies (*2*) of the activity of pyramidal tract (PT) neurons during conditioned hand movements in the monkey provide an excellent example of this type of approach and the kinds of data derived from its use. We now describe a somewhat different approach and present data which suggest that information about a given movement is carried not simply in the discharge patterns or spike trains of individual cortical neurons but to a significant extent by the temporal relations between them.

Our experiments are related to the general question of whether neuronal spike trains might be used for quantitative prediction of simple motor responses (*3*). In particular, we were interested (i) in determining which of the measurements associated with simple arm movements (such as arm

position, velocity, net force exerted about a joint, or the derivative of force with respect to time) appeared the most easily predictable from the activity of cortical cells and (ii) in the relative accuracy of predictions based on single neuron spike trains when compared with those derived from the combined spike trains of several cells. Accordingly we used a recently developed multiple microelectrode system (*4*) to record *simultaneously* from several, individually selected neurons in the motor cortex of the unanesthetized monkey as the animal performed simple arm movements.

We chose a behavioral task similar to that used by Evarts. The monkey was trained to insert his hand through a narrow plastic sleeve, grasp a vertically oriented handle, and move it from side to side by alternate flexion-extension of his wrist (Fig. 1A); a pulley system allowed us to weight the handle with various loads, which opposed either flexion or extension. Displacements of the handle and the force exerted upon it were monitored by appropriately placed transducers which yielded estimates of the net flexor or extensor torque exerted by the animal and the angular position of his wrist. The displacement signal was fed to a comparator circuit, which energized one of two small lights in front of the animal when he flexed or entended his wrist to within ± 5° of a 30° angular displacement. The monkey was required to energize one of the lights and to maintain that wrist position for 0.5 to 1.0 second before moving to the second position and obtaining a fruit juice reward (*5*). As the animal performed the required movements, five tungsten microelectrodes (2 to 4 μm in tip diameter) were inserted into a cortical area (2 by 3 mm), centered at the forearm region of the contralateral motor cortex (*6*). The electrodes were independently adjustable, and each was advanced until it recorded the activity of one or two distinct single units, with discharge frequency changes that appeared to begin prior to, but to cycle with, variations in the force and position traces. When all electrodes had been positioned, tape recordings of the unit and response data were obtained for 30 to 150 flexion-extension cycles under each of five different load conditions (0, 100, and 300 g opposing flexion and opposing extension).

The data were subsequently read into a computer, which counted the number of spikes per 50 msec for each

