DISCHARGE PATTERNS AND FUNCTIONAL ORGANIZATION OF MAMMALIAN RETINA*

STEPHEN W. KUFFLER
The Wilmer Institute, Johns Hopkins Hospital and University Baltimore, Maryland

(Received for publication December 11, 1951)

INTRODUCTION

THE DISCHARGES carried in the optic nerve fibers contain all the information which the central nervous system receives from the retina. A correct interpretation of discharge patterns therefore constitutes an important step in the analysis of visual events. Further, investigations of nervous activity arising in the eye reveal many aspects of the functional organization of the neural elements within the retina itself.

Following studies of discharges in the optic nerve of the eel's eye by Adrian and Matthews (2, 3), Hartline and his colleagues described the discharge pattern in the eye of the Limulus in a series of important and lucid papers (for a summary see 20). In the Limulus the relationship between the stimulus to the primary receptor cell and the nerve discharges proved relatively simple, apparently because the connection between sense cell and nerve fiber was a direct one. Thus, when stimulation is confined to one receptor the discharge in a single Limulus nerve fiber will provide a good indication of excitatory events which take place as a result of photochemical processes. Discharges last for the duration of illumination and their frequency is a measure of stimulus strength. Lately, however, it was shown by Hartline *et al.* (22) that inhibitory interactions may be revealed when several receptors are excited. On the whole, the Limulus preparation shows many features which are similar to other simple sense organs, for instance, stretch receptors. In the latter, however, instead of photochemical events, stretch-deformation acts as the adequate stimulus on sensory terminals and is translated into a characteristic discharge pattern.

The discharge from the cold-blooded vertebrate retina (mainly frogs) proved much more complex. Hartline found three main types when recording from single optic nerve fibers: (i) "on" discharges, similar to those in the Limulus, firing for the duration of the light stimulus, (ii) "off" discharges appearing when a light stimulus was withdrawn, and (iii) "on-off" discharges, a combination of the former two, with activity confined mainly to onset and cessation of illumination. The mammalian discharge patterns were studied in a number of species by Granit and his co-workers in the course of their extensive work on the physiology of the visual system (summaries in 13, 15). On the whole, they did not observe any fundamental differences between frog and mammalian discharge types (see later).

* This investigation was supported by a research grant from the National Institutes of Health, U. S. Public Health Service.

The present studies were begun several years ago with the intention of examining the retinal organization and particularly processes of excitation and inhibition. As a first step, the discharge patterns were re-examined. It was assumed, in line with other workers, that the deviations in vertebrate eyes from the simple Limulus, or "on" discharge type, are due to the nervous structures and to their interconnections between the rod and cone layers on the one hand and ganglion cells on the other. Therefore, an extension of such studies should shed further light on the functional organization of the retina.

A preparation was used which approached fairly satisfactorily the "normal" state of the cat's eye. The discharge patterns reported by Hartline and those extensively studied by Granit were readily obtained. Single receptive fields—areas which must be illuminated to cause a ganglion cell to discharge —were explored with small spots of light and thereby some new aspects of retinal organization were detected. Specific receptive subdivisions, arranged in a characteristic fashion and connected to the common ganglion cells, seem to exist within each receptive field. This finding made it possible to study in detail some of the factors which normally contribute to the changing discharge pattern during vision. The present set-up also furnishes a relatively simple preparation in which the neural organization resembles the spinal cord and probably many higher centers of the nervous system. Many analogies have been found with discharge patterns in the spinal cord which are currently under study.

METHOD

The experimental arrangement, particularly the details of the optical system, has been described in full in a preceding paper (31). The main instrument, the "Multibeam Ophthalmoscope," consisted of a base which carried a holder in which the cat's head was rigidly fixed. Above the head, and also carried by the base, was the viewing-stimulating apparatus, which could be freely rotated and tilted. It contained three light sources with independent controls. This optical system was aligned with the cat's eye which thus was in the center of a spherical coordinate system and the eye's ordinary channels were used for illumination of the retina. One light provided adjustable background illumination and thereby determined the level of light adaptation. It was also used as a source for observation of the retinal structures. A maximal visual magnification of about 40 was obtained. The background illumination covered a circle of not less than 4 mm. (16° for the cat) in diameter, centered on the recording electrode. Two Sylvania glow-modulator tubes were used for stimulation of restricted areas of the retina. They illuminated patterns, mostly circles of varying diameter, which were imaged on the retina. The smallest light spots were 0.1 mm. in diameter on the retina. Thus, two images could be projected and their size and location varied independently on the retina. All three light sources used a common optical path, led into the eye through a pupil maximally dilated by Atropine or Neo-synepherine.

Complications from clouding of the cornea were prevented by the use of a glass contact lens, while the rest of the eye's optical system, lens and vitreous, remained intact. The circulation of the retina was under direct minute observation and whenever the general condition of the animal deteriorated this was readily noticed. The eye, as judged by its circulation and its discharge patterns, remained in good condition for the duration of the experiments which frequently lasted for 15–18 hours. Dial-urethane (Ciba) anesthesia (0.5 cc./kg.) or decerebration was used. The effect of anesthesia on the discharges is discussed later.

The eyeball was fixed by sutures to a ring which was part of the microelectrode manipulator. This fixation was generally satisfactory and breathing or minor body movement did not disturb the electrode position on ganglion cells. Sudden movements, however, such

as coughing, jerking, etc., prevented continuous recording from single units. Occasionally a persistent slow nystagmus developed and, in order to abolish this movement, the tendons of extraocular muscles were severed at their insertions.

Microelectrodes were introduced into the eye protected by a short length of #19 hypodermic tubing which served to penetrate the scleral wall near the limbus. The unprotected electrode shaft, less than 1 mm. thick, then traversed the vitreous and made contact with the retinal surface and toward the tip it was drawn to a fine taper. The shadow of the electrode thus covered only a small portion of the receptive field. If hit by the narrow light beams the electrode shaft caused scattering. All these phenomena and the positions and imagery of the stimulating beams or patterns were directly observed during the experiments and thereby a subjective evaluation of illumination conditions could be formed.

Electrical contact with the retinal cells was made by 10–15 μ Platinum-Iridium wires which were pushed to the tip of the glass tubes. The metal was either flush with the surrounding glass jacket which was sealed around it, or it protruded several micra. The configuration of the electrode tip was purposely varied a good deal, especially when the ganglion discharge was to be blocked by pressure. The potentials varied in size, and the largest were around 0.6 mV. The position of the indifferent electrode could be anywhere on the cat's body. In technically satisfactory preparations no difficulty was encountered in finding ganglion cells in quick succession and individual units could be observed for many hours (see later).

The second beam of the oscilloscope was used as an indicator of the current flow through the Sylvania glow-modulator tubes. The current was proportional to the light output but the spectral distribution of the light varied with different current strength. Therefore, Wratten neutral filters were used when the white light of the stimulators had to be attenuated. For the purpose of the present experiments the wave length variation which occurred played no significant role in those cases where intensities were varied by current flow adjustment (see Figs. 7*III*, 8, 10). The accurate electronic control of the stimulating light sources made an adjustment of flash durations quick and convenient. The time base was also recorded on the second sweep by intensity modulation through a square-wave oscillator.

Illumination values are given in meter candles; the calibration was made for flux reaching the corneal surface above the pupil and calculated for the area which it covered on the retina. Losses within the eye's media are neglected. The maximal available background illumination was about 6000 meter candles at the retina and could be attenuated to any desired extent. Since 1 m.c. at the retina corresponds to 10 mL external brightness (see 31) the samples illustrated here were taken well within the photopic range. Discharge patterns were, however, also studied in the absence of background illumination. In most experiments the exploring spot's intensity was approximately 100 m.c.

Results

1. Some characteristics of single unit discharge

Differentiation between ganglion and axon potentials. As a recording electrode of 5–15 μ diameter at the tip made contact with the surface of the retina, a mass of potentials was usually recorded on illumination of the eye. Very light touch of the retinal surface rarely yielded differentiated single unit potentials. The latter could, however, be obtained with a slight further advance of the electrode, still without marked pressure against the tissue. Different degrees of "touch" and pressure were easily differentiated under close direct observation (see Method). The most common and most easily recorded potential seen in the retina was a polyphasic spike, starting with an initial positive deflection, similar to that shown in Figure 1*B*. Such potentials are generally set up by a small spot of light at some distance from the recording electrode. From this observation it follows that conduction to the

recording lead has taken place and that the potential is derived from a nerve fiber. The polyphasic shape is typical of conducted potentials in a volume conductor. Similar potentials are familiar from recordings in other parts of the nervous sytem where microelectrodes are employed. The propagated potentials in nerve fibers could be used in the present studies, but they were small and could not be kept under the electrodes for prolonged periods. In contrast, potentials were recorded which always originated under the electrode tip (Fig. 1*A*). These were simpler and larger and usually started with

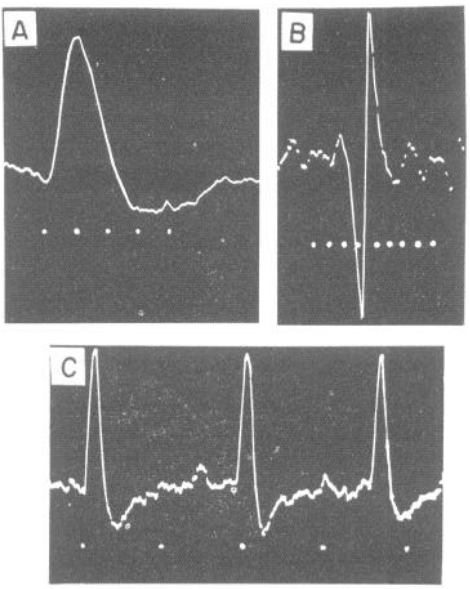

FIG. 1. Potentials from different retinal elements recorded with microelectrode. A: Ganglion cell discharge, caused by stimulation of retina in proximity of recording electrode. B: Nerve impulse in an axon, set up by retinal stimulation some distance from electrode. C: Three ganglion cell potentials from middle portion of a high-frequency discharge which is illustrated in Fig. 9c; potentials become progressively smaller at this rate. Negative deflexion of A and C 0.4 mV. and 0.1 mV. in B. Time intervals in A and B 0.1 msec. and in C 1.0 msec. Note that ganglion potential can also start with small positive inflexion if recording electrode is somewhat shifted.

a sharp negative inflection which was followed by a relatively smaller positivity. The potentials were generally about 0.3–0.4 msec. at the base, and their negative phase was of longer duration than in the potentials of Fig. 1*B*, where the whole triphasic complex is of a similar duration. The distortion of the real potential time course is due to the smallness of the effective interelectrode distance with the present electrodes. In a volume conductor the potentials which arise close to or under the electrodes start with a sharp negative inflection, as in Figure 1*A*. On such grounds this potential is likely to be a ganglion cell potential which lies in the vicinity of the electrode contact. Physiological tests furnish convincing evidence for such a conclusion. The area of the retina, which on illumination caused discharges in the "ganglion" cells, was found to be in the immediate neighborhood of the electrode tip; this also was the place where the lowest intensity light spot was effective in setting up discharges. As an exploring spot was moved further from the tip of the electrode, stronger stimuli were needed. The active unit lay in the approximate center of the "receptive field" (see later) and excitation apparently reached it through converging pathways from its immediate

neighborhood. Such an arrangement is typical of ganglion cells. Figure 1*C* illustrates three ganglion potentials which form part of the high-frequency discharge series of Figure 9*c*; the impulses follow at intervals of 2.0 and 1.7 msec. At these rates the potential heights decline.

