Unit Activity in Prefrontal Cortex During Delayed-Response Performance: Neuronal Correlates of Transient Memory

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Since the original investigations of Jacobson (22, 23) in primates, the granular cortex of the frontal lobe, also called prefrontal cortex, has been thought to play a role in short-term memory. This is an inference from the deficits that lesions of this cortical region cause on performance of delayed-response tests. A delayed-response trial typically consists of the presentation of one of two possible visual cues, an ensuing period of enforced delay and, at the end of it, a choice of motor response in accord with the cue. The temporal separation between cue and response is the principal element making the delayed-response procedure a test of an operationally defined short-term memory function. However, the neural processes involved in delayed-response performance, as the psychological processes participating in short-term memory, are probably many and complex. The precise role of the prefrontal cortex in these is not yet clear.

In contrast to the abundant documentation of the effects of frontal ablations, the literature on the neuroelectrical correlates of delayed-response behavior is brief and recent. In 1969, Stamm and Rosen (43) showed steady potential shifts in the cortex of monkeys during a delayed-response test. Their most significant finding was a slow negative potential on the surface of the prefrontal cortex at the beginning of the delay period, suggesting that at that time there is in that region an increase of neuronal excitability. Kubota and Niki (27) have more recently reported firing changes of units in the periprincipal area of the prefrontal cortex in relation to the performance of a delayed alternation task calling for successive alternate presses of two levers with interspersed 5-sec delays. Some units are more active immediately before lever-pressing than during the delays; others show the opposite pattern.

A previous investigation showed that bilateral or unilateral cooling of the prefrontal cortical convexity produces marked and reversible deficits of performance in animals trained at the direct-method version of the delayed-response test (16). It is because of these results that the same behavioral test was adopted for the present study: a microelectrode exploration of the prefrontal cortex of the monkey performing the delayed-response task.

To the three successive periods that constitute a single trial in the delayed-response task—cue period, delay, and response period—three fundamental processes of short-term memory function may be attributed: the acquisition, the retention, and the corollary use of information. Therefore, it is reasonable to inquire whether neurons in the prefrontal cortex manifest changes of activity temporally related to these test periods. A question of particular interest on which the present study was focused is whether prefrontal neuron discharge during the delay period differs from discharge in intertrial periods, even though the physical characteristics of the animal's environment are identical in the two conditions. Differences of prefrontal neuron activity between these two conditions might be related to a role of the prefrontal cortex in the retention of mnemonic information.
Some of the results of this study have been presented in a preliminary report (17) together with data from thalamic units.

**METHODS**

**Behavioral task**

The testing situation is represented in Fig. 1. The animal, restrained by a rigid plastic collar, sits in the interior of a booth facing a window which allows view of the test objects. These are two identical white wooden blocks covering shallow food wells on the right and left of the animal's field of vision. Two openings covered by spring-loaded doors give the monkey manual access to the objects and food wells. A one-way vision window permits the experimenter to observe the animal from above. Between trials, an opaque screen (blind) blocks the view of the objects and the two doors are mechanically locked.

A delayed-response trial is initiated by the lifting of the screen. For the first 2 sec, only the empty food wells and the test objects behind them are visible from the animal's compartment. A uniform piece of apple is then placed in one of the wells and the test objects are advanced to cover them. Immediately thereafter the screen is lowered. The raising and lowering of the screen mark the beginning and the end of the "cue period," which lasts 7 sec in all. The cue itself is the position of the bait, right or left, changed randomly from trial to trial.

The cue period is followed by a delay, during which the screen is down and the two doors remain locked. At the end of the delay, which can be determined either manually or automatically (by a preset timer), the screen is again raised and, simultaneously, the doors are unlocked. The animal is thus given the choice of one object, which he can reach and displace by using the corresponding hand and door. If the correct ("baited") object is chosen, the animal retrieves the food from under it; no reward is allowed if the choice is incorrect. The trial is terminated by descent of the screen and relocking of the doors.

**Subjects and training procedures**

Six adult male monkeys, *Macaca mulatta*, weighing between 4.5 and 6.0 kg, were used in the experiments. Before surgical preparation, three of the animals (F-4, F-6, F-7) were trained to perform the described task at a level of at least 85% correct response after delays of 18 sec. This was accomplished by approximately 10 training sessions of about 40 trials, starting with delay of minimum duration and gradually prolonging it as performance improved.

The other three animals were adapted to behavioral control procedures designed to assess the effects on unit discharge of stimuli, which are normally presented during the delayed-response task in conjunction with the cue. Two of these animals (F-10, F-12) were exposed to all stimuli of the cue period except the sight of food. They underwent 10 sessions of 40 mock trials, consisting of the elevation of the screen for 7 sec, during which time the test objects were simply placed over the food wells, an ensuing "delay" of 18 sec, and a second presentation of the objects for about 10 sec. Two additional adaptation sessions were administered after surgery. Following subsequent recording during mock trials these two animals were trained to criterion on the delayed-response test. New records were obtained during delayed-response performance.