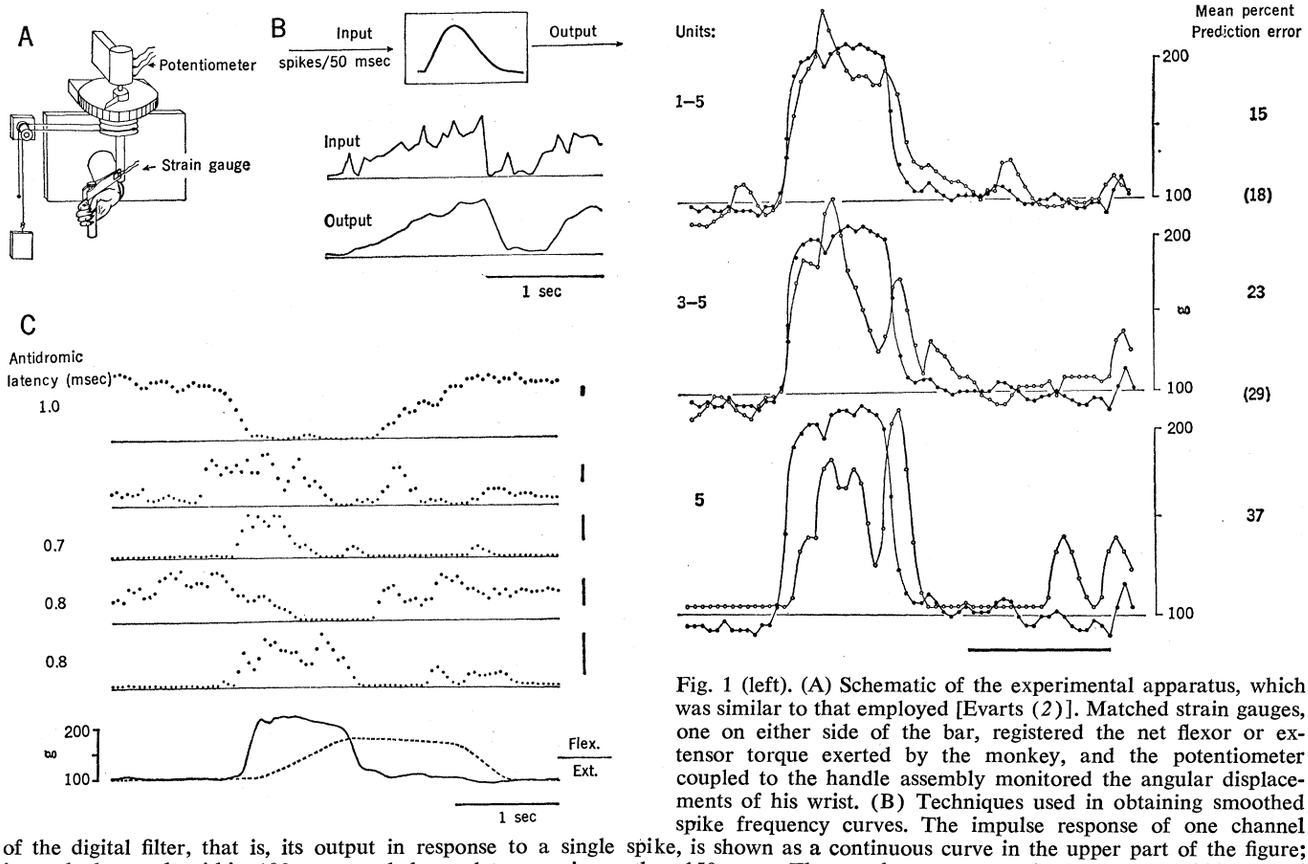

Fig. 1 (left). (A) Schematic of the experimental apparatus, which was similar to that employed [Evarts (*2*)]. Matched strain gauges, one on either side of the bar, registered the net flexor or extensor torque exerted by the monkey, and the potentiometer coupled to the handle assembly monitored the angular displacements of his wrist. (B) Techniques used in obtaining smoothed spike frequency curves. The impulse response of one channel of the digital filter, that is, its output in response to a single spike, is shown as a continuous curve in the upper part of the figure; it reached a peak within 100 msec and decayed to zero in another 150 msec. The two bottom traces show segments of the actual input and output spike frequency series for one PT cell. (C) Smoothed spike frequency is plotted against time (dotted curves) for one set of five simultaneously recorded cortical units during a single movement. The vertical bars to the right of each trace represent 10 spikes/sec. Four of the units were PT cells, and their antidromic response latencies are shown to the left. The lower curves are tracings of the force (solid line) and displacement (dashed line) measurements during the movement. In this case 100 g opposed flexion (upward deflection in displacement trace). Fig. 2 (right). An example of the accuracy in predicting the time course of the force trace as a function of the number of simultaneously observed spike trains used in the prediction equation. In each case, the dots represent the observed force values and the open circles those predicted. The movement is the same in each case, with the slightly different amplitudes resulting from the computer plotting routine which scaled the combined predicted and observed values in each case to the same peak to peak range. The numbers to the left indicate the units used in the prediction equation, which were the same as those yielding spike frequency curves 1 through 5 (reading from the top) in Fig. 1C. The numbers on the right show the average prediction error (percentage) in each case; for this particular movement, the numbers in parentheses represent the cases computed but not illustrated (predictions based on units 2 to 5 and 4 to 5, respectively). As in the previous figure, 100 g opposed flexion.