It follows from the relationship between receptive area and recording electrode that one can distinguish between conducted potentials in axons and those arising from ganglion cells. The latter may, however, also show a more complex shape, presumably when the recording electrode is some distance away from the cell body. The present technique favors the selection of larger ganglion cells but the extent of this selection is not known (see Discussion). The findings agree with those of Rushton (29), who by different methods showed the large single retinal discharges to arise from ganglion cells.

The potentials can also be easily distinguished by listening to their discharge in the loudspeaker. The ganglion potentials, which arise in the center of the receptive fields, have a lower pitch, apparently because of less high-frequency components than in the axon spike.

Evidence for single cell discharges. The conventional criteria of single cell discharges are usually potentials of uniform size which arise at a sharp threshold and do not vary in a step-like fashion with fatigue or injury. Such criteria are generally sufficient to insure that potentials do not arise from several cells which fire in unison. In view of later findings, however, it is especially important to know that one really deals with single cell discharges.

The following procedures, which were incidental to many experiments, gave additional convincing evidence on this point.

(i) During *progressive pressure* which was obtained by advancing the recording electrode by means of a micrometer control, the ganglion cell discharge could frequently be blocked. Electrodes which had a relatively thick jacket flush with the platinum tip, were most convenient for such pressure blocks. By these procedures, potentials could be separated into two components. The first component was variable over a very wide range and its height depended on the amount of pressure, while the other varied much less. The small potential had the characteristics of a local potential which precedes propagated spikes as described by Katz (25) and Hodgkin (23). Accordingly, whenever such a "prepotential" was sufficiently reduced the spike disappeared abruptly. These events leading up to pressure block are illustrated in Figure 2. Pressure itself frequently stimulated the ganglion cells and the ensuing activity was usually photographed by exposing a fast recurring sweep until a required number of impulses was obtained. In Figure 2*a*, at the beginning of pressure, an inflexion marked by arrows is seen on the upper half of the rising phase. In *b* the two phases are more marked, the spike taking off from the beginning of the falling phase of the prepotential. In *c* a critical level is reached and at the first arrow a pure prepotential appears. The second arrow indicates two potentials which, by chance, were accurately superimposed; in one case the prepotential causes a spike, in

the other it just fails to do so. In *d* the prepotential alone is seen. It should be noted that the time course of the potentials under pressure is slower than under normal (Fig. 1) conditions. This applies especially to the prepotential. While the microelectrodes give a distorted (shortened) time course of potentials, the difference between spike and prepotential seems significant. Decreasing the pressure restored the prepotential size and when it reached a critical height the spike suddenly reappeared; the process could then be repeated. With excessive pressure, however, the whole potential disappeared irreversibly. The constancy of the spike under such conditions of block, and recovery from block, confirm the assumption that it is derived from one ganglion cell only. It is unlikely that two cells should be so located in the vicinity of the electrode tip as to be affected in a quite similar and simultaneous manner. The origin of the variable prepoten-

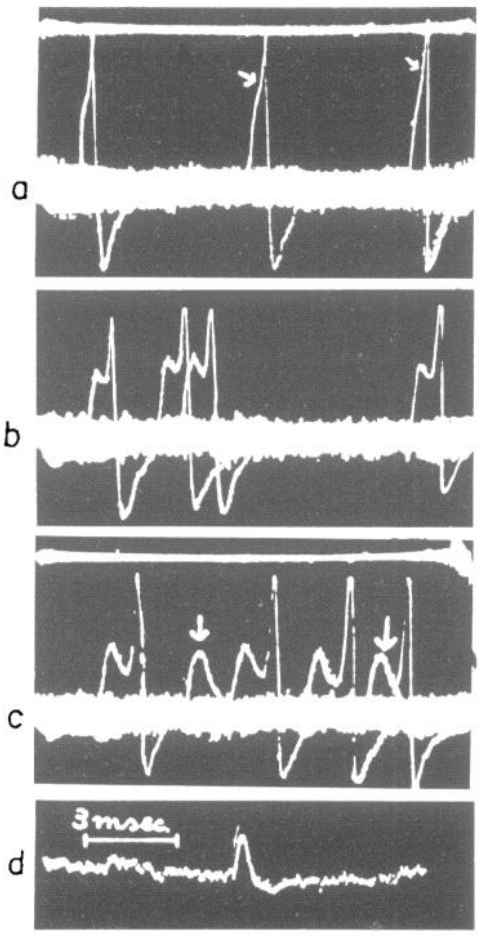

FIG. 2. Progressive pressure block of ganglion cell discharge. Exposures made with sweep recurring at high frequency. Four successive stages of pressure block. *a:* An initial inflexion (arrow) appears on upper portion of rising phase. *b:* A more discrete "prepotential" is seen. *c:* Prepotential is further reduced and occasionally (arrows) no spike appears. At second arrow a chance superposition of two apparently identical prepotentials occurred; one sets up spike, other fails to do so. *d:* spike is completely blocked, prepotential alone recorded on single sweep. Potential size in *a* is 0.3 mV.; note that also spike diminishes. Under progressive pressure potentials are of longer duration than normal (see Fig. 1).

tial was not studied in detail. It probably also originates in the ganglion cell, and such potentials may be set up there by the bipolars. It resembles some of the potentials obtained by Svaetichin (29) in spinal ganglion cells. Similar potential sequences are also seen at neuromuscular junctions or ganglionic synapses with curare or fatigue blocks.

(ii) The *potential size* of impulses at high frequencies is further evidence that single cell discharges are recorded. In the eye discharge, frequencies of 200–700/sec. and more are quite common. During these high-frequency bursts the potential size may decline, sometimes to about half of its original

size. The decline is generally smooth in its progression and therefore cannot be due to one or two units dropping out during the discharge (Figs. 9, 10). If one cell ceased to fire the potential should abruptly decrease. Alternatively it could hardly be assumed that several units should be so closely coupled. Variability of potential size in single peripheral nerve fibers has been observed at frequencies around 500/sec. when recording stretch receptor discharges (24). There seem to be some differences, however, in the potential height changes between axons and ganglion cells. The latter tend to show a fall in height at lower frequencies, a fact already studied by Renshaw in spinal motoneurons (27). In the present instances (*e.g.*, Fig. 9) the ganglion cell probably fires near its physiological limit, each impulse following in the relative refractory period of the preceding one.

The most convincing test of single unit discharge, however, was a functional one revealed in the mapping of the receptive fields. As will be shown below, discharge patterns are distributed in a characteristic fashion within receptive fields (*e.g.*, Fig. 6). That more than one ganglion cell should happen to have identical receptive fields with such a great regularity as was found in the present experiments would be a difficult assumption to make. Moreover, one would have to postulate that the cells always gave coupled high-frequency discharges at near-limit rates without, even occasionally, separating. Further, interaction, such as will be seen in the series of Figure 8, where regular mutual suppression of discharges occurs, could hardly happen if one recorded simultaneously from two or more cells.

2. Spontaneous retinal activity

Spontaneous activity in the mammalian retina has been regularly observed by Granit in dark-adapted cats (13). In the present preparation considerable background discharge was a dominant feature especially in dimly illuminated retinae (1–5 m.c. at the retina). In dark-adapted eyes it proved very difficult to investigate the detailed discharge patterns of single units, since they fired frequently at "resting" rates of about 20–30/sec. The "spontaneous" activity in the absence of illumination seems to be a normal feature for the following reasons: discharges due to injury of nerve fibers or ganglion cells under the recording lead, due to movement and pressure, could be excluded; spontaneous activity in many isolated units could be suppressed by illuminating the receptive fields some distance away from the recording lead (see also later); similarly, an electrode with a tip of 10–15 μ, if gently placed near the middle of the optic disc, recorded massed spontaneous discharges which originated elsewhere, since illumination of the whole eye suppressed a great portion of the discharge; injury discharges along nerve fibers could not be expected to be modified by illumination in such a fashion.

Spontaneous activity was particularly pronounced in decerebrate animals, but was also regularly seen under Dial-urethane anesthesia. The latter seemed to reduce the activity. Similarly, intravenous Nembutal, in amounts such as 20 per cent of the anesthetic dose, had an immediate and

prolonged effect in arresting or diminishing discharges from the retina. A similar effect with a slower onset was seen with intraperitoneal injections.

Since a great part of the present studies was done on cats under Dial-urethane the effect of the anesthetic will influence the findings to an unknown degree. All essential observations, however, were also repeated in decerebrate preparations.

As indicated above, spontaneous activity, when recorded from isolated dark-adapted units, was generally suppressed or decreased for varying periods after application of increased background illumination. In the course of light adaptation discharges usually returned gradually, or the slowed rates increased again. However, once a unit discharges in the light-adapted state, it is not possible to say how "spontaneous" the activity is.

Of particular interest are those discharges which were apparently not due to injury and were not appreciably modified by general illumination of the eye. No detailed study of their nature could be made since they were never recorded in complete isolation. It is possible that during a steady increased background illumination many units appear which have previously not discharged, while others drop out. Such switching of active units may make it impossible to decide whether certain units have been continuously active or not. This important aspect of retinal activity has yet to be explored. In many cats grouped discharges in numerous nerve fibers were seen. They could usually be suppressed by illumination of the eye, but again their origin was not studied.

While most features of "spontaneous" activity remain to be investigated, it is a noteworthy phenomenon, since it is upon such a high level of background activity that patterns of many visual events are superimposed. Rhythmic and "spontaneous" activity is common to the central nervous system in mammals and has also been observed in a variety of other visual systems (1, 4, 7).