The third animal (F-9) was adapted for 10 sessions to a situation requiring the withholding of response in the presence of food. The test trials consisted of the following: the blind was lifted, the reward was placed on the rim of one food well and, after a delay of 10–25 sec with reward in full view, the doors were unlocked allowing the animal to retrieve it. The signal to respond was a click produced by the unlocking mechanism of the doors. Reward position was changed in random order. After surgery and subsequent recording during such action-withholding trials, this animal was trained to perform the normal delayed-response task and recording was resumed.

**Surgery**

Under Nembutal anesthesia, one or two pedestals for a microelectrode positioner were implanted over cortical areas. These pedestals...
were similar to those utilized in previous studies (14), each allowing multiple and parallel microelectrode penetrations through the dura along a circumference of 3 mm diameter. All animals were fitted with unilateral or bilateral pedestals over prefrontal areas. Two of the animals (F-10 and F-12) were also fitted with a pedestal over parietal cortex.

Steel electrodes were inserted in the orbital ridges for recording eye movements (EOG). One Ag/AgCl electrode pellet was implanted epidurally on dorsolateral prefrontal cortex for recording the surface EEG. EOG and EEG leads were connected to a miniature plug attached to the skull by means of acrylic. In addition, four inverted bolts were implanted in the skull for subsequent fixation of the head by a method similar to that devised by Evarts (11). Antibiotics were topically and systemically administered for protection against infections.

Recording

Recording sessions were initiated 1 week after surgery at the earliest. The monkey was placed in the testing apparatus, the head fixed, the cable for EOG and EEG connected, and a hydraulic microelectrode positioner (14) mounted on a pedestal (Fig. 1). Tungsten microelectrodes were used, insulated with Isonol 31 (Schenectady Chemicals); their impedance in saline was 0.5–3.0 megohms at 1 kHz. Extracellular spike potentials were amplified beyond a cathode-follower stage and recorded on one of the channels of a tape recorder (Ampex FR-1300). Square pulses (2 v, 0.5 msec), generated by the spikes through a Schmitt-trigger, were recorded on a second channel. Throughout the recording from any given unit, both spikes and shaped pulses were oscilloscopically monitored to insure reliability of the conversion.

Other recording channels were used for the bipolar EEG and the monopolar EEG (Tektronix 122 low-level preamplifiers; coupling time constant, 1 sec). Movements of the animal, transmitted to the spring-mounted grid on which he was sitting, were detected by an electromagnetic device and also recorded on magnetic tape. Such a record, called kinetogram in this exposition, was sensitive to movements of any part of the trunk or limbs. Deviations from its isoelectric line indicated the presence of movement, although the record did not specify the nature of it.

Electrical pulses marking the test periods and the opening of the small doors by the monkey were recorded on another tape channel. A digital code on the same channel marked the sequence of records.

One microelectrode penetration was ordinarily carried out during each recording session. The descent of the microelectrode was remotely controlled by means of a microsyringe connected to the positioner with polyethylene tubing. Single-unit records were sampled on the basis of stability and uniformity of spontaneous action potentials as well as sufficient separation from background noise and lower-amplitude records of neighboring units. Initially negative spikes of duration longer than 0.5 msec were assumed to be recorded from the vicinity of cell bodies.

At irregular intervals an animal trained on the delayed-response task was given test trials with delays varying between 5 and 18 sec. When, in the course of the microelectrode's descent, the impulses from a unit were identified and isolated, the descent was halted and the animal subjected to a series of trials. Records of the spontaneous activity of the unit were obtained between test trials. Ordinarily, in animals performing the task, the activity of a given unit was recorded during 5–10 trials. An electronic programmer, operating on the screen and the door locks by means of compressed-air cylinders, allowed the automatic timing of a pretrial period of 20 sec, a cue period of 7 sec, and delays of either 18 or 32 sec. Mainly trials with delays of these two durations were administered during unit recording. Only mock trials with an 18-sec delay were administered to untrained animals.

Unit localization

Electrolytic marks were made in the cortex and in the underlying white matter by passing current (100 μa, 15 sec) through the tips of microelectrodes. After an animal was sacrificed the brain was extracted and fixed in formalin. Sections were obtained from the frozen specimen and stained with thionin. The tissue marks allowed the localization of the units sampled during the experiments.

Analysis of data

All the taped records were displayed on polygraph paper chart using a Schwarzer (type E-541) electroencephalograph. This allowed the study of simultaneous EEG, EOG, kinetogram and unit records. The study of units and their classification according to discharge changes in the course of test trials were facilitated by superimposed oscilloscopic photography of time-locked records from several trials with equal delay duration (or waiting period in the action-withholding test). The oscilloscope beams were triggered by a pulse at the start of the 20-sec pretrial period. Groups of five consecutive trial records were ordinarily superimposed in this
manner, although for exceptionally fast or slow units, respectively, smaller or larger numbers of trial records were pooled (minimum, 3; maximum, 10).