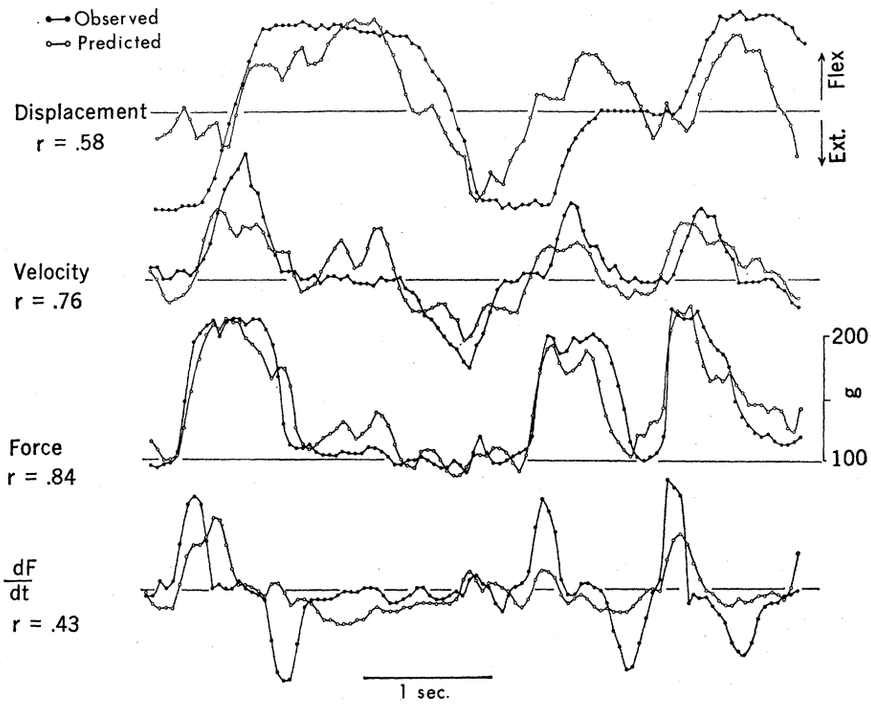

Fig. 3. An example of the predicted and observed response measurements during one and a half single-movement cycles with 100 g opposing flexion. The correlation coefficients between the combined neuronal spike train data (predicted curves) and the observed response measurements are shown to the left. The correlation was highest for the force trace, but it was also appreciable and statistically significant ($P < .001$, d.f. = 98) for velocity and displacement.

unit and sampled the values of the force and displacement traces at 50-msec intervals. The spike counts were then digitally filtered, yielding a smoothed version of spike frequency over time (t) for each unit (Fig. 1B). From cross-correlation functions (*7*) computed with some of our initial data, we found the spike frequency and response measurements to correlate most highly when the latter were shifted backward in time by an average of 100 msec. The displacement and force (F) measurements were shifted accordingly, and velocity and dF/dt were computed from these by numerical differentiation. From such data we attempted to derive empirical transfer functions that would allow a given set of spike trains to be used for real-time (but off-line) prediction of the simultaneously recorded response measurements. A number of techniques from time series analysis were used, including Wiener filtering procedures (*7*). However, none of these techniques yielded significantly more accurate predictions than those which were obtained by simply multiplying the smoothed discharge frequency of each cell by a constant coefficient and then summing the resulting values across the set of units at each point in time.

Optimal coefficients were determined by the use of multiple regression procedures (*8*) to find those constants (a_o,a_i) which maximized the prediction accuracy of the equation

$$\phi(t) = a_o + \sum_i a_i U_i(t)$$

for a particular set of cells. Here, t represents time, $\phi(t)$ the value of the response measurement to be predicted, and $U_i(t)$ the discharge frequency of the ith unit in the set. Sample distributions of coefficients were obtained for each response variable, with the use of only a few short segments of the initial data obtained under each load condition. Mean or modal values were derived from these and used with subsequently recorded data from the same set of cells to predict the concurrent response measurements (*9*). In several cases we used the coefficients calculated from data obtained under a particular load condition in estimating responses recorded 30 minutes to 1 hour later, when the animal was again subjected to that condition. By comparing predicted and observed measurements we could thus gain an indication of the accuracy of our procedures and whether the unit-motor response relations were relatively stationary over the period of observation.