3. Extent of receptive fields of cat's retina

The receptive field of a single unit was defined by Hartline as the area of the retina which must receive illumination in order to cause a discharge in a particular ganglion cell or nerve fiber. Hartline (17, 18) was the first to study the physiological characteristics of receptive fields of single optic nerve fibers in frogs in a precise and thorough manner, by exploring the area with a small spot of light. Since the retina is composed of a group of overlapping receptive fields, the extent of these is of obvious interest. By charting the boundaries of an area over which a spot of light sets up impulses in a ganglion cell or in its nerve fiber, one will obtain the configuration of the receptive field. The field size depends on stimulus strength, the size of the exploring spot and the state of dark adaptation. The latter will largely determine the level of sensitivity of the area. For instance, if an exploring spot is made smaller, or if the level of background illumination is increased, the intensity of the spot has also to be increased in order to set up responses

over as large an area as previously. The problems of determining receptive field sizes have been discussed in detail by Hartline (18), and his results on frogs were found to apply equally to the mammalian retina.

The receptive field definition may be enlarged to include all areas in *functional* connection with a ganglion cell. In this respect only can the field size change. The anatomical configuration of a receptive field—all the receptors actually connected to a ganglion cell by some nervous pathways—is, of course, assumed to be fixed. As will be seen below, not only the areas from which responses can actually be set up by retinal illumination may be included in a definition of the receptive field but also all areas which show a functional connection, by an inhibitory or excitatory effect on a ganglion cell. This may well involve areas which are somewhat remote from a ganglion cell and by themselves do not set up discharges.

The optical conditions in the mammal present additional difficulties for mapping of receptive fields, as contrasted to those in the opened frog's eye. Because of the imperfections of the optical system, an appreciable amount of light scattering occurs and the images will be less sharply focussed. The most advantageous situation for the full exploration of the receptive fields, which approximates the anatomical receptive field boundaries, is complete dark adaptation. During this state, however, most units discharge spontaneously, making threshold determination or detection of changes in response patterns difficult. The mapping was mostly carried out in different states of light adaptation, and even under such conditions a "steady" state cannot be maintained. As implied in the term "adaptation," thresholds change, drifting towards a lower value, and discharge patterns may also vary correspondingly. Such changes seem to be part of normal events in the eye. In spite of these factors some relevant data of the size of receptive fields can be obtained.

Figure 3 illustrates a chart of a retinal region which contains receptors with connections which converge upon one ganglion cell and cause it to discharge. The exploring spot was 0.2 mm. in diameter and the background illumination approximately 10 m.c. The smallest inner area was obtained by an exploring spot, about five times threshold for a position near the electrode tip. If the spot was moved outside this area (5×), no discharges were set up. If the spot intensity was increased 10 times, by removing a Wratten neutral filter, and thus making it 50 times threshold, it caused discharges within the larger area (50×). Further increase in the stimulus strength to 500 times threshold expanded the receptive field on three sides (500×) while the demarcation line on the left remained practically unchanged. This may indicate that light scattering was not a very great factor in this particular mapping. Otherwise such a fixed portion of the boundary, in spite of an increase in stimulus intensity, could hardly be obtained. Frequently a receptive field as shown here was charted and then the exploring spot was further increased in strength. The field suddenly expanded several times and then generally no distinct boundary demarcation was obtained. It is thought that this was

clearly due to scatter of light since a reduction of the stimulating spot size again resulted in a definite limit of the receptive field.

The present technique, using small exploring spots, is suited to detect relatively dense concentrations of receptors which feed into a single ganglion cell, and therefore provides only an approximate estimate of the actual anatomical receptor distribution. Evidence suggests that the density of receptors beyond the receptive field limit (Fig. 3) may be insufficient to produce more than subthreshold effects on a ganglion cell (see Discussion). Stimulation with larger spots may overcome the difficulties and extend the recep-

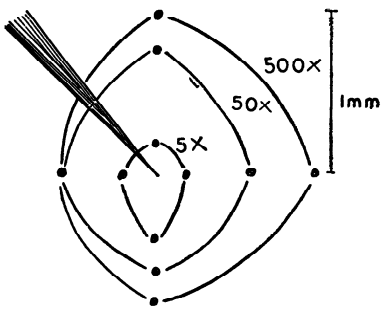

FIG. 3. Extent of receptive field obtained with exploring beam of 0.2 mm. in diameter at three different intensities. Electrode (shaded) on ganglion cell. Background illumination about 10 m.c. Inner line encloses retinal region within which light spot, about 5 × threshold at electrode tip, sets up discharges. Other boundaries of field were mapped at intensity 50 × and 500 × threshold. Note that on left, receptive field does not expand appreciably as stimulating spot intensity is increased.

tive field into areas where the receptor concentration is low. By increasing the spot size, in fact, receptive fields apparently 3–4 mm. in diameter were found, but scatter of light makes those findings unreliable. The experiment should be done by the use of a great variety of illumination patterns near threshold intensities which would allow a more exclusive excitation of the "surround," while the central region is not illuminated. Most determinations in the present study were made in the region of the cat's tapetum, a highly reflecting region where the anatomical features of the retina can be observed with greater accuracy through the optical system. Further, the tip of the recording electrode can be seen, the stimulating spot can be followed, and in this way conditions can be checked by direct observation, provided the background illumination is sufficiently bright. The receptive field diameters varied between 0.8 and 2.0 mm. with the present method. Small ganglion cells may have fields of different extent. No determinations have been made in the periphery of the retina (see Discussion).

4. Stimulation of subdivisions of receptive fields

(*a*) *Specific areas within receptive fields.* In Hartline's (17) experiments stimulation anywhere within a receptive field of the frog caused essentially the same discharge pattern in a given fiber; *i.e.*, either "on," "on-off" or pure "off" responses resulted. Accordingly the discharge type from the frog's receptive field seems relatively fixed (see, however, Discussion). This question was investigated in the present study.

It was found that the discharge patterns from ganglion cells whose receptive fields were explored varied with the specific subdivisions which were illuminated. Figure 4 illustrates such findings. A light spot, 0.2 mm. in diameter, was moved to different positions, all within an area of 1 mm. in diameter. In Figure 4*a* a discharge appeared during illumination; this "on" response was of a transient nature and although stimulation was continued at the same intensity, the discharge ceased within less than one second (see Section 6). In Figure 4*b* when the light spot was moved 0.5 mm. from the

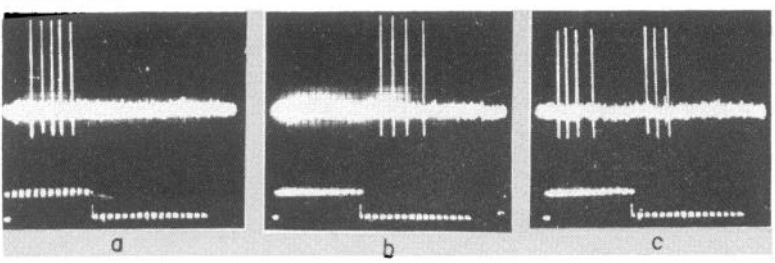

FIG. 4. Specific regions within receptive field. 0.2 mm. diameter light spot moved to three different positions within receptive field. Light flash to region near electrode tip in (a) causes only "on" discharges in ganglion cell, while same stimulus 0.5 mm. away is followed by "off" responses (b) and in an intermediate position an "on-off" discharge is set up (c). In this and subsequent records second beam signals intensity and duration of light flash; intensity modulation of 50/sec. gives time base. Impulses 0.5 mV.

first position no "on" discharge at all appeared and the response was of the pure "off" type, *i.e.*, discharges occurred after the cessation of illumination. At an intermediary position of the exploring spot, a combination of the first two responses resulted, and an "on-off" discharge is seen (*c*). All transitions in discharge patterns from those here shown were seen when the light spot of fixed intensity was moved to a number of positions within the receptive field, while the background illumination of the eye remained constant. Other illustrations of changes in discharge patterns with illumination of different areas within the receptive field are seen in Figures 7 and 8. Thus, the ratio and number of "on" or "off" discharges varied with the specific area which was illuminated. The changes in discharge type, caused by merely shifting an exploring spot, were not always striking in all units. To obtain the varied discharge patterns it was frequently necessary to change, in addition, the state of light adaptation, the stimulus intensity, or area of the stimulating light (see below).

It is concluded that within the receptive fields of single ganglion cells (or nerve fibers) there exist areas which can contribute differing discharge patterns. The discharge, as seen with stimulation of the whole receptive field, is the resultant of the contribution and interaction of all of these areas.

(*b*) *Distribution of discharge patterns in receptive fields.* All units had a central area of greatest sensitivity in which either the "on" or the "off" component predominated in the discharge pattern. Flashes of 0.5–1.0 sec. dura-

tion, for instance, to subdivisions of an area of perhaps 0.5 mm. in diameter around the ganglion cell would give "on" responses only. Within this area the "on" frequency decreased as the spot was shifted away from the most sensitive region in the center. This is shown in Figure 5. A spot 0.1 mm. in radius was projected onto the retinal region around the tip of the recording electrode which was placed on a ganglion cell. In this and nearly all other ex-

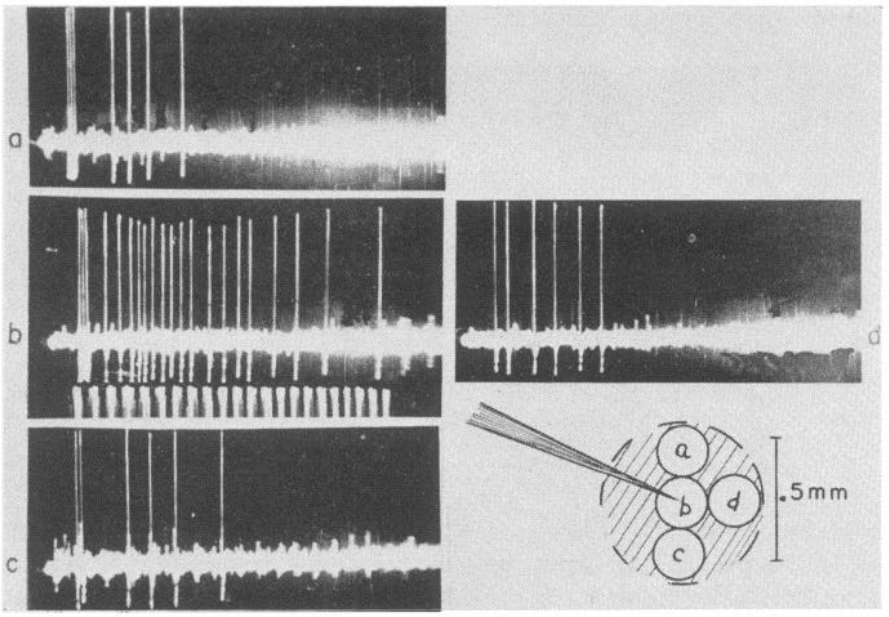

FIG. 5. Center portion of receptive field. Ganglion cell activity caused by circular light spot 0.2 mm. in diameter, 3–5 times threshold. Background illumination was about 30 m.c. Positions of light spot indicated in diagram. In *b* an "on" discharge persists for duration of flash. Intensity modulation at 20/sec. Movement of spot to positions *a*, *c*, and *d* causes lower frequency discharge which is not maintained for duration of light stimulus. Movement of spot beyond shaded area fails to set up impulses (see, however, extent of receptive field in similar unit with stronger stimuli in Fig. 6). Potentials 0.5 mV.

periments the region of electrode contact proved to be the most light-sensitive part of the receptive field. The area of lowest threshold and the geographical center of the receptive fields usually coincide. If the stimulating light spot was made 3–4 times threshold for the central location it evoked there a vigorous "on" response for the duration of illumination (Fig. 5*b*). A shift of the light spot, as illustrated in the scheme included in Figure 5, made it much less effective. The "on" discharges set up by the same stimulus became shorter and of lower frequency, and with further movement away from the center no discharges at all were set up. The boundaries of the receptive field with this relatively weak stimulus strength at a background of 30 m.c. are indicated by the broken circle.

