MAINTAINED ACTIVITY IN THE CAT'S RETINA IN LIGHT AND DARKNESS*

By S. W. KUFFLER, R. FITZHUGH, and H. B. BARLOW

(From the Wilmer Institute, Johns Hopkins Hospital and University, Baltimore)

(Received for publication, November 5, 1956)

INTRODUCTION

Maintained activity in the absence of obvious external stimuli has been observed in several sense organs of vertebrates and of invertebrates (Tasaki and Davis, 1955; Katsuki, Yoshino, and Chen, 1950; Bullock and Diecke, 1956; Cohen, 1955; Adrian, 1937; Löwenstein and Sand, 1940; Zotterman, 1953; Roeder, 1955; Autrum, 1952; see Granit's review, 1955). Such "spontaneous" discharges are of special interest in the retina because of its similarity in neuronal organization to the higher nervous centers in which continuous activity is well known (Bremer, 1949). The presence of maintained activity is also related to the problem of transmission of sensory information. It implies that visual stimuli are transmitted by modulation of ever present background discharges. The occurrence of a background discharge with a random component, or "noise," is essential evidence in support of the view that sensory thresholds should be regarded as signal/noise discrimination problems (Hecht, 1945; Tanner and Swets, 1954; Gregory and Cane, 1955; FitzHugh, 1957, Barlow, 1956), and it was therefore important to make sure that such noise was constantly present in the best preparations, and was not the product of experimental interference. In this paper the statistical properties of the maintained discharge are analyzed, and the changes in maintained impulse frequency with different levels of steady illumination and in the absence of light are described.

The cat's eye is well suited for these studies since it can be used unopened without appreciable surgical interference and with its circulation and optical pathways intact. Good physiological conditions are especially important in the registration of spontaneous discharges which are very sensitive to environmental changes. The great increase of sensitivity during dark adaptation gives an indication of the good condition of the present preparation. Further, the eye is isolated from possible efferent centrifugal nervous control and light can be excluded, thus providing a sense organ cut off from external stimuli.

* Supported by a research grant from the National Institutes of Health, United States Public Health Service.

The subsequent papers (Barlow, FitzHugh, and Kuffler, 1957 *a*, *b*) will deal mainly with changes of organization of the receptive field during dark adaptation and with the measurement of absolute threshold for the discharge of a single ganglion cell. The last paper will present a statistical analysis of threshold responses to short flashes (FitzHugh, 1957).

METHOD

Preparation

Unless otherwise noted, adult cats were decerebrated under ether or surital (thiamylal sodium, a barbiturate of short action) by opening the skull and cutting across the mesencephalon just anterior to the tentorium. After removal of the cerebrum, cranial nerves III, IV, V, and VI were crushed or cut intracranially to prevent movements of eye and facial muscles. In some decerebrate preparations subparalytic or paralytic doses of tubocurarine were used. Many preparations stayed in good condition for 8 to 15 hours.

The preparation was set up for retinal recording by the technique of Talbot and Kuffler (1952); the eye was closely fixed to a ring by the conjunctiva, a hypodermic needle pushed through the sclera just behind the ciliary body, and an electrode inserted through the needle until it touched the retina, as observed through the magnifying eyepiece of the multibeam ophthalmoscope (see below, under Equipment).

Experimental observations were started more than 3 hours after the cat came off ether, so that in this case residual anesthetic was unimportant. The same is probably true after surital, since a test showed that a cat was fully awake, but its muscular coordination was not quite normal, in 3 to 4 hours. Progressive physiological changes attributable to initial anesthesia were never noticed.

Equipment

The optical equipment and general experimental set up have already been described in detail (Talbot and Kuffler, 1952). The main instrument, a multibeam ophthalmoscope, had a base with a headholder for rigid fixation of the preparation. The eye was held fixed to a ring which also carried the microelectrode holder and electrode-advancing mechanism. Above the head was the optical viewing and stimulating system. This was aligned with the eye and could be freely tilted and rotated, to direct light beams to various retinal regions. The eye was thus in the center of the spherical coordinate system of the movable optical system. A tungsten filament lamp served for background illumination for light adaptation and at the same time provided light for viewing of the cat's fundus through a microscope eyepiece. The background light covered a roughly circular area 16 to 19° in diameter (equivalent to 3.7 to 4.5 mm. on the cat's retina). The intensity of this background was changed both with neutral filters and by altering the current flowing through the lamp, and its color was controlled by using Wratten filters. A second light source was a glow modulator tube (Sylvania R1131C) driven from an electronic stimulator. This gave square pulses of light of constant intensity and variable duration. This light passed through two Eastman circular neutral wedges giving an optical density variable through 7 log units, and, if needed, a Wratten color filter. A diaphragm was placed

in a position parfocal with the retina and could be revolved to give a series of concentric spots ranging in diameter from 14°3 minutes to 0°36 minutes (equivalent to 3.25 to 0.135 mm. on the cat's retina). Annular patterns with inside diameters ranging from 0°50 minutes to 3°30 minutes (0.80 to 1.90 mm. on the retina) were also available. The procedure for measuring the illumination of the retina in light and energy units from these two beams will be described in a following paper (Barlow, FitzHugh, and Kuffler, 1957 *a*).

Light from the stimulator passed through a contact lens on the cat's cornea and the natural pupil which was dilated by section of nerve III. Since the contact lens was not suited to every cat, an additional spectacle lens was frequently needed to focus accurately, but as it was of low power and close to the eye, it did not change the magnification appreciably.