Unit records were submitted to spike-train analysis by means of a PDP-12 computer. Statistical parameters of spike discharge were determined using a program developed by Wyss (49). Graphic displays of spike trains and frequency histograms were obtained with the computer and a Calcomp plotter.

RESULTS

Spontaneously active units were found in all the cortical areas explored, but firing rates varied widely within any given domain: some units only emitted isolated impulses at intervals of several seconds, whereas others discharged at frequencies of 20 impulses/sec or higher. The majority fired at frequencies within the 1–10/sec range. While the animal was at rest in the testing environment, the pattern of discharge of most units in prefrontal cortex was irregular and lacked any obvious rhythmic characteristics. It differed in this sense from the pattern of spontaneous discharge of thalamic units which, under the same set conditions, show a tendency to fire in rhythmic clusters or groups of spikes (15).

The majority of units investigated were situated in cortical area FD according to the cytoarchitectonic map of von Bonin and Bailey (5); 328 units from this area were sufficiently studied for characterization of their modes of discharge in the course of delayed-response performance. In addition, 102 units from the same area were investigated during mock trials in untrained animals and another 46 during performance of the action-withholding test. The results of studying these 476 units from prefrontal area FD will be presented in the first five sections of the results. The sixth and last section will deal with the activity of 93 units of the parietal cortex during delayed-response performance.

1. Unit activity in delayed-response trials; reaction types

The majority of prefrontal units showed changes of firing frequency in relation to the events of a delayed-response trial. For any given unit these changes were relatively consistent from trial to trial in both magnitude and duration.

Figure 2 shows typical examples of unit discharge in delayed-response trials. As the figure illustrates, a wide diversity of spike activity patterns were encountered. Nevertheless, it was possible to classify all units into a limited number of categories. Using the discharge rate between trials as the base line and considering the temporal characteristics of deviations from this base line during the trials, six basic types of units were distinguished. These are schematically represented in Fig. 3.

Type A is characterized by a transient excitatory reaction to the lifting of the screen at the onset of the cue period (Fig. 2:1).  

Throughout this exposition, excitation (or activation) and inhibition are used as descriptive terms for spike-frequency changes, without necessarily implying any particular kind of synaptic processes.
This reaction begins within 1 sec of the screen movement and usually lasts less than 2 sec, terminating before the baiting procedure. A brief firing increase may also be seen in units of this type at the lowering of the screen which initiates the delay period. A final activation occurs at the end of the delay, when the screen is again raised and the objects are presented for choice. It is also brief and begins before the response of the animal.

Units of type B show increased discharge throughout the cue period, between the rise and the descent of the screen (Fig. 2:4). A second activation occurs on termination of the delay.

The firing increases of A and B units in the cue period began simultaneously with an EEG reaction characterized by transition to lower amplitude, faster frequency waves which prevailed for the duration of this period and the early part of the delay. This EEG change was most conspicuous when the pretrial record was “synchronized” (high voltage, low frequency).

During the cue period, there was also an increased incidence of eye movements. By observing the animal it was evident that this was due to the occurrence of searching eye movements directed to the objects and events in the testing compartment. The elevated discharge of A and B units, especially the latter, appeared generally related to his phenomenon. However, no clear correlation could be established between unit discharge and eye movements spontaneously occurring during intertrial or delay periods.

The principal characteristic of type C units is an activation beginning in the cue period and carried over into the delay. In some, this activation began abruptly on initiation of the cue period (Fig. 2:3); in others, the beginning was gradual (Fig. 2:4). Firing frequency normally reached its maximum in the latter part of the cue period or early part of the delay, and then declined slowly toward base line. This decline was smooth and gradual or irregular and fragmented. The higher level of discharge lasted in some units for the entire duration of delays longer than 60 sec and base-line firing was not reattained until after the response of the animal. Some C units showed a final surge of discharge at the time of response.

Some animals responded faster than others to the presentation of the test objects for choice. Moreover, the motor reaction time of any given animal varied from trial to trial. However, the vast majority of motor responses, as could be determined by the kinetogram, started within 0.2–1.0 sec of the second screen-raise. Although the reactivation of A, B, and C units usually began before the motor response, considerable overlap occurred between this reactivation and the movements of the animal. No evidence was found of a relationship between the degree of reactivation and the side of response. Occasionally the animal failed to respond, presumably because of uncertainty about the position of the reward; unit reactivation was nevertheless present in such instances. None of the units activated at response time failed to show a preceding activation in the cue period.

Units of type D were inhibited during most or all of the cue period (Fig. 2:5). Toward the end of this period or at the beginning of the delay, these units showed a reversal of impulse frequency to a level higher than the intertrial base line. Firing then slowly declined in the course of the delay as in units of type C. Elevated dis-
charge sometimes persisted for the duration of long delays (Fig. 4). A final inhibition occurred on re-presentation of the objects.