To date, we have recorded from 29 sets of three to eight simultaneously observed units ($N = 135$) in three monkeys. Cells were identified as PT or non-PT by antidromic stimulation of the medullary pyramid in only one monkey. Of the 43 units recorded from in this animal, 23 were classified as PT cells by the usual criteria (*10*). All of these had antidromic response latencies of less than 3 msec, and were thus among the larger, more rapidly conducting members of the PT cell population. By extrapolation, we would estimate that a third to one-half of the units in our sample consisted of such cells. It has been our experience, however, that non-PT (possibly extrapyramidal) neurons may exhibit activity as highly correlated with a movement as do PT cells, and for our purposes the magnitude and reliability of these correlations were the variables of interest (*11*).

The spike frequency curves from one set of five simultaneously recorded units are shown for a single movement in Fig. 1C. Although each of these units exhibited changes in discharge frequency during certain phases of the movement, the correlations between these and the various response measurements were often neither impressively high nor stable. For example, from correlation matrices computed for each of 20 flexion-extension cycles, the largest mean correlation was found to be that between the discharge frequency of the first unit and the force trace; although its value (-0.62) was statistically significant ($P < .01$, d.f. = 58), the coefficients for individual movements varied considerably (range $= -0.31$ to -0.86, S.D. $= 0.18$). Thus, the discharge pattern of each of these units provided a reasonably good indication of the occurrence of a movement; but none, in isolation, yielded accurate or reliable estimates of a given response measurement over the course of several movements. Our statistical analyses of single spike trains have consistently yielded such results, and for several reasons they might have been anticipated. Although it was not emphasized, the records published in previous similar studies (*2*) show quite clearly that the quantitative relations between single-unit spike trains and a given response parameter are apt to vary considerably from one movement to the next. Moreover, even a simple flexion-extension of the wrist requires the coordi-

nated interplay of several muscles, and our response measurements provided only a rough estimate of the net result. Finally, thousands of cortical neurons must be involved in control of such a movement, and one would hardly expect the activity of any one of these to reflect the time course of the population response.

In view of these considerations, the results of our multiple unit analyses were somewhat surprising. Although we have not subjected all of our data to a complete statistical analysis, extensive computations have been performed with the spike trains of at least one set of simultaneously recorded units from each monkey. The results were comparable in every case. By simply weighting and summing the discharge frequencies of each cell in the set we were able to predict the time course of certain response measurements with unexpected accuracy. Moreover, the accuracy was an increasing function of the number of simultaneously observed units, *provided* (i) that each exhibited a discharge pattern correlated with some phase of the movement, and (ii) that the patterns were not highly intercorrelated (*12*). To illustrate these points, we used different numbers of the five cells yielding the data in Fig. 1C to compute predictions of the force trace. The optimal regression coefficients were determined separately for each subset of cells, with the use of the initial 10 seconds of record obtained with 100-g opposing flexion. The resulting values were then used in predicting force measurements obtained subsequently under the same condition (*9*). The results are shown in Fig. 2 for a single, randomly selected movement. As can be seen, the prediction accuracy (100 percent — mean percent error) increased significantly, though by progressively smaller amounts, as the discharge frequencies of more units were added to the regression equation. When the spike frequencies of all cells in the set were included, the predicted and observed measurements agreed remarkably well (*13*). It is possible, of course, that more complex (and time-dependent) transfer functions might have allowed predictions of comparable accuracy from the spike trains of only one or two cells. Our results suggest, however, that such complicated formulations may be unnecessary if the simultaneous activity of several neurons, each bearing some relation to the movement, can be taken into account. In brief, we take such findings to suggest that the temporal relations between the spike trains of separate cortical cells may be a significant variable in the control of movement and as important, if not more so, than the particular type of firing pattern exhibited by any single neuron.