Nerve or ganglion cell potentials were recorded by electrodes of three types: glass-insulated platinum-iridium wires of 10 to 15 μ in diameter of the type used by Granit (1947), by indium-filled, gold- and platinum-plated, glass capillaries with a tip to 3 to 6 μ as recently described by Dowben and Rose (1953), and by micropipettes of less than 1 μ tip diameter, filled with 3 M KCl. The electrodes were advanced by a fine screw or by a hydraulic mechanism remotely controlled by a micrometer screw. The electrode holder could be moved on a ball-and-socket joint so as to reach different points on the retinal surface. At each position, the electrode was slowly advanced so as to touch or penetrate the retina. The platinum electrodes were allowed merely to touch the retina lightly, while the indium and micropipette electrodes penetrated the internal limiting membrane. Suitable single units (ganglion cells) were usually found only after trying a number of positions on the retina when platinum or indium electrodes were used, giving potentials of 100 to 500 microvolts. With micropipettes the potentials were sometimes many millivolts and were obtained at practically any point on the retina. The usual criteria for a single unit discharge were used: uniformity of size, shape, and sound of the action potentials. Multiunit discharges were not used. The stimulus spot was centered on the tip of the electrode above the ganglion cell, which lies in the center of the receptive field, and the response to a flash there indicated whether the cell was an on- or off-center unit. The metal electrodes were connected to a conventional physiological amplifier, cathode ray oscilloscope, and loud-speaker. The 3 M KCl-filled capillaries were connected through a negative capacity feed back input stage (MacNichol and Wagner, 1954) to a direct coupled amplifier.

Counting of Nerve Impulses

The nerve impulse signal from the amplifier was used to trigger an electronic counter which periodically counted the number of impulses occurring during a 10 second period, three times a minute. If the action potentials were large relative to the noise level in the baseline, the triggering voltage of the counter could easily be adjusted so that all the impulses but none of the baseline variations were counted. However, if the action potentials were relatively small, the variation in their heights due to the noise could cause some of the impulses to be missed by the counter. In the earlier experiments, a check of counting accuracy was made periodically by displaying the output pulse from the counter on one beam of the cathode ray oscilloscope,

the action potential on the other, and seeing if any missed impulses could be detected with a fast repetitive sweep. In the later experiments, a device to brighten the cathode ray oscilloscope trace of the impulse at the instant of triggering served the same purpose.

RESULTS

A. General Properties of the Maintained Discharge

Maintained activity in single ganglion cells of the cat has frequently been observed by Granit (1947) and Noell (1953), and has been regularly seen by Kuffler (1952; 1953). Experiments, however, were needed to establish specifically the physiological nature of the background discharge. Ganglion cell activity was influenced, for instance, by electrode pressure and eye movement, poor circulation, distortion and manipulation of the eyeball, anesthetics, and changes in ventilation and temperature.

Pressure.—The most important source of artefact in the present preparation was pressure on the ganglion cells. The platinum wire electrode, to record best, must lie over and quite near the ganglion cells, and around its area of contact it always presses to some extent on the cells. Once a ganglion cell was located, a constant light flash caused a reasonably reproducible response. In platinum electrodes the fine wires are surrounded by a relatively thick glass jacket and appreciable pressure can be exerted without piercing the retina. If the electrode was advanced and obvious pressure exerted (detected by dimpling of the retinal surface), the following sequence of changes was frequently seen. The background activity rate was increased and usually the frequency waxed and waned. If the pressure was great enough the discharge attained a rate of several hundred per second and was followed by an abrupt cessation of activity, obviously due to injury. This high pitched injury discharge of an agonized cell, familiar to most investigators, is readily recognized in the loudspeaker. Pressure with the electrode, if carefully and gradually applied, may lead to a reversible block of the spike in the ganglion cell soma, leaving behind a local potential (*cf.* Fig. 2, Kuffler, 1953).

In many ganglion cells advancing the electrode did not cause the changes mentioned above, although obvious pressure was exerted. It is assumed that in such instances the electrode was not located directly above the active cell. Iridium electrodes (see Method) with an outer tip diameter of 3 to 4 μ, or the smaller fluid electrodes, penetrated the different retinal layers without difficulty. Ganglion cell discharges appeared at definite depths of the advancing electrode. Activity in individual cells was often readily and repeatedly obtained at a definite depth during fine up-and-down adjustments. Under these conditions the electrode clearly did not injure the cell since the average maintained discharge frequency did not change.

Another way of studying maintained background discharge is by recording

from optic nerve fibers. In such a case, electrode pressure cannot affect the activity, which originates in a receptive field some distance from the electrode. The same is true when an electrode touches lightly the surface of the retina at any place (except in the area centralis, where there are few nerve fibers—unpublished data). The triphasic axon discharges recorded simultaneously from

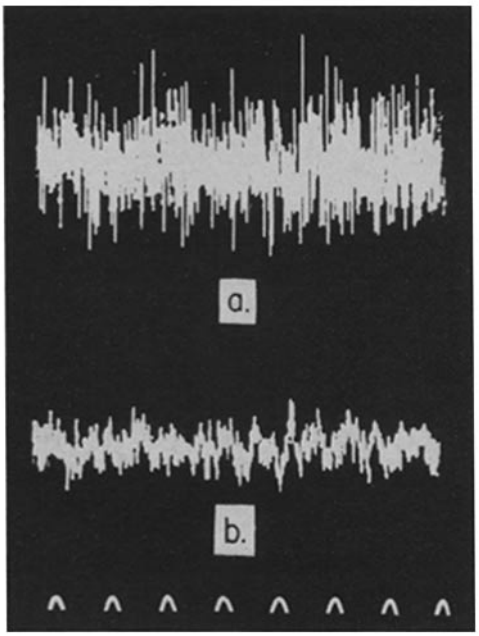

FIG. 1. Potentials recorded from the unopened eye of the cat with a platinum electrode and with no retinal illumination. (*a*) Massed spontaneous discharge from nerve fibres, electrode touching the surface of the retina. (*b*) Noise level with electrode tip withdrawn into the vitreous. Time signal 10 msec. The largest action potentials in (*a*) have been retouched; the smaller ones are not seen individually but rather as a broadening of the baseline as compared with (*b*).

many fibers are readily distinguished from those of single ganglion cells. It is impossible with present methods to record for prolonged periods from individual nerve fibers and to estimate whether units are active continuously or perhaps in relays only. Nerve fiber records (Fig. 1), however, clearly show, in agreement with records from single ganglion cells, that massive continuous nervous activity is a feature of the light- or dark-adapted retina. The activity in Fig. 1 *a* is affected by illumination of the eye while the record in Fig. 1 *b* is not changed. The increased baseline disturbance on touching the retina is therefore not due to an increased input resistance.

After a single ganglion cell was obtained, the discharge rate and rhythm sometimes changed suddenly, as a result of movement of the eyeball, due to coughing, jerking, etc. On occasions the discharge followed the rhythm of the heart beat. All these changes were due to pressure effects and could be avoided by different placement of the electrode, better fixation of the eye or, in a few instances, by curarization.