Type I includes all units that presented inhibitory changes and no elevation of firing above base line. Some units (subcategory Iₜ) only showed an inhibition circumscribed to the cue period and another at the end of the delay (Fig. 2:6). Others (Iₜ) showed a long depression of firing, beginning in the cue period and lasting through the delay (Fig. 2:7).

Units which showed no deviations from base-line discharge during delayed-response trials are designated as units of type 0.

Although the above classification has descriptive value, it should be noted that the separation between different categories is not sharp but gradual. This is especially true of the separation between contiguous categories of reactive units as arranged in the schema of Fig. 3, which share certain common features. While most units clearly conformed to the types described, some units of transitional character were observed and their classification was somewhat arbitrary, based on the most prominent features of the averaged discharge from several trials.

Table 1 shows the incidence of units of the different types in prefrontal area FD. Units showing some form of excitatory reaction (A through D) are clearly in the majority. Among these, the largest contingent (50% of all units) is made up of units that underwent elevated discharge during the delay, whether this was preceded by activation or inhibition in the cue period (G and D types).

The magnitude of spike-frequency changes varied between units of the same type. While some only showed minor deviations from spontaneous rate, best seen by averaging spike activity through several trials, others showed firing increases to levels more than 100% higher than the spontaneous rate or declines to the point of complete cessation of spikes.

No differences were found in unit discharge as a function of the cue per se, namely, the position of the reward. Thus, none of the units showed firing activation or inhibition depending on whether one or the other object was baited, nor were differences in magnitude of firing change observed.

Whereas no differences were seen in relation to cue side, the discharge of some

**Fig. 4.** Unit of type D during five delayed-response trials with 32-sec delay. Spikes are represented by vertical lines in a graphic display obtained by computer method. The notation next to the arrow at the end of each trial's delay refers to the accuracy (C, correct; I, incorrect) and side (R, right; L, left) of the response. The series of single-trial records in this figure—as in subsequent figures—is made of records from consecutive trials.
units during the delay was clearly dependent on the antecedent presentation of the cue at the time when this normally occurred. This was demonstrated by use of dry-run trials in trained animals. Such trials were identical to regular trials, except that during the cue period no reward was placed in either of the food wells, though these were covered with the test objects in normal fashion.

On dry-run trials, units of types A and B showed reactions similar to those observed in normal trials (Fig. 5). The reactions of C, D, and I units, on the other hand, were characteristically abortive. Most remarkable was the absence of the delay component of a normal reaction (Figs. 6 and 7).

Inasmuch as none of the animals was seen to adopt any consistent postural attitude in

![Diagram](image-url)

**Fig. 5.** Unit of type B during eight trials with 18-sec delay. The second and eighth are dry-run trials (no bait.)

the course of the delay, the sustained activation or inhibition of C, D, and I units during this period did not appear related to the tonic contraction of any given muscle or muscle group. This also applies to eye muscles, for no habitual gazing in any particular direction was observed (e.g., toward the side of the reward).

During the delay, as between trials, the animals ordinarily sat still, showing only eye movements and, occasionally, minor movements of the extremities in apparently random fashion. However, restlessness was sometimes present in the form of frequent unsystematic movements. This was neither a sufficient nor a necessary condition for firing change. Very marked firing changes were observed during delayed-response trials while the animal was completely quiescent and, conversely, spontaneous bursts of motor activity occurred in intertrial periods without concomitant alterations of firing rate. In no case was it possible to relate firing changes to any specific movement.

In the course of testing, all the animals rapidly developed high proficiency at delayed-response performance. Incorrect re-
responses were scarce even after delays longer than 1 min. For this reason, it was difficult to obtain from any given unit enough records through incorrect response trials to allow a satisfactory study of possible correlations between the unit's activity and the level of performance of the animal. Nevertheless, some records obtained over long series of trials provided evidence of a relation between reactive unit discharge and performance level, even in the presence of only occasional errors. In general, unit reactions were relatively small in trials terminated by incorrect response, as well as in some of the preceding trials. This was especially evident in units whose typical re-

![Diagram of unit activity](image)

**FIG. 6.** Unit of type C. Note absence of sustained activation on dry-run trials (fifth and seventh).

![Diagram of unit activity](image)

**FIG. 7.** Six trials in the course of recording from a unit of type I. First and third are dry-run trials. Observe sustained inhibition of firing during the delay of normal delayed-response trials. Bottom three lines represent a continuous record from a trial with a delay nearly 4 min long. (It was manually terminated by the experimenter on a resurgence of discharge with the expectation that this might be followed by incorrect response: it was not.)
Prefrontal units and delayed response

2. Action extended into the delay (Fig. 8). At times, the trial succeeding an error curiously showed the most marked firing change of a series, as if such a change reflected an effort of the animal to ensure success after a failure.