The records of Figure 5 show only a central area of a receptive field similar to the one which is within the inner circle of Figure 3. If the small exploring spot is made 100–1000 times threshold, a more complete picture of the discharge pattern distribution in receptive fields can be formed. The chart of Figure 6 was obtained from a unit under a background illumination of about 25 m.c. It is characteristic in a general way of the majority of units which have been studied. The crosses denote "on," the open circles "off" responses, and the "on-off" discharges are indicated by the cross-circle com-

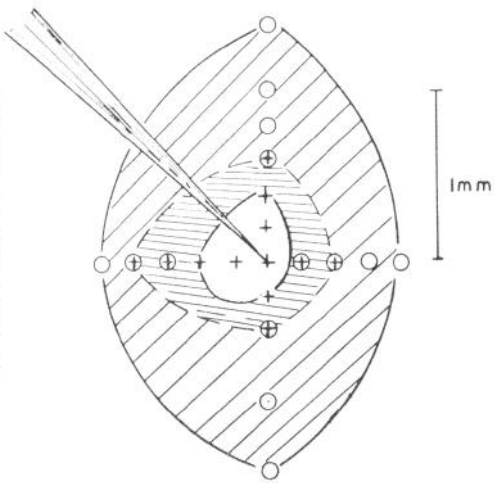

FIG. 6. Distribution of discharge patterns within receptive field of ganglion cell (located at tip of electrode). Exploring spot was 0.2 mm. in diameter, about 100 times threshold at center of field. Background illumination approximately 25 m.c. In central region (crosses) "on" discharges were found, while in diagonally hatched part only "off" discharges occurred (circles). In intermediary zone (horizontally hatched) discharges were "on-off." Note that change in conditions of illumination (background, etc.) also altered discharge pattern distribution (see text).

binations. The different shaded areas give an approximate picture of the predominant areal organization within the receptive field, *i.e.*, of receptors and neural connections (see Discussion). The center-surround relationship may be the converse in other units, with the "off" responses predominating in the center; the area ratio between center and surround also fluctuates greatly. Further, the discharge pattern distribution shifts with changing conditions of illumination (see below).

Not in all units was the field laid out in a regular concentric manner as in Figure 6. The areas were frequently irregular. In some instances there appeared "gaps" between regions; *i.e.*, isolated spots in the periphery seemed to be functionally connected to a ganglion cell.

(*c*) *Factors modifying discharge patterns and size of receptive fields.* As indicated above, the discharge patterns arising in single receptive fields may vary, if conditions of illumination are altered. The four upper records of Figure 7 show "on" discharges produced by a 0.2 mm. diameter light spot. In the lower records is seen a corresponding series of "on-off" discharges which were obtained from the same unit by changing different parameters of illumination. In *I* the area of the stimulating spot was increased so as to include the whole receptive field and thereby the "on" was converted into

an "on-off" discharge. In *II* the same effect was obtained by decreasing the background illumination while leaving all other conditions unchanged. In *III* merely the intensity of the testing spot was increased, while in *IV* the spot was moved to another portion of the receptive field, without altering its intensity or area. It follows from these observations that a modification of any of these variables of light stimulation, alone or in combination, will in turn lead to modifications of the discharge pattern. In addition to the factors illustrated in Figure 7, the duration of stimulation also plays a role. The direction of the changes can usually be predicted. If, in a composite discharge pattern, one of the components—for instance, the "on" portion—predominates strongly, a reduction of stimulus strength will cause the relatively weak

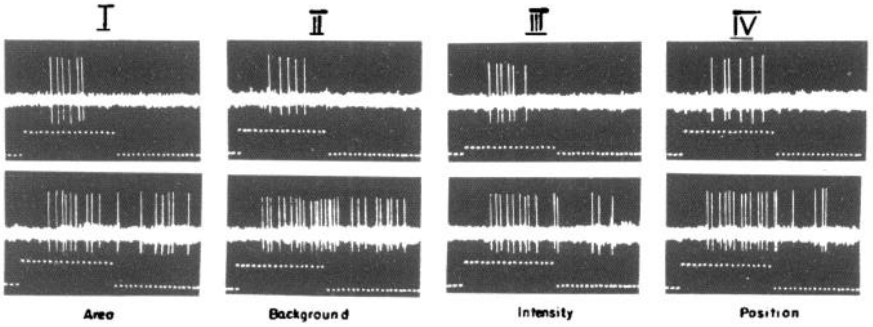

FIG. 7. Change in discharge pattern from "on" response (upper records) in single ganglion discharge into an "on-off" response (lower records). In I stimulating spot of 0.2 mm. diameter in central region of receptive field set up "on" discharge. Increasing spot diameter to 3 mm. set up more "on" impulses and brought in an "off" component. Same result was obtained in II by merely decreasing background illumination from 19 m.c. to 4 m.c. and in III by increasing stimulus spot intensity (intensity scale in III different). In IV exploring spot was shifted by about 0.4 mm. from central into more peripheral part of receptive field. Intensity modulation 50 p.s.

"off" fraction to disappear, while the "on" may be only little affected. The same result can generally be obtained by merely increasing the background illumination or reducing the area of the stimulating spot. Conversely, a combination of a weak "on" and a strong high-frequency "off" component can be changed into a pure "off" response by reducing the stimulus strength or increasing the background illumination intensity. Discharge patterns can frequently be altered by variation of background and stimulating light intensities even when the whole receptive field is illuminated. However, results are usually not as clear-cut as with fractional activation of the receptive field.

The *effect of background illumination* deserves more detailed analysis since it is one of the most potent factors in altering discharge conditions. As the background illumination is increased, the boundaries of the receptive fields "contract" and also the discharge pattern distributions change. The response type which is characteristic of the surround (diagonally hatched area of Fig. 6) tends to disappear and the pattern of the center (non-hatched re-

gion) will predominate. In fact, some units even with careful exploration, using small 0.1–0.2 mm. light spots under photopic conditions, gave only pure "on" or "off" responses within the limits of the receptive field which might be only 0.5 mm. in diameter. If the area of the stimulating spot was increased without changing its intensity—for instance, by illuminating a retinal patch 1 mm. in diameter—then the stimulus occasionally brought in an additional weak response which was characteristic of the "fringe" or surround. Thus, an "on" type of response would be converted into an "on-off" as the spot size was increased (see also Fig. 7*I*). The characteristic response of the surround could always be made evident by using a dim background, or after a short period (several minutes) of complete dark adaptation. Decreasing the background illumination first expanded the area from which center-type responses could be elicited, then brought in "on-off" responses around its boundary and eventually disclosed discharges which were characteristic of the surround. Whenever a careful search was made, both "on" and "off" components were seen in all receptive fields.

It should be noted that increased background illumination changed the receptive field in a similar manner in all units which were studied. The surround type of response, involving a presumably less dense contribution of receptors (see Discussion), was always suppressed first, independently of whether it consisted of a predominantly "on" or "off" response. This will have to be considered in discussions of the contribution of rods and cones to discharge patterns.

The great range of flexibility at the level of the single unit discharge is of particular interest, since all the factors which were found to affect the discharges play a role under normal conditions of vision.

5. *Interaction of different areas within receptive field*

It may be presumed that one of the basic contributions of interneurons within the retina (cells between the photoreceptors and the ganglion cells which give rise to the optic nerve fibers) consists in modifying the pattern of discharges which are set up by excitation of rods and cones. The impulses emerging through the optic nerve show the result of a complex series of events which have taken place in the retina, such as spatial interaction and processes of facilitation and inhibition. These problems have already been considered by Adrian and Matthews in their classical investigations on the eel's eye (2, 3) and by Hartline in the early studies of the organization of the receptive field (17, 18, 19). A wealth of data on the functional organization of the retina has also emerged from Granit's laboratory (13, 14, 15).