Circulation, Respiration, Temperature.—In our preparation the circulation of the retina can be examined during the experiment. Deterioration in the cat's condition could frequently be detected in this way prior to the onset of more obvious general symptoms. Slowed movement of red cells in the capillaries and finer vessels was sometimes seen, frequently accompanied by "clumping and sludging," a phenomenon also seen in other organs when they were poorly circulated; *e.g.*, in the surface vessels of muscles. In some experiments the circulation to the eye was purposely cut off for varying periods by clamping the carotids. This was promptly followed by a decrease of volume of the retina resulting in a slight withdrawal of the retina from the electrode. By advancing the electrode, contact could be reestablished and frequently ganglion cell discharges continued for several minutes before becoming irregular and dropping out. No serious attempt was made to study in detail the effect of poor circulation on retinal activity, but the impression was formed that units can recover well after temporary arrest of blood flow. As a result of impaired circulation the general level of activity, *i.e.* the number of active units as estimated by nerve fiber potentials, seemed to be decreased and in some units the background discharge eventually slowed or stopped altogether. In the eyes of decerebrate cats which had apparently good circulation, all ganglion cells showed maintained activity after a period in darkness or during steady illumination (see later).

Some cats were hyperventilated by a respirator or were made to breathe into a balloon. Again, these experiments were carried only so far as to make reasonably certain that these factors were not responsible for or did not greatly influence maintained activity as studied in these investigations. The same was true for cooling of the eyeball by cold saline and letting the body temperature fall to about 34°C. from the usual 37–38°C.

Anesthetics.—The most convenient way of experimenting on the eye is during deep anesthesia; dial-urethane or pentobarbital sodium (nembutal) was frequently used. Some features of the retinal discharge were, however, affected by anesthetics. The effect of nembutal on retinal discharges was tested by injecting it intravenously into decerebrate preparations while recording from a ganglion cell. The action of a dose, about one-fourth that needed for general anesthesia, may be summarized as follows. (1) The maintained activity was decreased or suppressed but generally returned to its original level within a few minutes. When it was reestablished, however, it often seemed to have different characteristics from the normal maintained discharge. Sometimes the

discharge became less regular, but it is also noteworthy that the most regular discharge we ever recorded was from an anesthetized preparation. Perhaps the most constant finding was that impulses tended to be grouped and various rhythms appeared; it would demand an elaborate statistical analysis to describe these completely. (2) The response to a light flash of 0.5 to 1.0 seconds, which in the absence of the drug did not outlast the stimulus, became prolonged and increased in frequency. For instance, an on-response might be followed by an after-discharge lasting 5 seconds. On occasions the discharge was then suddenly cut off.

When dial-urethane or nembutal was used alone, without decerebration, their specific effects were not easy to assess. It seemed that some features of the responses during prolonged flashes, of 1 to 10 seconds or longer, were changed. In decerebrate cats, maintained illumination of a small central area in an on-center unit produced an increased discharge rate for many seconds or minutes rather than the shorter transient on-responses generally seen in anesthetized animals. Further, the maintained on-discharge could also be obtained more readily by stimulating the surround of an off-center unit with an annular pattern of light that did not fall on the center of the receptive field.

The present experiments lead to the definite conclusion that all retinal units which are accessible by the present recording methods are in continuous irregular activity when dark-adapted and when light-adapted.

B. Changes in Discharge Rates at Different Levels of Illumination

After a single unit was found, only the background light, covering the entire receptive field, was altered. The maintained activity was present in all units whether in light or dark-adapted and was measured by counting impulses, either during ten 1 second periods per minute or during three 10 second periods per minute. Both procedures gave similar results.

This method of counting impulses did not resolve any transient changes of frequency that occurred within several seconds following a sudden change of illumination. The cell population was selected without regard to type (on- or off-center), generally from the upper half of the retina in the region of the tapetum. No distinctive pattern of maintained discharge behavior has emerged but some representative samples will be described. Most of our observations were started at a light-adapted level, and we tried to record from a unit for about 30 minutes before the background illumination was changed, or until at least a steady initial baseline of frequency was reached. Those units which gave irregular discharges with wide fluctuations of frequency were arbitrarily discarded because in many instances the changes in discharge rates were accompanied by some detectable movements of the eye. After a change in background illumination there was generally a large transient change in frequency, lasting between 5 and 15 minutes, followed by a fairly constant frequency level.

Fig. 2 illustrates an off-center unit which discharged at about 15/second for

30 minutes while under 3.0 f.-c. retinal illumination, recorded with a platinum electrode. In this and the following figures, there were too many readings to be plotted as separate points. Instead, the solid line was drawn through the points to indicate mean frequency; the dotted lines above and below enclose all but a few of the readings. When the illumination was reduced to 0.03 f.-c. there occurred a sudden high frequency burst of activity (not plotted) following which the frequency returned to 80 to 90/second within a minute. After another 15 to 20 minutes the discharge frequency levelled off to a rate near 55/second

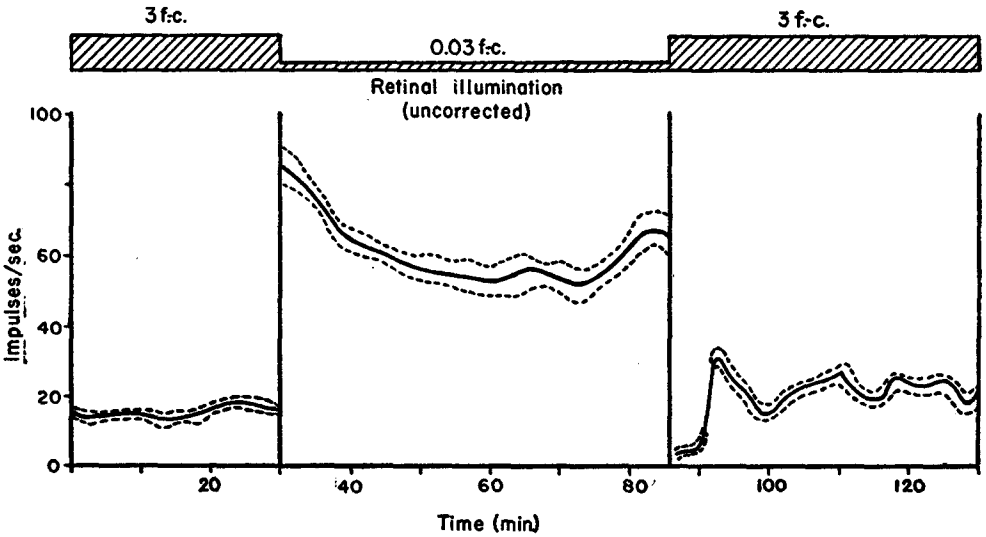

FIG. 2. Frequency of maintained discharge during changes of retinal illumination. Off-center unit recorded with platinum electrode. Values of retinal illumination are uncorrected for all losses in the ocular media. Solid line indicates mean frequency; dotted lines are limits of variation.