2. Anatomical distribution

All units were within a band of cortical area FD, about 8 mm wide, extending from the lower lip of the sulcus principalis in the lateral aspect of the hemisphere to the lower lip of the sulcus cinguli in its medial aspect. The majority were from three subregions that were most intensively explored: a) the cortex lining the middle third of the sulcus principalis, b) the cortex of the dorsolateral convexity above the sulcus principalis, and c) the cortex lining the most anterior portion of the sulcus cinguli.

No interhemispheric differences were found in proportions or distribution of unit types. All unit types were found rather diffusely and unsystematically represented in area FD. However, some indications of organization were apparent:

1) Units of any given type were commonly found in close proximity to each other; thus, several units showing similar reactions were frequently encountered in succession and at short intervals in the microelectrode's course.

2) Units of C and D types were generally found to be more common in cortical layers V and VI than in more superficial layers. They were particularly abundant in the depth of the sulci.

Figure 9 shows in sections of the right hemisphere the distribution, by type, of all the units investigated in area FD. Those from the left hemisphere have been projected onto homologous points of the right one.

3. Effects of stimuli unrelated to task: distraction

Visual and auditory stimuli unrelated to the task were presented to trained animals during unit recording. Such stimuli were purposely diverse and unstructured in order to test the effect of their novelty on unit activity. They consisted, for instance, of a moving or stationary light beam projected through the observation window on the animal's eyes, sound produced by striking a tense spring, whistling, etc.

Novel stimuli produced significant firing changes in 47 of 127 (37%) prefrontal units on which they were tested during intertrial periods. Excitatory reactions to such stimuli were more common in A, B, and C units than in units of other types. Inhibitory reactions were only observed in D and I units. The reactions did not appear to be modality specific, although in some units responsiveness to acoustic stimuli was more prominent than to visual stimuli, while in others the reverse was true. The effects on unit discharge were generally brief and showed a tendency to habituation, i.e., diminution of magnitude with reiteration of the same stimulus.

Natural acoustic stimuli were tested on 36 units. These stimuli were vocal sounds from monkeys stereophonically recorded in the animal quarters at feeding time and played back to the experimental animal through a pair of loudspeakers situated in the interior of the testing booth. They were presented at various times in the course of delayed-response performance primarily with the purpose of distracting the animal from the task.

Presentation of distracting stimuli during cue and delay periods generally increased the incidence of incorrect responses and often led to failure of the animal to respond. The most remarkable effects of distracting stimuli on spike activity were observed in C and D units. These stimuli usually attenuated the discharge elevation that such units exhibit in the delay period, in some cases lowering firing to a level below that of the pretrial baseline (Fig. 10).
Fig. 9. Type and approximate location of all units studied in the prefrontal cortex. Units are plotted on four diagramed sections of the right hemisphere made at an angle of 70° with respect to the horizontal stereotaxic plane. Notation for each section indicates distance in millimeters from the genu of the corpus callosum. SA, sulcus arcuatus; SCI, sulcus cinguli; SP, sulcus principalis.
Distracting stimuli were not seen to antagonize the protracted inhibition of $I_2$ units during the delay.

4. Untrained animals; mock trials

Of 102 prefrontal units investigated during mock trials in two untrained animals, 68 showed no modifications of activity on presentation of the visual and auditory stimuli that are spatially or temporally contiguous to the delayed-response cue (screen movements, test objects, experimenter's hands, etc.). The remaining 34 units showed firing changes, which by their time course resembled those observed in trained animals. Most common among these were units that by such changes might be assigned to A, B, or $I_1$ categories. A few showed some carryover of activation into the delay or a mild rebound activation at the beginning of this period after being inhibited during presentation of the stimuli. In general, however, reactions running into the delay were considerably shorter and of smaller magnitude than those observed in trained and performing animals. No sustained activations or inhibitions were observed lasting for the duration of the 18-sec delay.

After the two animals had been trained to perform the delayed-response task, a second series of cortical penetrations were made placing the microelectrode carrier in identical settings with respect to the cortical surface as those used for pretraining penetrations. A second sample of unit records was thus obtained from regions previously explored, viz., the cortex of the prefrontal convexity and the cortex lining the sulcus principalis. The 79 units of this sample were classified as follows: 4 A (5%), 11 B (14%), 28 C (35%), 12 D (15%), 7 I (9%), and 17 of type O (22%). When compared with the sample obtained before training, each of 16 new microelectrode tracks was estimated to deviate less than 0.5 mm from one of another set of 16 followed before training: a new set of electrolytic marks was made for ascertaining unit

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3 Before recording, both animals had been exposed to such stimuli in a number of habituation sessions (see METHODS).

4 Each of 16 new microelectrode tracks was estimated to deviate less than 0.5 mm from one of another set of 16 followed before training: a new set of electrolytic marks was made for ascertaining unit.
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the second sample was distinguished by two basic characteristics: 1) a higher number of reactive units of all types (78% vs. 33%; \( \chi^2 = 31.4; P < .001 \)); and 2) the presence of units showing sustained deviations from base-line firing during the delay.