An example of the predicted and observed values for all response measurements is shown in Fig. 3; for comparison, the predicted curves are based on the five units whose individual discharge patterns are illustrated in Fig. 1C (*14*). As illustrated, and for a particular load condition, predictions were most accurate for the force trace. This has been a consistent finding, and our results with multiple spike trains might appear to agree with previous suggestions (*2*), based on single unit recordings, that the activity of cortical PT cells is the most highly related to muscular force. There are certain aspects of our data, however, that prevent us from drawing this conclusion. Given a particular set of units, and fixed regression coefficients, we could obtain relatively accurate predictions of the *time course* of the force trace across all load conditions. However in order to properly scale the predictions for increasing loads we found it necessary to scale the values of the regression coefficients in a directly proportional manner. In brief, we found little in the activity of a given set of cells that would allow quantitative estimates of the steady force exerted in supporting a given weight, or of the peak amplitude of the force excursions during movement. This result may simply reflect some bias in our unit recording procedures, a possibility that we cannot rule out (*15*). On the other hand, it should be noted that Evarts (*2*) found that only a small percentage of the cells he observed exhibited clear changes in discharge frequency during postural fixation against different loads, although marked changes occurred in most cases during movement; as a result, he suggested that the activity of many of these cells might be most highly related to dF/dt. Our results are generally consistent with these findings, but we would give them a somewhat different interpretation. Although we have recorded simultaneously from units with discharge patterns that appeared, individually, to be related to dF/dt, the particular period during the movement at which the discharge frequency changes began or reached a peak varied from one cell to the next. Perhaps as a result, the combined activity of a set of these cells has typically yielded quantitatively better predictions of the time course of the force trace, or of some other variable, than of dF/dt. Moreover, by combining the activity of a set of simultaneously observed units we have been able to derive estimates of displacement and velocity that, for a given load condition, were only slightly less accurate than those for force (for example, Fig. 3), and which, for a set of fixed regression coefficients, proved to be more accurate across load conditions. Thus, our data allow no simple answer to the question of the response variable most highly related to, and therefore possibly "controlled" by, the activity of cortical neurons. Instead, our results suggest that answers to such questions must be based on quantitative grounds, and that these answers may well depend not only on the types of units observed, but also on whether or not they are observed simultaneously so that the important temporal relations between their discharge patterns can be taken into account.

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3. This question is of more than academic interest. If it can be shown that neuronal spike trains can be used for quantitative prediction of simple motor responses, then the use of such signals for control of prosthetic devices becomes a less remote and a more interesting possibility. [See K. Frank, *Ann. Otol. Rhinol. Laryngol.* **77**, 761 (1968), for a discussion of this concept.]
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5. This procedure reduced the occurrence of rapidly alternating movements, and, as in Evarts' experiments (*2*), served to dissociate the static components of force and displacement. For example, with a load opposing flexion the animal had to continuously exert a net flexor force in order to control the position of the handle, regardless of the direction of displacement. Neither our procedures nor those employed by Evarts, however, were effective in separating the dynamic phases of force and displacement, that is, their momentary departures from some background level during the onset of movement. A distinction between dynamic and static components was not made clear in Evarts' studies, and our results suggest that it may be an important one.
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9. We adopted this procedure for an important reason. With multiple regression procedures, it is possible to combine a number of nonredundant variables in such a way that, over a short segment of time, the result can be made to match any curve with similar frequency components. However, unless two sets of variables are reliably related, in a statistically stationary sense and for relatively long periods of time, it is highly improbable that the use of regression coefficients computed from their values at one period in time would result in accurate prediction when used with subsequent measurements. It is for this reason that we used the procedures described in the text.
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12. The addition of several highly intercorrelated spike trains will not produce predictions of significantly greater accuracy than those derived from the activity of a single unit, because each contains essentially the same information.
13. Predictions based on the multiple unit data were not only more accurate than those derived from single spike trains, but more reliable as well. For example, over the 20 flexion-extension cycles mentioned previously, the mean correlation ($\bar{r}$) between the combined unit data and the force trace was higher than that for any single unit, and the coefficients for individual movements varied considerably less ($\bar{r} = 0.82$, range = 0.76 to 0.92, S.D. = 0.08).
14. In this short report we deemed it preferable to base our illustrations on data from a single, representative set of cells, so that the types of discharge patterns underlying the predicted curves and the improvements in accuracy resulting from addition of these patterns, could be clearly illustrated. We have made more detailed analysis of the spike train data from this and other sets of simultaneously observed units (D. R. Humphrey and E. M. Schmidt, in preparation).
15. It is probable that our experiments suffered from the limitations inherent in many microelectrode studies, namely, the tendency for the majority of successful recordings to be obtained from larger cortical neurons, with relatively few from the smaller, but more numerous, cells.
16. We thank K. Davis for technical assistance, W. Pearson for reproduction of the figures, and Drs. K. Frank and R. Wurtz for reading the manuscript.