which was maintained for more than half an hour. The background illumination was then increased once more to 3.0 f.-c. This time the discharge disappeared briefly and then returned, during 10 to 15 minutes, to a maintained level near 20/second which was counted for another 30 minutes until the unit was lost.

While in the previous experiment the discharge rate increased as the illumination was reduced, the opposite result is shown in Fig. 3. This ganglion cell, an on-center unit, had a low discharge rate of 4 to 8/second for a 40 minute period at an illumination of 0.3 f.-c. A tenfold increase of illumination to 3.0 f.-c. increased the discharge rate to 14 to 17/second, which remained constant for 35 minutes. It should be noted that there was no marked transient discharge shift during the first 5 to 15 minutes after increasing the illumination; the new maintained rate was established practically at once. This unit was recorded with a KCl-filled micropipette of about 0.5 μ tip diameter.

A third example is plotted in Fig. 4, an on-center unit which was recorded for 3 hours with a KCl-filled electrode. Observations were started with the

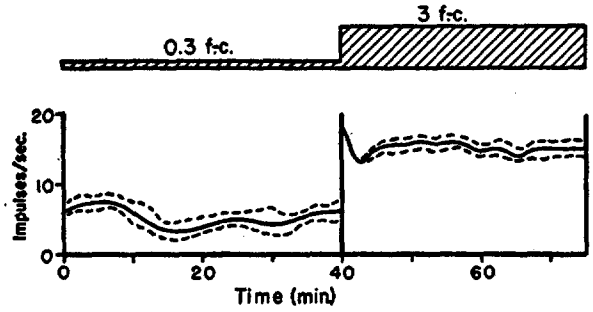

FIG. 3. Frequency of maintained discharge during a change of illumination. On-center unit recorded with micropipette. New maintained discharge rate at 3.0 f.-c. established almost immediately, in contrast to Fig. 2.

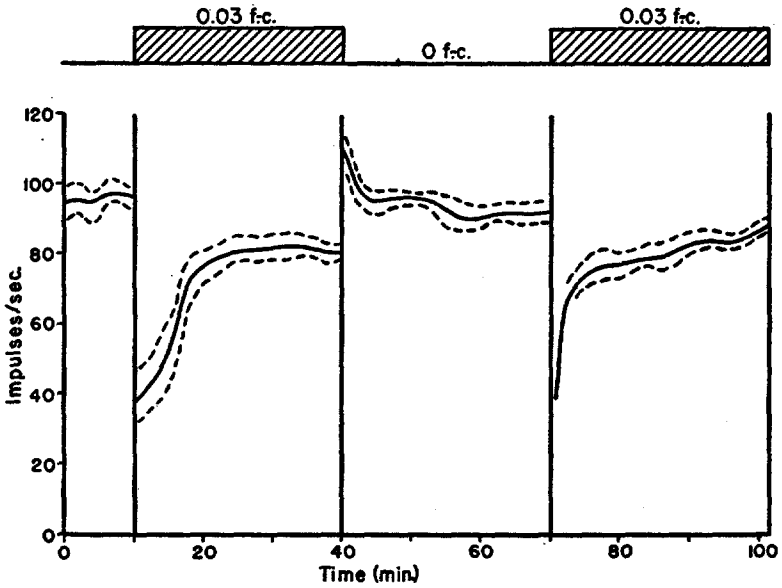

FIG. 4. Frequency of maintained discharge during changes of illumination. On-center unit recorded with micropipette. Maintained frequency decreased with increased illumination, in contrast to Fig. 3.

retina in total darkness and a discharge rate of 92 to 100/second. Following illumination of 0.03 f.-c. the first recorded frequency was decreased and it was 10 to 15 minutes before a new steady level of 80 to 85/second was established. After 30 minutes the light was turned off again, resulting in a transient increase of frequency lasting 5 minutes, followed by a steady rate of 90 to 95/second.

After a return to the 0.03 f.-c. background, the previous pattern was repeated. It should be pointed out that from Fig. 3 the opposite result might be expected from an on-center unit; *i.e.*, an increased maintained rate when the illumination is increased. If there was an increase in frequency, resulting from stimulation of the central zone, this was too brief to produce an appreciable effect on the first count which started about 10 seconds after changing the illumination.

Finally, in Fig. 5 an off-center unit is shown in which backgrounds of 3.0 and 24 f.-c. were used alternately for 10 minute periods. There were large transient

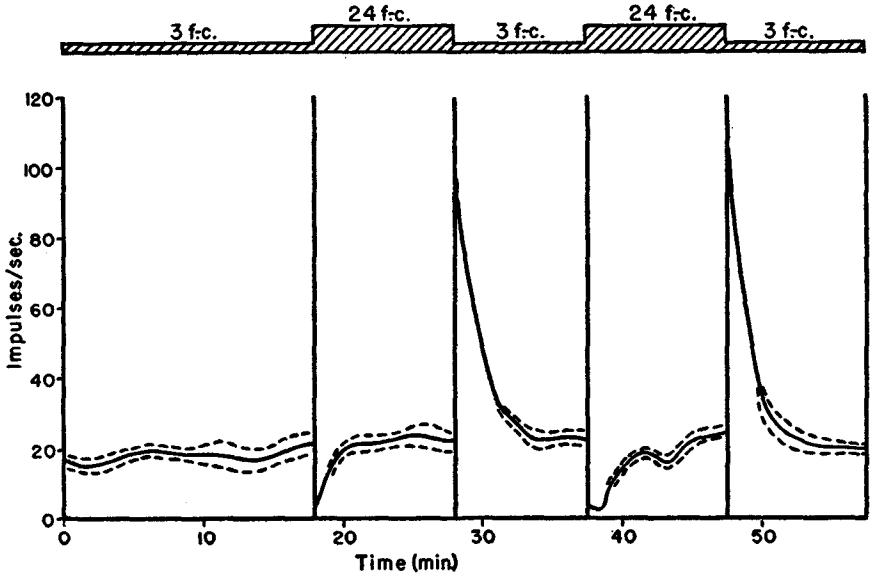

FIG. 5. Frequency of maintained discharge during changes of illumination. Off-center unit recorded with indium electrode. No permanent change of frequency resulted from change of illumination.

frequency changes lasting less than 5 minutes, after which the frequency returned each time to a nearly constant frequency.