5. Action-withholding test

Records were taken during performance of the action-withholding test in order to assess the relative importance for unit discharge of two combined factors which are presumably involved in the delayed-response task: anticipation of food and delay of purposive movement. In an action-withholding trial the food was placed in view but the animal prevented from reaching it for a period of time comparable to the delay of a delayed-response trial.

Forty-six units were isolated in eight penetrations of the prefrontal cortex of an animal while being tested in the action-withholding paradigm. The firing of most of them (63%) did not appear affected by the procedure. Some showed excitatory changes beginning with the raising of the screen or the ensuing presentation of the food, but the activation of firing was, in most cases, transitory. Nine of the units showed inhibition of firing throughout the waiting period.

After delayed-response training, a new sample of units was obtained by eight additional penetrations of the same cortical region (see footnote 4). (Table 2). The most notable difference between the two samples was in the number of units that were activated at any time in the course of test trials (74% in delayed-response vs. 15% in action-withholding trials; \( \chi^2 = 23.5, P < .001 \)). C and D units account for most of the difference.

6. Units in parietal cortex

Ninety-three units were isolated in the superior and inferior parietal lobules (areas PE and PG, ref 5) of two monkeys trained positions. However, the number of units isolated on each penetration was variable and, in addition, it was difficult to match the cortical depth of units explored on different occasions. Therefore, comparability of the two samples is somewhat limited by inequalities of topographic distribution.

<table>
<thead>
<tr>
<th>Action Withholding</th>
<th>No. units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation</td>
<td></td>
</tr>
<tr>
<td>&lt; 7 sec</td>
<td>4 (9)</td>
</tr>
<tr>
<td>&gt; 7 sec</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Inhibition</td>
<td></td>
</tr>
<tr>
<td>&lt; 7 sec</td>
<td>1 (2)</td>
</tr>
<tr>
<td>&gt; 7 sec</td>
<td>9 (20)</td>
</tr>
<tr>
<td>No change</td>
<td>29 (63)</td>
</tr>
<tr>
<td>Total</td>
<td>46 (100)</td>
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</table>

<table>
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<tr>
<th>Delayed Response</th>
<th>No. units</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4 (10)</td>
</tr>
<tr>
<td>B</td>
<td>3 (8)</td>
</tr>
<tr>
<td>C</td>
<td>12 (32)</td>
</tr>
<tr>
<td>D</td>
<td>9 (24)</td>
</tr>
<tr>
<td>( I_1 )</td>
<td>2 (5)</td>
</tr>
<tr>
<td>( I_2 )</td>
<td>0 (0)</td>
</tr>
<tr>
<td>O</td>
<td>8 (21)</td>
</tr>
<tr>
<td>Total</td>
<td>38 (100)</td>
</tr>
</tbody>
</table>

Subdivision of units according to whether the reaction was shorter or longer than 7 sec, length of the cue period in the delayed-response test. Figures in parentheses are percent.

for the delayed-response test. The purpose was to compare the activity of neurons in posterior association areas with that of neurons in the frontal association cortex.

Parietal units were generally smaller and more difficult to isolate than prefrontal ones. Their spontaneous activity was more phasic and irregular; consequently, a steady state of spontaneous discharge was more difficult to determine.

The main feature of parietal cell firing was its relationship to movement. In this the parietal cell population was clearly different from the prefrontal one. Parietal cells showed changes of firing closely correlated with the execution of specific movements. Simple observation of the animal in the course of recording was frequently sufficient to identify a particular form of movement
associated with sharp increases of firing by a given unit. During delayed-response testing, this association was most evident in certain units whose discharge was related to movements of the upper extremities, as these were involved in the performance of the task. This allowed the observation in parietal units of a phenomenon never observed in prefrontal units: firing changes contingent on the side of the reward (Fig. 11). A related and distinctive characteristic of parietal units was the occurrence of firing changes circumscribed to the response period and not preceded by earlier changes in the cue or delay periods.

The firing of some units in the cortex of the intraparietal sulcus was related to eye movements. Because of this relationship, such units showed, during the cue period, reactions similar to those of the B units of the prefrontal cortex. However, unlike prefrontal units, some of these parietal cells showed a preponderance of activation on presentation of one of the two alternate positions of the reward.

Sustained reactions in the absence of movement and consistent from trial to trial, as exhibited by prefrontal units, were not seen in the parietal cortex.

**DISCUSSION**

This study has demonstrated temporal correlations between cell firing in the prefrontal cortex and performance of a delayed-response task. Since performance of the same task was markedly and reversibly impaired by cryogenic depression of this cortical region (19), it seems a priori highly probable that these correlations are direct manifestations of a physiological role of prefrontal neurons in the performance. This inference is also in accord with evidence from ablation studies showing that the integrity of the prefrontal cortex is essential for delayed-response tests (21–23, 46). In the present investigation, however, unit discharge related to delayed-response performance has been found beyond the limits of the cortical area directly affected by cooling in the cryogenic experiment and also beyond the limits of the particular region which, from results of ablation work, has been considered critical for delay tasks, i.e., the region of the sulcus principalis (4, 7, 19). Especially interesting in this respect are the changes of unit discharge observed during delayed-response trials in the anterior cingular portion of prefrontal area FD. Some lesion experiments (26, 39) have indicated involvement of this region in de-
layed-response performance. Furthermore, anatomical studies show that the anterior cingular area is profusely and reciprocally connected to the principalis area (35, 37).