An additional approach is made possible by the present findings that certain areas within a single receptive field make a predominant "on" or "off" contribution to the discharge pattern. Two spots of light were projected onto the retina; each came from a separate light source, and the location, size, brightness and duration of illumination of both were controlled independently. The two light beams could be shifted on the retina in relation to

each other and their temporal sequence was controlled electronically. There are numerous possible variants under which the experiments could be done. The first and simplest is illustrated in Figure 8. One of the exploring spots, (A), with a radius of 0.1 mm. was placed near the tip of the recording electrode in the center of the receptive field, and it caused a high-frequency "on" response during illumination. The other spot, (B), twice the diameter of the

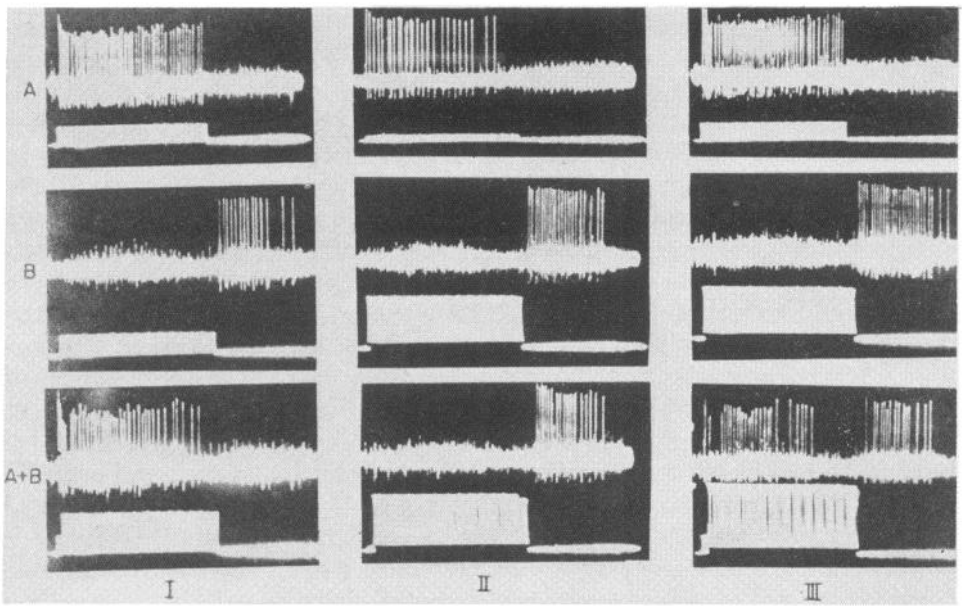

FIG. 8. Interaction of two separate light spots. Single ganglion cell discharge during background illumination of 20 m.c. Spot A, 0.1 mm. in radius, was placed in center of receptive field at tip of recording electrode. Spot B, 0.2 mm. in radius, was 0.6 mm. away in surround. Flashed separately they set up "on" (A) and "off" (B) responses. With a simultaneous flash, A+B in column I, "off" response was suppressed and at same time number of "on" discharges in A+B is slightly reduced as compared with A. In II, intensity of spot A was reduced, while spot B was increased (note flash strength indication on second beam). As a consequence B suppressed "on" discharge of A. In III, both spots were "strong." When flashed together (A+B) they reduced each others' discharges. Flash duration was 0.33 sec., potentials were 0.3 mV.

first, with its center 0.6 mm. from the ganglion cell and the recording electrode, was in the surround and set up a simple "off" discharge in this unit. When both spots were flashed on the retina simultaneously, the "off" response was suppressed (Fig. 8*I*, A+B). At the same time the number of "on" impulses was somewhat reduced as compared with the control response to stimulation of spot (A). Such situations could be produced regularly with two spatially separated light spots within a receptive field, *i.e.*, illumination of one area could suppress discharges arising from stimulation of another. The reverse situation from Figure 8*I* could be produced in the same unit as is shown in Figure 8*II*. Spot (A) was made less intense while (B) in the sur-

round was made stronger. When these stimuli were given together (A+B), the "on" effect of (A) was completely suppressed, while the off discharge was but little affected. An intermediate situation between Figure 8*I* and *II* could also be created by altering the intensities so as to make the effects from spots (A) and (B) equally "strong." When flashed simultaneously in Figure 8*III* (A+B), they simply reduced each other's effect, setting up a relatively weak "on-off" response. In order to make certain that increased scatter of light with two spots was not responsible for the effects, the two light beams were superimposed. In such cases their effect on the discharge was simply additive. Figure 8 illustrates only a few of the possible variants in discharge which can be produced by two interacting light patches. Instead of changing the intensities of the stimulating spots, results similar to Figure 8 could also be obtained by merely varying the areas of spots (A) and (B) so as to produce the required amount of "on" or "off" discharge. Alternately, shifting the location of the light stimuli or altering the background illumination would balance the "on" and "off" relationship in any required direction.

In many experiments one light spot was fixed and the other was moved around it in the manner of a satellite. In this way a systematic study was made of the interacting regions within a receptive field. As might be expected from the above results, one could produce all combinations of response types and variants of the "on-off" ratio. Once the receptive field with its boundaries and discharge patterns within that area was plotted (see Fig. 6), the result of interaction of two spots could usually be predicted. It is worthy of note that in the present experiments not only the excitatory result of a light stimulus, such as an "on" discharge, could be inhibited, but also the "off" discharge—itself a consequence of inhibitory processes—could be suppressed. As a rule, then, when two light stimuli within the receptive field interact, *both* become modified, but if the effect of one is much "stronger" than the other, its discharge may not be appreciably affected.

Suppression of "off" responses could also be seen some time after stimulation of an "on" area. The time course of this inhibitory effect, presumably caused by persistent excitation after previous illumination, could be studied in the following manner. In units similar to that shown in Figure 8*I* the duration of the stimulus to the "on" area (A) could be progressively shortened while (B) was kept constant. It was found that beam (A) could suppress (B) for varying periods after (A) had been turned off. The time course of the inhibitory after-effect depended on the duration and intensity of (A). There was a transition from complete suppression of the "off" discharge to partial suppression and to a mere delay in the onset of the "off" discharge.

In these investigations it was surprising that frequently a ganglion cell, which gave an "off" effect, was largely unresponsive to stimulation of an "on" area during the period of the "off" discharge. Further, in the tests where the interaction of two "on" areas was studied, lack of addition of excitatory influences frequently developed. Since these observations on interaction phenomena have a bearing on functional organization of the retina a

more thorough analysis will be presented in a separate publication.* Particularly the combination of spatial and temporal effects opens up some further approaches. These instances are mentioned here because they present a wider picture of factors which play a role in the production of discharge patterns. Further, they tend to explain some "anomalous" observations, such as lengthening of latent periods with stronger stimuli, or increased discharge frequencies with weaker ones (Figs. 11, 12).

6. Characteristics of "on" discharge

(a) Transient and maintained "on" response. From analogies with the Limulus eye there may be reason to suspect that the maintained "on" response in mammals, which keeps discharging for long periods during illumination, is set up in receptors which have a fairly "direct" connection from photoreceptors to bipolars and to ganglion cells. On the other hand, the "on" which is part of the frequently occurring "on-off" type may be set up in units where the receptive field has different neuroanatomical connections.

In the preparations studied there were units which gave only the Limulus type of "on" response when the whole retina was stimulated under photopic or scotopic conditions. Under careful scrutiny, when restricted subdivisions of the receptive field were stimulated, with dim background illumination, it was always observed that these "on" units also received "off" contributions from the periphery (see Section 4). More frequent were those units which gave a transient "on" response lasting about 1–2 sec. with diffuse maintained retinal stimulation. These were generally followed by an "off" response, depending on the background illumination (see above). The most frequent units were those with "on-off" discharges, the "on" lasting 1 sec. or less. The following modifications of the transient "on" responses were of special interest because they revealed some further aspects of receptive field organization: (i) When the central portion of some receptive fields was illuminated by a spot of 0.1–0.2 mm. in diameter an "on" discharge resulted lasting for seconds or, in several instances, even minutes. Either increasing or decreasing the stimulus strength frequently shortened the duration of the "on" discharge. (ii) Moving the stimulating spot as little as 0.1–0.2 mm. from the center of the receptive field greatly shortened the discharge and at the same time the onset of the discharges could be delayed (Fig. 10b). Further, units were observed which gave a maintained "on" response at the center, "off" responses in the periphery and transient "on" responses coupled with "off" discharges in intermediate regions of the receptive field. This required the selection of an appropriate background illumination, stimulating intensity and size of the exploring spot. (iii) In some units a small central spot gave maintained "on" responses and, as the area of the illuminating patch was enlarged to include the surround, the discharge became of the transient type (see also 17). (iv) One isolated instance in which, however,

* These questions are discussed more fully in *Cold Spr. Harb. Symp. quant. Biol.*, 1952, *17*.

the unit gave easily repeatable responses for several hours deserves mentioning. Under a background illumination of 10–20 m.c. the unit showed an "on" response which could not be maintained for longer than 1–2 sec. at any available intensity of the stimulating spot which was 0.2 mm. in diameter and directed onto the central region. When the background illumination was increased (60–100 m.c.) this discharge was converted into a maintained "on" type although the stimulus was of the *same* intensity as that which gave the shorter "on" response before. This situation was the reverse of the more common one since, by increasing the background illumination, a given stimulating intensity usually becomes less effective. One may surmise that in this unit the background illumination preferentially suppressed inhibitory influences from the "off" areas.

The above findings suggest the following interpretation: the maintained "on" discharge is converted into the transient type by activation of elements which converge onto the same ganglion cell from the periphery of the receptive field. Accordingly a unit which is so organized that it has a strong "on" center and a weak "off" surround will tend to give a well-maintained discharge even with illumination of the whole eye. The discharge will shorten in proportion to the peripheral "off" contribution. Such a view is also supported by the interaction experiments in which a simultaneous second spot in the surround weakens and shortens the discharge set up by the central one. The duration of the "on" discharge then will depend on how many "off" pathways to a ganglion cell are active in relation to the "on" fraction. It is realized that the inhibitory "off" action starts approximately simultaneously with the "on" action. Therefore, if both continued simultaneously at the same strength, one would expect merely a reduction of the "on" discharge frequency scale and not a shortening when a certain "off" component is added. Such a reduction of an "on" discharge is seen in Figure 8*III*. However, the "on" discharges which are generally observed start at a relatively high frequency which subsequently tends to decrease. With reduction of the stimulus strength producing such an "on" discharge, the initial high frequency will be reduced while the later discharge of lower frequency may drop out completely (see also 18). Therefore a similar "weakening" of an "on" discharge by an inhibitory action may lead to a shortened "on" response. Further, the suppressing effect from "off" zones does not necessarily start with its full force, but may increase with prolonged stimulation as can frequently be seen in its action of stopping "off" discharges. The presence of inhibitory contributions in many pure "on" elements has already been shown by Donner and Willmer (9).

(*b*) *Latent period and discharge frequency of "on" responses.* Generally one can cause increased excitation, as measured by frequency of response and shortened latent period, by (i) increased stimulus intensity, (ii) increase in stimulated area, (iii) decrease in background illumination (or increased dark adaptation), (iv) moving the stimulating spot toward the center of the receptive field.