In a few experiments, portions of the receptive fields were illuminated separately for varying periods. Thus in an on-center unit a spot of light of about 0.5 mm. in diameter increased the maintained discharge rate. Illumination of the surround of the same field by a ring of light which did not shine on the central region suppressed or decreased the discharge. When the whole receptive field was illuminated (as in Figs. 2 to 5) the discharge rate was intermediate between the activity produced by exclusive excitation of the center or of the surround. These results suggested that the discharge evoked by light, though it adapted rapidly to begin with, did not adapt completely. The maintained discharge could then be explained as the residue of the light-evoked discharge,

and its net amount should be the result of the interaction of the non-adapting residue of the excitatory and inhibitory influences converging on the ganglion cell. Although this is a relatively simple hypothesis it would not work out so simply in practice because the receptive field organization itself changes when the level of illumination is changed (Barlow, FitzHugh, and Kuffler, 1957 *b*). Thus an increase of illumination over the whole field might cause an increase or a decrease of frequency depending on whether the on-region or the off-region of the receptive field was dominant, or there might be no change if they were evenly balanced. Furthermore, which was dominant would vary, not only from ganglion cell to ganglion cell, but also with the level of the adapting light and the past history of the cell. The results of Figs. 2 to 5 do not, therefore, prove or disprove the hypothesis: to settle the matter it would be necessary to follow the change in receptive field organization together with the change in level of maintained activity, and this has not been done.

It might be thought that the level of maintained activity would change during the course of dark adaptation. As described elsewhere, many units were successfully held throughout a period of dark adaptation, but no correlation between dark adaptation levels and discharge frequencies was noticed, apart from the transient changes in the first few minutes.

C. Statistical Properties

There were four steps in the statistical analysis of the apparently random discharge. (*a*) The intervals were measured and arranged in histograms. (*b*) The hypothesis that the probability of occurrence of an impulse is constant and does not vary with the time of the preceding impulse was tested; the hypothesis leads to the expectation of an exponential distribution of intervals, and this was shown not to fit the data. (*c*) The gamma distribution was shown to fit the data adequately. (*d*) By using serial correlation coefficients it was shown that the impulses do not occur at random from this distribution, but there is a slight but significant tendency for short intervals to be followed by long ones and *vice versa*.

Fig. 6 shows a typical maintained discharge from a single off-center ganglion cell; the intervals between successive impulses vary in an apparently random fashion over a wide range. Such a record resembles at first sight a discharge of pulses from a Geiger counter near a radioactive source; in the latter case the different pulses are statistically independent events, except that the counter has a brief "dead time," following each pulse, during which it is unresponsive. The distribution curve of intervals (t) in a Geiger counter discharge with dead time d is closely approximated by an exponential function (Alaoglu and Smith, 1938):

$$\begin{aligned} f(t) &= 0 && 0 \leqq t < d \\ &= me^{-m(t-d)}, && d \leqq t < \infty \end{aligned} \tag{1}$$

In order to compare the ganglion cell discharge statistically with that from a Geiger counter, the values of the intervals were measured from records of the maintained discharge from six different units from three cats. The number of intervals measured for each unit was between 144 and 400. First, histograms were plotted to show the experimental distribution of interval durations. Figs. 7 *a* and *b* show two such histograms, labelled "experimental." In an attempt to fit formula (1) to the histograms, the parameter d was chosen as

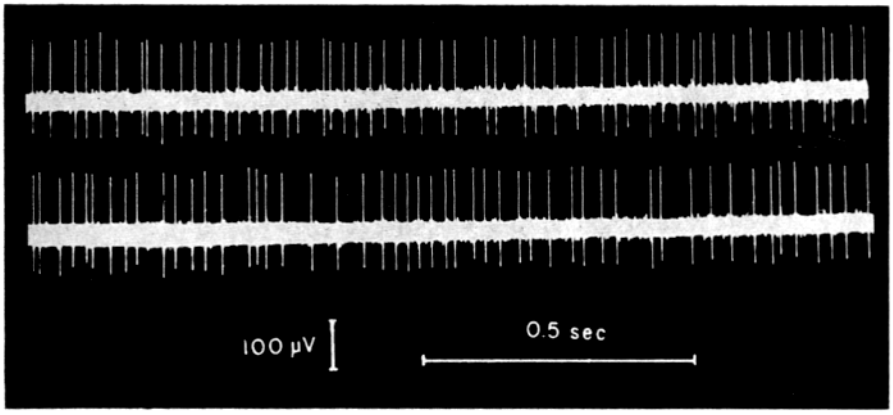

FIG. 6. Maintained discharge in a single off-center ganglion cell, showing fluctuations in the durations of successive intervals. Retouched photographic records.

the minimum interval by inspection of the histogram, and m calculated from the following formula, which can be derived from (1):

$$m = 1/(\bar{t} - d) \tag{2}$$

Here $\bar{t}$ is the mean value of t. The broken curve in Fig. 7, marked "exponential," represents equation (1) as fitted to the data. Chi-square tests showed that all six histograms departed significantly from the exponential curve. (The number of degrees of freedom for the chi-square test was taken as two less than the number of classes into which the data were grouped, since two parameters, d and m, had been fitted to the data.) Assuming for the moment that the exponential curve is the true one, let P be the probability that a random sample would give a worse fit than that actually found; then the smaller P is, the poorer the fit. For unit 1, P was 4.5 per cent; for the other five units, P was 0.1 per cent or less. Since a P of 5 per cent or less is usually taken to indicate a significantly poor fit, all the histograms did not fit the exponential distribution; this shows that, in contrast to the case of the Geiger counter, the probability of the cell's firing during any infinitesimal interval of time dt did depend on the time of occurrence of the preceding impulse, even for intervals much greater than the absolute refractory period (about 1 msec.) of ganglion cells.