The source of nerve impulses to prefrontal cells during delayed-response performance is not clear. However, enough is known about input connections to the prefrontal cortex to allow tentative postulations on this matter in the light of the present findings. Two principal categories of such connections have been demonstrated in the monkey: a) afferents from the nucleus medialis dorsalis (2, 45), and b) afferents from sensory association areas (3, 24, 28, 36, 37). In addition, electrophysiological studies have shown that the prefrontal cortex is accessible to particularly strong influences from nonspecific subcortical structures (6, 44). Such influences can be, at least in part, mediated by medialis dorsalis (10, 20).

The firing changes of prefrontal units during the cue period of delayed-response trials appear, in many respects, to be phenomena of sensorial attention. Changes that occur immediately at the start of a trial, before the actual presentation of the cue, may be manifestations of the orienting reflex (29, 42) and mediated by nonspecific subcortical input. This view is supported by the finding that such changes coincide with an EEG arousal reaction and can also be elicited by a variety of sensory stimuli unrelated to the delayed-response task. Sustained alterations of firing during the cue period, which are generally accompanied by increased frequency of searching eye movements, may be, to some extent, functionally related to the attention that the animal directs to the testing field. This interpretation is consistent with the finding that these sustained firing changes are also present during dry-run trials and the observation of similar changes in some units recorded from untrained animals during mock trials.

A possibility suggested by these findings is that the higher discharge of prefrontal units during cue presentation is a manifestation of a role of the prefrontal cortex in visual attention. Efferent connections from the area under study to the periarcor-

ate region (Broadman's area 8) (35, 37), which is implicated in eye-movement control (41), might be involved in the performance of this role.

Considering the visual character of the cue, parastriate and inferotemporal cortical areas seem very likely sources of input determining and maintaining the enduring changes of discharge observed in the majority of prefrontal units during delayed-response trials. This supposition is based on the importance of those areas for visual discrimination (32) and on their efferent connections to the prefrontal cortex (24, 28, 37). Input from medialis dorsalis probably contributes also to enduring activity changes of prefrontal cells. Such contribution may be of special functional importance because medialis dorsalis, in addition to diencephalic and mesencephalic afferents, receives afferents from limbic structures, notably the amygdala (10, 33). Since these structures, the amygdalar complex in particular, have been implicated in the evaluation by the organism of the motivational significance of external stimuli (18, 25, 47), input of limbic origin may be, to some extent, responsible for the firing changes induced by the cue on prefrontal cells. In accord with this is the fact that many units in medialis dorsalis show similar changes (15, 17).

The majority of reactive units isolated in the prefrontal cortex showed a peak of activation at the end of the cue period or beginning of the delay. Therefore, it appears likely that the slow negative potential detected at that time by d-c recording (43) is an electrocortical phenomenon of summed cellular depolarizations.

Elevated discharge during the delay is the most prevalent and characteristic effect of the cue display on prefrontal cells (C and D types). Apparently, this effect is largely contingent on the significance of the cue as a memorandum for prospective action. The evidence for this mainly derives from: a) the finding that the effect disappeared or was greatly diminished on dry-run trials; b) the near absence of a temporally comparable effect on units studied during the action-withholding test, where food was presented for delayed response without the re-
requirement of mnemonic retention; and c) the relation observed in some units between the level of discharge during the delay and the level of correct performance.

Because of the reciprocity of connections between medialis dorsalis and the prefrontal cortex (2, 31, 45), the two structures may play a joint role in delayed-response performance. An attractive possibility is that, between cue and response, they are bound by reverberating activity essential for retention of information about the cue. However, the absence of a relationship between unit reactions and laterality of the cue suggests that information about the latter is not retained in such a system. It would appear more likely that, during the delay, this frontothalamic system tonically facilitates the function of structures more directly involved in visual discrimination; namely, the visual association cortex and limbic formations, which are not only sources of afferents to the system but targets of some of its efferents (1, 34, 35, 37). This hypothesis incorporates the function of the frontothalamic system in a simple and plausible view of short-term memory, i.e., the view that this form of memory basically consists of the continued facilitation of neural activity in cerebral structures recently engaged in sensory processing.

The mechanism and significance of the inhibitions of firing observed in some units (D and I) are obscure. It is noteworthy that these inhibitions mirror in their temporal aspect the activation of B and C units. Conceivably, D and I units manifest phenomena of collateral inhibition but, in the absence of intracellular records and better knowledge of cortical organization, this is merely speculative.