A fairly typical effect of stimulus strength on the latent period and discharge frequency is seen in Figure 9. A spot 0.2 mm. in diameter was flashed at four different intensities onto the "on" center of a receptive field, increasing in steps of 10 from *a* to *d*, with the eye under a background illumination of about 2 m.c. This illustration is of particular interest because it shows how short the latent period can be and how high the discharge rate can become in the cat's retina even with moderate intensities of stimulation. In *a* the stimulus is near threshold and the latent period is 93 msec. In *b* the latency is 36 msec. and the average discharge rate for the first 8 impulses is about 180/sec. In *c* the discharge frequency is 300/sec. for the first 13 discharges and the latency is 22 msec. and in *d* a peak frequency of over 800/sec. is reached between the 4th and 10th impulse, the latency being 15 msec. This rate of discharge is much higher than is customarily obtained from nervous structures under physiological conditions. A pause as in *d* is common, both after "on" or "off" bursts. Increasing the area of stimulation within the center of the receptive field, starting with a relatively weak stimulus, also caused higher discharge frequency and latency shortening in this unit (see, however, below). The latent period of 15 msec. in Figure 9*d* is shorter than hitherto seen in mammals, presumably due to restriction of the stimulus to a predominantly "on" area (see below).

Figure 10 illustrates a unit which gave an "on" discharge lasting several seconds with illumination of the whole eye and a somewhat longer one with illumination confined to its "on" center. In *a* it showed a high-frequency initial burst of 575/sec. for the first 8 impulses with the potential size sharply declining (followed by a pause). The latent period of 15 msec. in *a* was lengthened and the discharge frequency and duration was reduced in the subsequent three records by the following: (i) in *b* the light spot of the same intensity as in *a* was moved from the center of the field by 0.1 to 0.2 mm.; (ii) in *c* with the light spot in the center again, the background illumination was increased; and (iii) in *d*, the stimulus intensity was reduced. Reducing the stimulating area or shortening the duration of the light flash (not illustrated) had a similar result as shown in *b–d*. The findings of Figures 9 and 10 are in general agreement with the early work of Adrian and Matthews (2), Hartline (17) and Granit (13).

Some notable exceptions to the general "rules" as discussed above were also observed—and, in fact, could frequently be produced by appropriately arranging the conditions of the experiment. Thus, in contrast to the usual results, the latent period of discharge was actually prolonged in the unit of Figure 11 when the area of stimulation on the retina was changed from a patch 0.2 mm. in diameter (a) to one of 3 mm. (b). Similarly, increasing the light intensity could have the same effect. One may assume that stimulation of the larger area brought in a strong "off" component from the surround, causing a delay in the "on" response. Such a situation could actually be produced frequently by stimulation of two separate small "off" and "on" areas. Another "exception" is seen in Figure 12 where an "on-off" response is con-

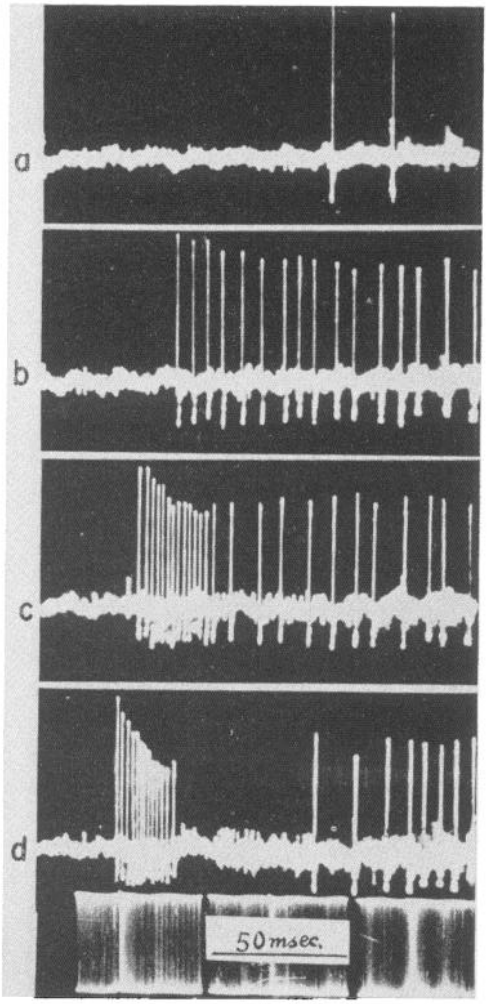

FIG. 9. Effect of stimulus strength on latent period and discharge frequency. 0.2 mm. diameter spot projected onto "on" center of a receptive field at illumination background of about 2 m.c. Between *a* and *d* stimulus was increased in steps of 10. Latent periods were 93, 36, 22 and 15 msec. Peak frequency in *d* was over 800/sec. Transient pause after a high-frequency burst occurred regularly, as did decline of potential size. Impulses 0.4 mV.

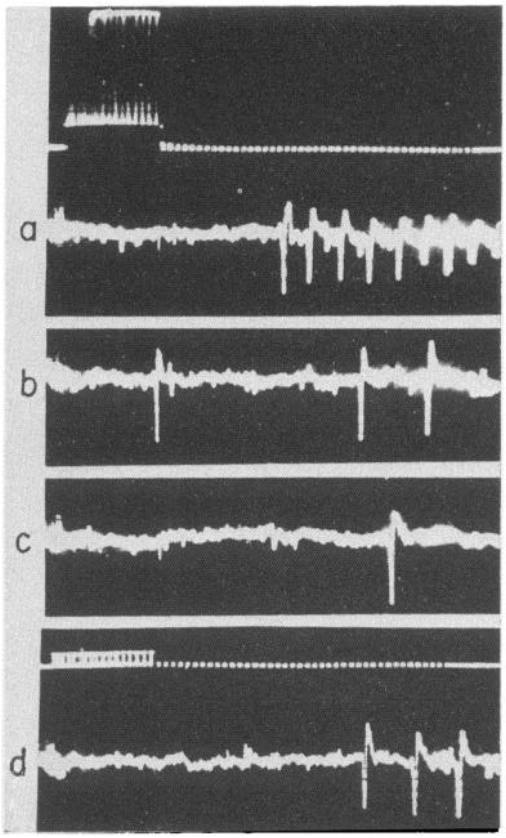

FIG. 10. Ganglion discharge with spot (0.2 mm. diam.) illumination. *a*: flash of 6.5 msec. in duration to center of receptive field set up response with initial frequency of 575/sec. and latent period of 15 msec. Prolonging illumination did not change latent period but caused an "on" response for 2 3 sec. *b*: same flash; image moved 0.1 0.2 mm. from central position. Latent period 21 msec., only two impulses set up. First impulse on sweep was "spontaneous" and not related to flash. *c*: same flash as *a*, but background illumination increased. Only one impulse set up. *d*: conditions as in *a*, but stimulus intensity decreased. Latent period 22 msec., discharge burst shorter. Effects seen in *b d* were also obtained by shortening flash or reducing spot size. Intensity modulation 2000/sec.

verted into an "off" by reducing the stimulus strength. Surprisingly, however, the latent period of the "off" response is shorter and the number of impulses is greater with this weaker illumination (see also 9). Again, an explanation can be sought in the antagonism of "on" and "off" influences. The weaker stimulus, by failing to excite the "on" fraction, caused less inhibition

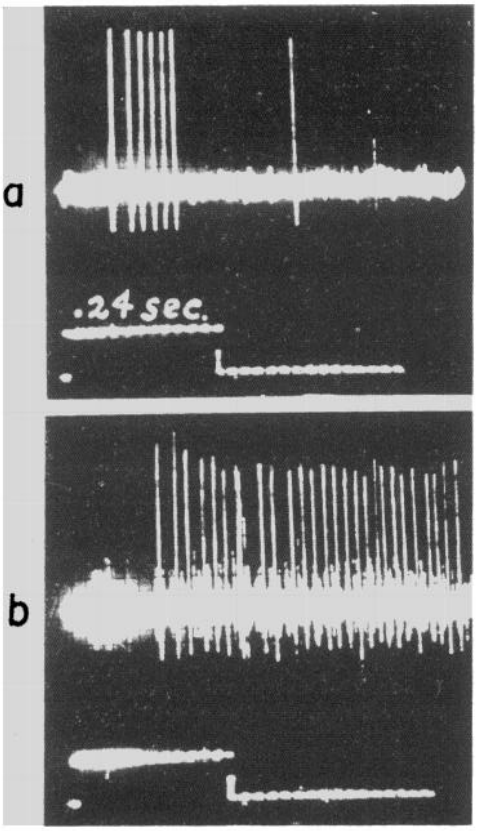

FIG. 11. "Anomalous" effect of change in stimulus area on latent period. *a:* ganglion discharge set up by 0.2 mm. diam. spot within central region of receptive field. *b:* spot size increased so as to include whole field. Note the greatly prolonged latent period of "on" component. Potentials 0.6 mV.

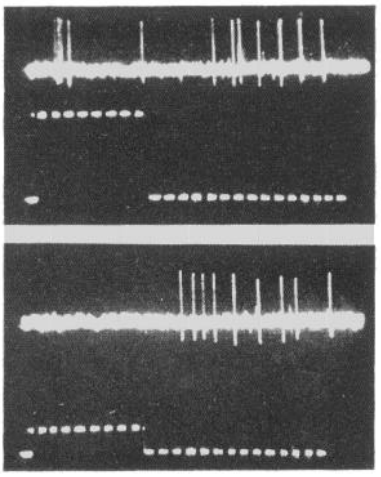

FIG. 12. "Anomalous" effect of change in stimulus intensity on discharge. Upper record: "on-off" ganglion discharge. Below: with stimulus intensity decrease "on" component drops out. Note, however, the shorter latent period and increased number of impulses in "off" discharge (see text). Frequency 50/sec.

of the "off" component. In all these "anomalous" instances it must be noted that a non-homogeneous population of receptors is activated and the discharge pattern depends on the proportion of "off"- and of "on"-oriented receptors which are excited.

7. *"Off" response*

As appears from Section 4, no pure "off" units were found when the receptive fields were explored with small spots of light and suitable background illumination. Those units which gave an "off" response alone with illumination of the whole eye were always found to have an "off" center and "on" surround, while units giving "on-off" responses could have either type

of center. The "off" activity of an area could be tested by the ability of a light stimulus to set up impulses when its intensity was reduced or the light turned off, or by the suppression of spontaneous activity.