Since the histograms were all strongly skewed (asymmetrical about the mean

value) and since the interval t is a positive number, the gamma distribution (Pearson's type III: Kendall, 1945) was tried:

$$f(t) = \frac{k^a t^{a-1} e^{-kt}}{\Gamma(a)}, \qquad 0 \leqq t < \infty \tag{3}$$

A satisfactory fit was found, although this expression does not contain the minimum interval d. The two parameters a and k were calculated from the

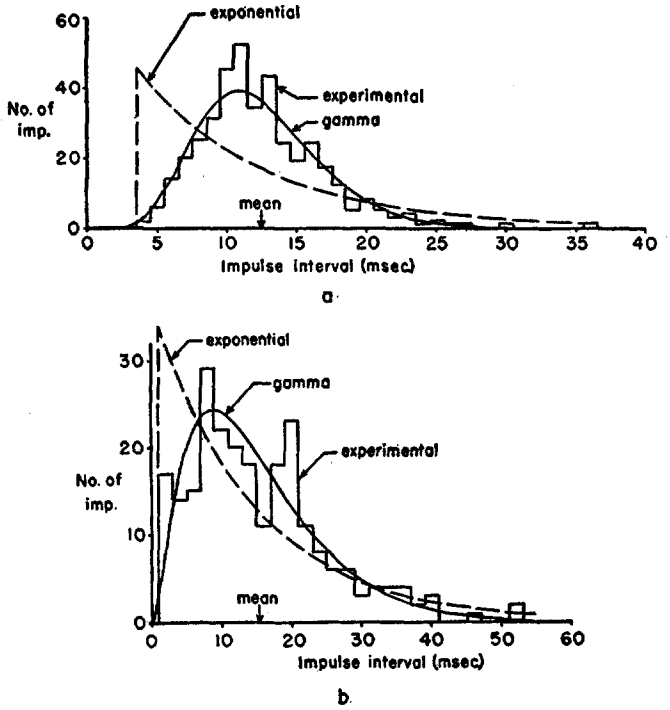

FIG. 7. Two distributions of impulse intervals from different ganglion cells. (*a*) shows unit 3 (see Table I), and (*b*), unit 4. Two theoretical curves, the exponential and the gamma distributions, are shown. Only the gamma gives a satisfactory fit.

experimental mean $\bar{t}$ and the standard deviation σ_t by the following formulas, which can be derived from equation (3) (Weatherburn, 1946):

$$k = \frac{\bar{t}}{\sigma_t^2}, \qquad a = k\bar{t} \tag{4}$$

Two of the fitted distributions are shown in Fig. 7, labelled "gamma." Chi-square tests gave values of P between 12 and 70 per cent for five out of the six histograms, indicating good fits. The other histogram (unit 5) had a P of less than 0.1 per cent for both the exponential and the gamma distributions, showing that neither distribution fitted. This histogram was unusually irregular and

not typical, but the same unit had typical serial correlation coefficients (see below).

When the chi-square test was used to test the fit of the gamma distribution, the number of degrees of freedom was reduced by two, as before, since two parameters were estimated from the data. Equations (4), it is true, do not provide a maximum likelihood estimate for the two parameters (as was true for the exponential distribution), although this condition may actually be necessary to justify our procedure (Kendall, 1945). However, any errors in estimating k and a from this source would only increase the value of chi-square and make the fit worse than that provided by the maximum likelihood method.

TABLE I

Unit No.	Type of unit	Retinal illumination	Period of adaptation	Gamma distribution			Serial correlation coefficients				
				$\bar{t}$	k	a	r_1	r_2	r_3	r_4	r_5
		f.-c.	*min.*	*msec.*	*msec.*$^{-1}$						
1	?	?	?	15.7	0.114	1.80	−0.174	+0.046	−0.071	−0.108	+0.050
2	On-center	45	30	25.1	0.163	4.08	−0.117	−0.093	−0.064		
3	On-center	45	7	12.4	0.653	8.08	−0.200	−0.152	−0.037		
4	Off-center	0	48	15.3	0.159	2.43	−0.102	+0.107	+0.019		
5	On-center	0	180	34.2			−0.257	−0.095	+0.036		
6	On-center	0	47	33.5	0.070	2.36	−0.188	−0.122	−0.060		
Mean							−0.173	−0.052	−0.030		

Since, however, except for unit 5, the fits found by the above method were satisfactory, our conclusions remain unchanged.

Table I shows the values of $\bar{t}$, k, and a for the six units (columns 4 to 6). There was no apparent relation between any two of these parameters, and k and $\bar{t}$ did not depend on the state of adaptation (retinal illumination given in column 3), but a was greatest for the two light-adapted units.

The gamma distribution provided a good empirical description of the distribution of intervals in the maintained discharge, but other formulae might have fitted our data just as well. The gamma distribution was chosen partly because it is simple enough mathematically to lend itself to further theoretical studies. Hagiwara (1954), studying afferent discharges from muscle spindles, used a different formula, but his curves look similar to the gamma distribution.

It has been found that the firing probability at any time depends on the

firing times of previous impulses. It is therefore of interest to know whether the firing probability of a given impulse is affected by the times of any impulses earlier than the immediately preceding one. For this reason some of the serial correlation coefficients of the intervals between impulses were computed. The jth serial correlation coefficient r_j is a measure of the correlation between the duration of any interval and that of the jth preceding one (Kendall, 1945; Yule and Kendall, 1950).

The calculated values of some of the serial correlation coefficients are given in Table I. All values of r_1 are negative, and they have a mean of -0.173. This mean value was found to be significantly different from zero by the t-test (Fisher, 1948), which gave a P of less than 0.1 per cent. The mean values of r_2 and r_3, however, were not significantly different from zero (P = 28 per cent, 17 per cent). It may be concluded therefore that there is a negative correlation between the durations of pairs of successive intervals, but that for more widely separated intervals we have found no evidence of correlation. This means, for instance, that a longer than average interval tends to be followed by a shorter than average one. Hagiwara (1949), studying the discharge of human motor units during voluntary contraction, found an r_1 of about -0.5; the higher order coefficients were nearly zero. However, in the frog muscle spindle afferent discharge Hagiwara (1954) found no significant serial correlation.