A finding to be emphasized is that many units (O, L, and C) did not show immediate and appreciable firing changes at the signal prompting the animal’s response in the delayed-response task (second screen-raise). However, in those units that did show a change in reaction to this signal, such a change was similar to the antecedent change at the initiation of the cue period. This was true whether the change was excitatory or inhibitory, whether the motor response occurred or not and, if it did, whether it was correct or incorrect. This again points to a relation of these changes to attentive processes—the orienting reflex and visual attention—which are presumably mobilized on both occasions.5

Using the delayed-alternation test, where a motor response constitutes the memorandum for the next response, Kubota and Niki (27) found that 67 and 33% of 49 reactive prefrontal units were, respectively, excited and inhibited by the signal to respond. By considering firing changes at the transition from delay to response period in the delayed-response task, one obtains roughly comparable proportions. Therefore, despite the obvious differences between the two tasks,6 it seems that the type of units designated by those authors as E (excited in the response period) may include A, B, and some C units as described in the present study, whereas their type D (inhibited in the response period) may include our D and also some of our I units.

The movement-related changes of firing observed in parietal units may be largely attributable to impulses of somatosensory origin. In view of recent evidence (9), proprioceptive input from joint receptors would seem to be involved in the activation of units in parietal area PE.

The observation that distracting stimuli interfere with the sustained activation of prefrontal units while also interfering with correct delayed-response performance provides another indication of the importance of that activation for securing an appropriate functional linkage between cue and response across the temporal breach of the

5 Although in the delayed-response task prefrontal units were not seen to respond differentially in relation to the direction of movement (right or left), it is noteworthy that H. Niki (unpublished observations), in a current study on monkeys in a delayed-alternation task, has found some units behaving in this fashion.

6 In addition to the differences between behavioral paradigms, other methodological differences make the comparability of results from the two studies difficult. The most noteworthy difference is that in the delayed-alternation study, the results are principally based on the comparison of firing rate between delay and motor response period, whereas in the present study the firing rate within any given period of a delayed response trial is compared to a base line determined by spontaneous firing outside of trial periods.
delay. Perhaps the continued facilitation of sensory processes by the prefrontal-medialis dorsalis system, as postulated above, is the basis of an innerly directed form of attention essentially involving the rehearsal of information for prospective use. This is consistent with findings of several studies in which certain modifications of testing procedures (13, 30, 38) or pharmacological agents (38, 48) have been used as aids to elucidate the nature of the prefrontal deficit. These studies indicate that the animal with a prefrontal lesion suffers primarily from an impairment of attention: in delayed-response tests, both the ability to attend to the cue and the resistance to distraction during the delay appear diminished by the lesion.

Extrapolating to man any inferences from the findings of this study can only be done with extreme caution and at the risk of oversimplification. Nevertheless, it is interesting to relate these findings to certain clinical observations in frontal lobe patients. Of special interest is the fact that disturbances of attention have long been reported to be prominent feature of frontal syndromes (8, 12, 29, 40). Reports of this kind commonly emphasize that most severely curtailed in frontal patients is the capacity to persistently maintain attention on mnemonic material, this apparently being a basic function of the human prefrontal cortex. Some of the patterns of nerve cell discharge presented here are possibly phenomena of such a function in a primate lower than man.

SUMMARY

Spike discharge was recorded from 328 units in prefrontal cortex of monkeys performing a delayed-response task. Units were classified into six basic types according to firing-frequency variations related to the principal periods of delayed-response trials (cue, delay, and response periods). The majority of units (80%) showed excitatory or inhibitory changes during the cue period. Similar changes were observed in 33% of 102 prefrontal units from untrained animals during mock trials. Unlike the mock-trial sample, the sample obtained during delayed-response performance contained a large proportion of units showing firing deviations that extended into the delay period. The most common and characteristic phenomenon was a sustained elevation of firing during this period. This activation, especially prevalent in unit records from the deeper cortical layers, had the following features: 1) It was present in normal delayed-response trials but not in dry-run trials (no cue). 2) It was attenuated by distracting stimuli that frequently lead to incorrect responses. 3) It appeared related to accuracy of performance. 4) It was not observed among 93 units from parietal cortex during the delayed-response task.

Sustained activations of comparable duration were not observed among 46 prefrontal units during a test that required withholding of purposive action in the presence of food.

Temporal correlations between prefrontal unit firing and delayed-response trials are viewed as manifestations of a functional involvement by neurons of the prefrontal cortex in delayed-response performance. This interpretation is in accord with behavioral results of cryogenic depression and ablation of this cortical region. The findings are examined in relation to present knowledge on afferent and efferent connections of the prefrontal cortex.

Considering the patterns of prefrontal unit discharge in reaction to sensory stimuli related or unrelated to the delayed-response task, it is suggested that the firing changes observed during delayed-response trials are attributable to a role of the prefrontal cortex in processes of sensorial and mnemonic attention, which are critical for short-term memory.

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