The interaction between separate stimuli to "on" and "off" areas was shown in Figure 8; in Figure 13 a similar experiment is illustrated with both

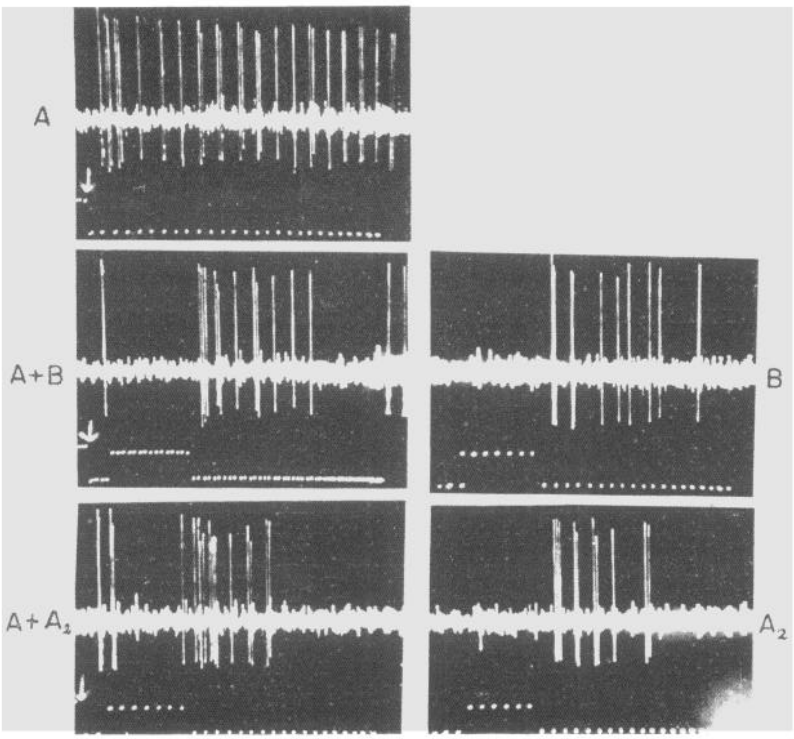

FIG. 13. Inhibitory action of light on "off" response. Light beams A and B projected onto separate areas, each 0.2 mm. in diameter, in central region of a receptive field near tip of recording electrode. Both regions give "off" responses only. Background 18 m.c. A: "Off" discharge produced following termination (arrow), at beginning of sweep, of stimulation by beam A. A+B: Beam B, applied during "off" discharge, suppresses impulses. B: spot B alone. A+A_2: Stimulus to spot A ceases near beginning of sweep, as above, but same area is re-illuminated by second flash. Not only is there suppression of "off" discharges during flash of A_2, but also subsequent "off" response duration is reduced as compared with A. A_2: second flash of beam A alone. Note that "off" discharges set up in one region of receptive field can be suppressed by stimulation of another "off" region, or by restimulation of same area. The grouped discharges occurred in many units of this experiment. Time base 100/sec. in A+B, 50/sec. in all other records. Potentials 0.3 mV.

light stimuli to an "off" region. A Spot caused a strong "off" response by illumination of an area 0.2 mm. in diameter in the central portion of a receptive field, just about 0.1 mm. away from the area of lowest threshold at the electrode tip. The illumination was started before the sweep and only the cessation of the light signal appears on the record (marked by arrow). Grouped discharges similar to those in this figure were frequently seen and have been also noted by others during the "off" effect (16, 17). Spot B was

the same distance from the electrode tip as A, but on the opposite side. This stimulus was shorter and by itself caused a briefer discharge (Fig. 13B). When B followed A, it suppressed the "off" discharge for the duration of its flash (Fig. 13A+B). When both stimuli were given to spot A in succession, the second (A_2) also suppressed the "off" impulses. A_2, being a shorter flash than the preceding A, set up a shorter "off" response than A alone. It is noteworthy, however, that the "off" discharges of A were not reinforced at the end of flash A_2. In this unit it seems that A_2 during its flash not only suppressed the impulses, but also the processes which "survived" after cessation of A. The inhibitory action of light on the "off" discharge by reillumination of the whole eye is well known from the work of Granit and Therman (16) and Hartline (17). Suppression of "off" discharges, set up in one region of the receptive field, by subsequent excitation of another "off" area is to be expected from the experiments on interaction (Section 5) and have also been seen by Hartline (20a).

The duration of the latent period of the "off" responses was studied, since it is a measure of the processes which have preceded the discharge. It is known that the latent period shortens and the discharge frequency increases as a function of the intensity and duration of the preceding illumination (13, 20). Again, however, exceptions to this rule occur. In some experiments latent periods as short as 10–15 msec., similar to the shortest periods for "on" discharges, could be seen. Another indication concerning the processes which are involved in inhibitory activity can be obtained from a determination of the time which is taken up between stimulation of the receptors and the first sign of suppression of activity at the ganglion cell level. Some conclusion may then be drawn regarding the mechanism of excitation spread within the retina. The speed and mode of this spread will be important in the competitive situation when both "on" and "off" areas are excited simultaneously, as must occur normally in the eye when stimulation is not confined to subdivisions of a receptive field. Such latent periods of inhibitory action are obtained by measuring the time it takes for a second flash (B or A_2 in Fig. 13) to suppress a discharge. The time between the onset of the "off" flash and the first suppressed impulse would clearly be the most accurate determination. This method will be most precise if the suppression is tested and measured on a well-maintained and regular high-frequency discharge. By such determinations the shortest latent periods of inhibition were around 10 msec. These times may, in fact, be too long since they do not indicate the actual onset of inhibitory action at the ganglion cell. The processes may start acting well before they become evident by their action of suppressing a discharge. Further information in this connection will be presented in a study of the inhibitory and excitatory pathways which converge on ganglion cells.

Discussion

Sampling of units within retina. An advantage of the present technique is the ease of recording retinal activity and the intactness of the eyeball which enables the normal optical channels to be used for illumination and

observation. The method, however, will tend to select the potentials from the larger ganglion cells. On the other hand, since one generally can find suitable cells for recording within any small area of the retina, such as 1 square mm., it is quite likely that these cells can be smaller than the "giant" cells described by Rushton (27). Further, nearly all the work was done on cells within a radius of about 5–8 mm. from the optic disc, particularly in the two quadrants above the disc within the highly reflecting region of the tapetum. No positive evidence has been found that within these areas there are specific subsections which give different discharge patterns. The cat has no fovea but there exists a region on the visual axis of the eye, called centralis (6), about 1–2 mm. temporal from the disc, which has an especially dense representation in the visual cortex (30). This region was included in the present studies and found to show no qualitative differences from other areas. No activity of bipolars has been recorded and therefore all the discharge patterns which are described, while derived from ganglion cells, represent also the discharges in the optic nerve fibers.

Since in each preparation the discharges from numerous units can be observed in quick succession, *e.g.*, 30–40 within an hour, it is possible to collect statistical data on discharge types. It was, however, found more informative to obtain detailed results from a relatively small number of units and frequently these were kept on the electrodes for 5–6 hours. Only those cases are presented which, at the present stage, seem more representative or important. The great majority of experiments were done well within photopic levels, with the background illumination between 1 and 50 m.c. All the essential features of discharge pattern behavior, however, were also present under scotopic conditions in the absence of background illumination. It should also be noted that in this study relatively short flashes were used and no "equilibrium" conditions were attained.

Fluidity of discharge patterns. The most outstanding feature in the present analysis is the flexibility and fluidity of the discharge patterns arising in each receptive field. Stability of discharge type can be obtained in the present preparations in units under certain conditions, especially with a relatively strong background illumination, when the surround is suppressed. A constant "on" or "off" response may then be seen even with spot illumination. Such stability, however, disappears when one or more of several parameters, such as the adaptation level, stimulus intensity, and area of illumination, are changed singly or in combination. In the absence of a fixed pattern from the whole receptive field, it does not appear accurate enough to speak of "on," "on-off" or "off" fibers in the cat's retina. The difference in retinal discharge pattern distribution between frog and cat is worthy of note, particularly since the analyses in frog were made by Hartline (18) with a well-controlled and accurate technique. Although he reported the discharge patterns in receptive fields fixed, he points out many exceptions and reports occasional units in which a change in discharge patterns did occur. He also presents data which may be interpreted to indicate the presence of inhibitory surrounds, such as a decline of discharge frequencies with strong stimuli

or with large areas of excitation (17, 19). The difference between cat and frog may turn out to be largely a quantitative one. A less flexible system of discharge in frogs may perhaps not be surprising. By using a different approach, such as pharmacological techniques (12) and passing current through the eye or varying the wave-length and intensity of the stimulating light, shifts in discharge patterns have already been observed by Granit and his colleagues. They also repeatedly pointed out the lability of certain portions of the discharges, particularly in connection with work concerning the on-off ratios (8, 14, 15). Donner and Willmer (9), working with dark-adapted cats and stimulation of the whole retina, also observed a great range of variability in ganglion response patterns during stimulation at different intensities. They have shown that visual-purple-dependent receptors can give rise to both "on" and "off" discharge components.

Functional organization of receptive fields. There seems to exist a very great variability between individual receptive fields and therefore a detailed classification cannot be made at present. Some features, however, of general organization were found common to all. In all fields there exists a central region giving a discharge pattern which is the opposite from that obtained in the periphery. The center may be either predominantly "off," the surround "on," or vice versa. A transitional zone is in between (see Fig. 6). The essential character of discharge within the centers cannot be changed by altering any of the parameters of illumination, *i.e.*, an "off" center cannot be converted into an "on" center. It must not be inferred, however, that the centers are quite uniform and receive no contribution which is characteristic of the surround. In view of the fixed nature of the center discharge, it may be convenient to classify receptive fields into "on" center and "off" center fields. In line with this the respective elements may be similarly designated as "on" center or "off" center units. No accurate record of distribution has been made in hundreds of units which were investigated. The "off" center units seemed to occur more frequently. Functionally the center and surround regions are opposed, the one tending to suppress the other. The ganglion cell is subjected to multiple influences from its receptive field and its discharge will express the balance between these opposing and interacting contributions. In view of the relative ease with which the peripheral receptive field contribution can be altered (see later), and thereby the balance within the unit changed, the discharge pattern fluidity is readily appreciated.

From a functional point of view, then, the important aspect of the present findings is not that one unit can give under special conditions either "on" or "off" responses but that there exists a mixture of contributing receptors, perhaps with their specific pathways (below). In proportion to their activation they can produce all shades of transitions from one response pattern to another. In any event, illumination of the whole receptive field will always produce a push-pull action as the opposing components are thrown into activity.

Specific neural pathways. The nervous organization of all the elements

functionally connected to a ganglion cell constitutes an example of the complexity of the central nervous system, well known from the studies of Cajal (5) and lately especially of Polyak (26). It is natural that a specific organization should be suggested for excitatory and inhibitory pathways for which there is physiological evidence. Experiments seem to show (Fig. 6) that excitation of a certain number of receptors by restricted illumination causes one type of response only. Presumably a given pathway is utilized by a given group of receptors. The principal reason for a change in response type seems to be that either additional receptors have been brought in or receptors have been eliminated (see below). Suggestions as to the specific neural connections, based on present evidence, are clearly speculative and grossly simplified. One may think of a neural arrangement which parallels the roughly concentric functional pattern, with relatively uniform connection types between receptors in the center and the ganglion cell and a differing pathway set-up from the surround receptors to the ganglion cell. The in-between region may present the zone where the pathway types are more mixed than anywhere else. A correlation of greater significance between neural pathways and discharge patterns may perhaps be obtained from a study of animals with a fovea, *e.g.*, monkey, where the neuroanatomical connections are simpler and better known. It may be predicted, accordingly, that the foveal paths are associated with specific discharge behavior.