The statistical properties found for the maintained discharge of the ganglion cell may be summarized as follows. The interval durations are not distributed exponentially, and therefore the preceding impulses have an influence on the firing probability that considerably outlasts the absolute refractory state. The gamma distribution, however, fits the data well in most cases. The serial correlation coefficients of the impulse interval durations show that a momentary fluctuation in impulse frequency tends to be compensated for immediately by a fluctuation in the opposite direction, and the firing probability at any time is affected principally by the time elapsed since the immediately preceding impulse, and to some extent by the time between that one and its predecessor, but not significantly by the times of earlier ones. Restated in physiological terms, each impulse is followed by a transient depression of excitability which may outlast the succeeding impulse.

DISCUSSION

Although the existence of "spontaneous" activity in the central nervous system and in sense organs has been accepted for some time, a rigorous proof of spontaneity is difficult to obtain in most cases. Some environmental factors providing "stimuli" are difficult to exclude, even in isolated cells. In sense organs it seems reasonable to apply the term spontaneous to nervous activity which occurs in the absence of a specific or adequate stimulus. An eye which remains in its normal environment and puts out afferent nerve impulses in

the absence of light stimuli thus possesses "spontaneity." Such spontaneity is of special interest in the eye because it related to the threshold-noise problem (Barlow, 1956; FitzHugh, 1957) and may be at the basis of "visual grey" perception.

If a light is shone on the receptive field of an active nerve fiber its discharge may be only transiently changed and in some cases the impulse rate which persisted in the dark may return to its original level. Activity in such a unit during illumination cannot be called spontaneous and therefore the term "maintained," or background, activity is preferred. Furthermore optic nerve fibers which do not alter their discharge rate permanently after changes in illumination, are by themselves not capable of signalling absolute levels of brightness. In other units the frequency changes may be in different directions and would have to be interpreted differently by the brain. In any event since all units seem to be continuously active it is the modulation of the maintained rate in either direction which provides the information of changed background illumination.

We were not able to predict the direction of the change in frequency of the maintained discharge from the discharge characteristics of its receptive field, and we cannot help suspecting that the sensory impulses that convey information about the absolute intensity of the light falling on the eye travel in fibers from units which we have not analyzed. The sustained, reflex constriction of the pupil leaves no doubt that the cat's nervous system receives such information.

Statistical Properties.—A study of the statistical properties of an afferent cell discharge is relevant to two problems: the origin of the discharge in cells and the analysis of the nerve fiber message by the central nervous system.

The first problem has been studied for the afferent discharge from the frog's muscle spindle by Buller, Nicholls, and Ström (1953) and Hagiwara (1954). Some of their interval distributions closely resemble ours. These authors advance the hypothesis that corresponding to each degree of stretch there is a randomly fluctuating level of depolarization of the nerve cell, and that whenever this depolarization rises above threshold, it excites the cell. Following an impulse, the threshold falls from the high value it assumes during the relative refractory period back to its resting value. When it falls sufficiently low, the cell is reexcited. The average interval duration between two successive impulses is determined both by the time course of the recovery curve and by the average level of the stimulating depolarization. The variation in the interval duration is the result of the random fluctuations of the depolarization. In this model no correlation between successive intervals could occur, unless the low frequency components of the noise voltage were so large as to cause a positive correlation. Such a hypothesis thus appears to be too simple to explain the significantly negative values of r_1 for the retinal ganglion cell discharge.

However, if this model were extended to include a temporary decrease of sensitivity following each impulse, a negative serial correlation could occur.

Unfortunately, our statistical analysis enables us only to describe the fluctuating maintained activity in darkness; it cannot tell us where it originates. It might arise either in the photoreceptors, by the spontaneous decomposition of visual pigment, or in an excitation of fine dendritic terminals by random electrical noise of the sort proposed by Katz (1950). Alternately, the self-excitatory process might have a regular rhythm, but be modified by a random process originating elsewhere. For instance, the ganglion cell might have an intrinsic rhythm which is modified by the random arrival of impulses from the bipolars, or the bipolars might all fire with regular rhythms, but at different frequencies and asynchronously, so as to produce a statistically fluctuating state of excitation at the ganglion cell.

The second problem mentioned above, the analysis of the nerve fiber message by the brain, is similar to the engineering problem of detection of a signal in a noisy communication channel. This problem will be treated in greater detail in a separate paper (FitzHugh, 1956), but one aspect of the present data is significant here, namely the negative first serial correlation coefficient (-0.10 to -0.24) between successive impulse intervals. This would make short intervals more likely to be followed by long ones and *vice versa*, and changes in frequency which last for several impulses would be less likely to occur than if there were zero serial correlation. Since such momentary changes in frequency, in the form of short bursts or pauses, characterize the response of a ganglion cell to a short flash, this would appear to be a mechanism making the inherently "noisy" retina detect a flash of light more effectively. However, since the values of the serial correlation coefficients are rather small, this effect may not be very important physiologically.

SUMMARY

Nervous activity has been recorded from the unopened eye of decerebrate cats. Recordings were made with metal electrodes or with small micropipettes from ganglion cells or nerve fibers.

Continuous maintained discharges were seen in all ganglion cells during steady illumination of their receptive fields, as well as in complete darkness. Possible artefacts, such as electrode pressure, abnormal circulation, anesthetic, and several other factors have been excluded as the source of the maintained discharge. Visual stimuli are therefore transmitted by modulating the ever present background activity.

Discharge frequencies were measured following changes of retinal illumination. No consistent patterns of frequency change were found. The maintained discharge frequency may be permanently increased or decreased, or may remain practically unchanged by altering the steady level of illumination. In

addition, there were often transient frequency changes during the first 5 to 10 minutes after changing illumination, before a final steady rate was established.