10 June 1970; revised 1 September 1970

Fluorescent Labeling of Chromosomal DNA: Superiority of Quinacrine Mustard to Quinacrine

We have recently reported a new technique for the selective labeling of chromosomal DNA with fluorescent DNA-binding agents in plant and mammalian metaphase chromosomes (*1–3*). The sharply defined, reproducible fluorescent bands are presumably indicative of biochemically different regions along the chromosomes. Although a number of fluorochromes have been studied in this investigation, most of them acridine derivatives, fluorescent alkylating agents are without question clearly superior to fluorochromes without alkylating groups. The best fluorochrome we have found is quinacrine mustard (*4*).

We have now applied this technique to the fluorescent labeling of human metaphase chromosomes (*5, 6*), and we are currently investigating the identification of human metaphase and interphase chromosomes in normal and pathological states, including neoplasias. The method also promises to be useful in studying the fine structure of human chromosomes and in karyotyping them. Visual identification of the 3, Y, and one of the D group chromosomes (*5*) and fluorometric differentiation of many other chromosomes, such as those in the B and C groups, have been reported (*6*). More recent papers on the fluorescent labeling of human chromosomes deal with the Y chromosome in interphase (*7*), the visual and fluorometric identification of abnormal chromosome 5 in the *cri du chat* syndrome (*8*), and the positive fluorometric identification of all human chromosomes in the normal metaphase plate (*9*).

Using this technique, other investigators have confirmed our studies on metaphase (*10*) and interphase (*11*) chromosomes and have also studied meiotic chromosomes (*12*). Several of these authors used quinacrine dihydrochloride as the fluorochrome, apparently for the reason that it is more readily available than quinacrine mustard. Quinacrine can be employed successfully in this technique (*2, 3*). However, we should like to point out several advantages of quinacrine mustard in contrast to quinacrine for chromosome fluorescence studies: the fluorescent bands produced by quinacrine are less clear, less stable, and possibly less reproducible; in plant chromosomes the quantitative fluorescence ratio (fluorescence of heterochromatin to that of euchromatin) achieved by quinacrine is substantially less than that resulting from quinacrine mustard (*2*); and quinacrine fluorescence, but not quinacrine mustard fluorescence, fades rapidly on continued irradiation of the chromosomes with ultraviolet light. These observations are all consistent with the evidence that quinacrine mustard, which can form covalent bonds, binds approximately 20 times more strongly to DNA than quinacrine does (*3*).

Although quinacrine mustard is not as stable as quinacrine, refrigerated aqueous or ethanolic stock solutions of quinacrine mustard dihydrochloride can be used reliably for at least 1 week. This minor disadvantage is more than offset by the qualitatively and quantitatively superior results obtained with quinacrine mustard, and by the possibility that since the optimum concentrations of quinacrine (5 to 10 μg/ml) compared with quinacrine mustard (50 μg/ml) are in the ratio of 100 to 200, problems of washing out excess quinacrine from the chromosome preparations may be encountered.

In summary, although quinacrine is a useful fluorochrome, we caution against the indiscriminate use of quinacrine in preference to quinacrine mustard for fluorescence studies of plant, animal, or human chromosomes.

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