A statistical analysis of the impulse intervals of the maintained discharge showed: (*a*) the intervals were distributed according to the gamma distribution (Pearson's type III), (*b*) the first serial correlation coefficient of the intervals was between -0.10 and -0.24, with a mean value of -0.17, which is significantly different from zero, (*c*) the higher order serial correlation coefficients were not significantly different from zero. Thus the firing probability at any time depends on the times of occurrence of the two preceding impulses only, and in such a way as to indicate that each impulse is followed by a transient depression of excitability that outlasts the following impulse.

The possible sites at which spontaneous or maintained activity may originate in the retina are discussed.

We are indebted to Dr. K. T. Brown and Dr. T. N. Wiesel for their assistance in many of the experiments and to Dr. P. Meier for his advice on the statistical analysis. We also wish to thank Mr. A. Goebel and Mr. R. B. Bosler for much technical assistance.

REFERENCES

1. Adrian, E. D., Synchronized reactions in the optic ganglion of *Dytiscus*, *J. Physiol.*, 1937, **91**, 66.
2. Alaoglu, L., and Smith, N. M., Statistical theory of a scaling circuit, *Physic. Rev.*, 1938, **53**, 832.
3. Autrum, H., Über zeitliches Auflösungsvermögen und Primärvorgänge im Insektenauge, *Naturwissenschaften*, 1952, **39**, 290.
4. Barlow, H. B., Retinal noise and absolute threshold, *J. Opt. Soc. America*, 1956, **46**, 634.
5. Barlow, H. B., FitzHugh, R., and Kuffler, S. W., Dark adaptation, absolute threshold, and Purkinje shift in single units of the cat's retina, 1957 *a*, *J. Physiol.*, in press.
6. Barlow, H. B., FitzHugh, R., and Kuffler, S. W., Change of organization in the receptive fields of the cat's retina during dark adaptation, 1957 *b*, *J. Physiol.*, in press.
7. Bremer, F., Considerations sur l'origine et la nature des 'ondes' cérébrales, *EEG. Clin. Neurophysiol.*, 1949, **1**, 177.
8. Buller, A. J., Nicholls, J. G., and Ström, G., Spontaneous fluctuations of excitability in the muscle spindles of the frog, *J. Physiol.*, 1953, **122**, 409.
9. Bullock, T. H., and Diecke, F., Properties of an infrared receptor, *J. Physiol.*, 1956, **134**, 47.
10. Cohen, M. J., The function of receptors in the statocyst of the lobster *Homarus americanus*, *J. Physiol.*, 1955, **130**, 9.

11. Dowben, R. M., and Rose, J. E., A metal-filled microelectrode, *Science*, 1953, **118**, 22.
12. Fisher, R. A., Statistical Methods for Research Workers, New York: Hafner Publishing Co., Inc., 10th edition, 1948, 99, 119.
13. FitzHugh, R., The statistical detection of threshold signals in the retina, *J. Gen. Physiol.*, 1957, **40**, in press.
14. Granit, R., Sensory Mechanisms of the Retina, Oxford University Press, 1947, 95.
15. Granit, R., Receptors and Sensory Perception, New Haven, Yale University Press, 1955, 81.
16. Gregory, R. L., and Cane, V., A statistical theory of visual threshold, *Nature*, 1955, **176**, 1272.
17. Hagiwara, S., On the fluctuations of the interval of the rhythmic excitation. I. The efferent impulse of the human motor unit during the voluntary contraction, (in Japanese, with English summary), *Bull. Physiograph. Sc. Research Inst. Tokyo Univ.*, 1949, **3**, 19.
18. Hagiwara, S., Analysis of interval fluctuation of the sensory nerve impulse, *Japan. J. Physiol.*, 1954, **4**, 234.
19. Hecht, S., Energy and vision, *in* Science in Progress, (G. A. Baitsell, editor), New Haven, Yale University Press, 1945, series 4, 75.
20. Katsuki, Y., Yoshino, S., and Chen, J., Action currents of the single lateral-line nerve fiber of fish. 1. On the spontaneous discharge, *Japan. J. Physiol.*, 1950, **1**, 87.
21. Katz, B., Action potentials from a sensory nerve ending, *J. Physiol.*, 1950, **111**, 248.
22. Kendall, M. G., The advanced theory of statistics, London, Griffin and Co., 1945, **2**, 402.
23. Kuffler, S. W., Neurones in the retina: organization, inhibition and excitation problems, *Cold Spring Harbor Symp. Quant. Biol.*, 1952, **17**, 281.
24. Kuffler, S. W., Discharge patterns and functional organization of mammalian retina, *J. Neurophysiol.*, 1953, **16**, 37.
25. Löwenstein, O., and Sand, A., The individual and integrated activity of the semicircular canals of the elasmobranch labyrinth, *J. Physiol.*, 1940, **99**, 89.
26. MacNichol, E. F., Jr., and Wagner, H. G., A high-impedance input circuit suitable for electrophysiological recording from micropipette electrodes, *Research Report, Naval Med. Research Inst.* (Bethesda), 1954, **12**, 97.
27. Noell, W. K., Studies on the electrophysiology and metabolism of the retina, United States Air Force School of Aviation Medicine Project No. 21-1201-0004, Report No. 1, Randolph Field, Texas, 1953.
28. Roeder, K. D., Spontaneous activity and behavior, *Scient. Month.*, 1955, **80**, 362.
29. Talbot, S. A., and Kuffler, S. W., A multibeam ophthalmoscope for the study of retinal physiology, *J. Opt. Soc. America*, 1952, **42**, 931.
30. Tanner, W. P., Jr., and Swets, J. A., The human use of information. I. Signal detection for the case of the signal known exactly, *Tr. I.R.E.*, Vol. PGIT-4, September, 1954, 213.

31. Tasaki, Y., and Davis, H., Electric responses of individual nerve elements in cochlear nucleus to sound stimulation (guinea pig), *J. Neurophysiol.*, 1955, **18,** 151.
32. Weatherburn, C. E., A First Course in Mathematical Statistics, Cambridge University Press, 1946, 149.
33. Yule, G. U., and Kendall, M. G., An Introduction to the Theory of Statistics, New York, Hafner Publishing Co., Inc., 14th edition, 1950, 638.
34. Zotterman, Y., Special senses: thermal receptors, *Ann. Rev. Physiol.*, 1953, **15,** 357.