Receptor density in receptive fields. Any given small area of the retina which has been studied presumably has a dense and fairly uniform receptor population (26). Histological data also show that adjoining receptors, or even the same receptors, may have connections to different ganglion cells. Further, we know that the same receptor may connect to different ganglion cells in differing ways. This is the neuroanatomical basis for overlapping receptive fields. The present study gives some information about the density distribution of receptors which are *functionally* connected to one ganglion cell. The following type of experiment supports the assumption that the central portion of receptive fields hold a denser population of receptors per unit area than do the peripheral regions: units which have an "on" center and "off" surround, when tested with stimuli 100–1000 times threshold (Fig. 6), may be excited in their most sensitive central region by a 0.2 mm. diameter spot of near-threshold intensity. At this strength "on" discharges of quite short duration are evoked within a small central area (as Fig. 5). Such a small spot is well below threshold for the outlying portions of the receptive field. Placing a ground glass in front of the eye, thereby reducing, and in addition scattering, the light beam, will produce "on-off" or pure "off" responses. This suggests that receptors dispersed in peripheral regions have been reached and summation has occurred in their pathways leading to the ganglion cell. The experiment also shows that the receptors which contribute the "off" component do not have a lower threshold than those in the central "on" region. Threshold differences, however, within the receptor population are not contra-indicated by such results. Presumably because of

the density of receptors, the center is found to be more sensitive when tested with small beams.

The scatter of receptors in the periphery makes obvious the difficulties of receptive field mapping with small light beams, since one has to assume that a sufficient number of receptors must be activated to evoke a ganglion cell discharge. Receptors, functionally connected but located in the periphery, will be missed and an error in underestimating the field size is likely to be made. When mapping is done in units with an "off" surround, while they show spontaneous activity, the field periphery can be delineated by the area over which a small spot will produce slowing or stoppage of firing. This method is more sensitive than the one described in Section 3 and receptive fields extending over 3–4 mm. (12–16° in cat), could occasionally be obtained. The effect of light scatter, however, could not be estimated closely enough to make these findings reliable.

The low density of receptors in the surround also makes readily understandable the observed shrinkage of receptive fields with augmented background illumination. If tested with a *small spot*, the dropping out of receptors by raised thresholds in the field surround will be of more consequence than in the dense central region, since the outer receptor family, being scattered, operates nearer to the margin for firing the ganglion cell. Even with illumination of the *whole field* the peripheral contribution, if it depends more on facilitation and summation, should be more affected if a portion of component pathways is put out of action. The background changes should affect an "on" or "off" surround equally, in line with present observations.

Changes in receptive field contribution to ganglion cells. A special nervous organization of receptive fields alone could not account for all the observed discharge pattern changes under diverse conditions of illumination. There is a great body of evidence for a diversity of receptor properties in respect to thresholds, adaptation, wave-lengths, etc. For instance, in view of the changes in receptive field size at low or high levels of light adaptation, it is clear that under such changing conditions a largely differing set of receptors will be thrown into activity with a given stimulus. Hence, this alone will bring a different set of active connections with a ganglion cell into being. The differing connections, in turn, are likely to cause changes in discharge patterns. Since steady states cannot be attained, a shift in the active receptor population is likely to go on continuously even in the dark-adapted eye, as indicated by the background activity, unless the latter is entirely due to spontaneous rhythms in the neural elements.

Psychophysical aspects. A transference of information about discharge patterns, as obtained here, to psychophysical data is obviously based on speculation. However, the data must be used with all their imperfections since they provide the components which form the basis of the message content reaching the higher centers. The most potent stimuli, those causing the greatest nervous activity, are relatively sudden changes. These may be either changes of the general illumination level or such changes as occur during

movement of images (see also 17). In the latter case the antagonistic arrangement of central and peripheral areas within receptive fields seems important since the smallest shift can cause a great change in the discharge pattern. This should be advantageous in the perception of contrast and in acuity. In view of this the importance of small eye displacements, as would occur in any scanning movement, is clear. It should be noted that zonal gradients within fields, between center and surround, will change with different levels of adaptation as the receptive field shrinks or expands. It may also be tempting to consider in this connection well-known suppression phenomena like lateral inhibition which has been studied by many investigators. Particularly the interaction of two light patches with facilitation and inhibition as observed in humans over small distances on the retina may be considered in view of the extent of the cat's receptive field (10, 11). It is clear, however, that even the smallest light beams used in the present experiments do excite a great number of ganglion cells through their overlapping receptive fields. It is not known how the latter are functionally related to each other. For instance, it would be of interest to know whether the same receptor can be connected to one ganglion cell through an excitatory and to another cell through an inhibitory pathway.

Regarding the information content carried by a single ganglion cell or its connected nerve fiber, the following two phenomena may be briefly considered: (i) If a ganglion cell can discharge at one time during illumination and then be converted into one which signals only when a light stimulus is withdrawn, one may assume that it does not carry merely the information which is suggested by a "simple" interpretation of the discharge pattern. The higher centers may receive identical impulse patterns in both cases. (ii) A unit giving "on-off" discharges in a given situation, according to a "simple" interpretation, sends information first about an increased and then about a decreased level of luminosity. The identical discharge pattern may, however, be evoked by turning a light on, and then instead of turning it off, one may further increase its intensity. Such fibers then merely signal change, but not necessarily the direction of change, such as brightness or darkness.

In view of the massive continued nervous activity in eyes "at rest" or during illumination it is difficult to think of information content in terms of single unit contributions. One may rather have to consider that groups of fibers modulate activity levels and patterns by superposition or subtraction. The latter—for instance, transient cessation or diminution—is likely to be as meaningful as the opposite in terms of message content. Further, similar discharge patterns at different background illuminations may convey a different meaning, since they are superimposed on a different background activity. These examples merely illustrate some of the difficulties inherent in this type of analysis. They indicate that on the basis of the single unit discharge a 1:1 agreement between discharge patterns and information should not always be sought. At the same time it should be recalled that there is

agreement between psychophysical measurements such as the visibility curves and the analogous curves, obtained in different mammals (13), or the Limulus (20), from nervous discharges.

In this study the influence of transient light changes on discharge patterns has been emphasized; in view of the importance of background activity the effect of steady levels of illumination on discharge behavior must be analyzed in great detail.

Summary

Discharge patterns from the unopened cat's eye have been studied by recording from single cells in the retina. Small electrodes, inserted behind the limbus, traversed the vitreous and made light contact with different regions of the retina. The normal optics of the eye were used for stimulation by two independently controlled light beams. Circular stimuli of various dimensions, duration and intensities were applied to different areas of the retina. A third light source provided the background illumination, determining the adaptation level, and also served for simultaneous direct observation of the fundus.

1. The discharges arising in nerve fibers and ganglion cells can be readily distinguished through differences in their time course and the location of their respective receptive fields. The present study was done on ganglion cell activity.

2. Ganglion cells can be blocked reversibly by pressure and the potentials can be split into "prepotentials" and "spikes."

3. Under dim background illumination and during dark adaptation the cat's retina is dominated by generalized spontaneous activity. The latter is reduced by illumination and anesthetics such as Dial or Nembutal. Certain discharges do not seem to be influenced appreciably by illumination. These observations are in general agreement with Granit's findings.

4. The configuration of receptive fields—those areas of the retina which must be illuminated to cause a discharge in a ganglion cell—were studied by exploration with small spots of light. The fields are usually concentric, covering an area of 1–2 mm., or possibly more, in diameter. The boundaries and extent of receptive fields cannot be delineated accurately. They shrink under high background illumination and expand during dark adaptation.

5. The discharge pattern from individual ganglion cells is not fixed. "On," "off" or "on-off" discharges can be obtained from one ganglion cell if specific zones within its receptive field are stimulated by small spots of light. The discharge pattern from a ganglion cell depends, amongst others, on the following factors: Background illumination and state of adaptation, intensity and duration of stimulation, extent and location of area which is stimulated within a receptive field. Each of these parameters can alter the discharge pattern by itself or in conjunction with the others.

6. The general functional organization of each receptive field is the following: There exists a central area of low threshold as tested by a small spot

of light. The discharge pattern of the central region is the opposite of that found in the periphery or surround. The center may give predominantly "off," the surround "on" discharges, or the reverse. An intermediary region gives "on-off" discharges. The units which carry discharges from "center on" or "center off" receptive fields may accordingly be classified as "on" center or "off" center units. A conversion of one type into another by changing conditions of illumination has not been possible.

Experiments indicate that receptors in the periphery of receptive fields are less dense per unit area than in the central regions.

7. Interaction of different regions within single receptive fields was studied by simultaneous excitation by two small beams of light. Depending on a number of factors, "off" areas may suppress the discharge from "on" regions, or vice versa. All degrees of mutual modification can be obtained. It is assumed that specific areas give rise to predominantly inhibitory or excitatory pathways to a given ganglion cell.

8. The discharge pattern of a ganglion cell, set up by illumination of an entire receptive field, depends on the summed effects of interacting pathways converging on the cell. The ratio of functionally opposing center and surround regions varies greatly in different receptive fields. Under diverse conditions of illumination the balance of active inhibitory and excitatory contributions changes within the same receptive field. This seems to be responsible for the varied discharge patterns.

9. The character of "on" components in discharge patterns was also studied. The maintained "on" response discharges for the duration of illumination while the transient "on" adapts quickly. Transitions between these two "on" types were found in the same receptive fields. It is suggested that the transient "on" discharges are the result of various amounts of addition by the "off" surround to the "on" center.

10. The latent periods of "on" or "off" responses were found to be shorter than hitherto observed, presumably because of more exclusive stimulation of their specific receptive areas. High discharge frequencies of 200–800/sec. were found to be within the normal range in the cat's eye.

11. Some "anomalous" observations, such as lengthened latent periods with increased stimulus intensities or higher frequency discharges with weaker stimuli, are interpreted in the light of receptive field organization.

ACKNOWLEDGMENT

I am grateful to Dr. S. A. Talbot for his help, particularly in the design of the optical and electronic instruments, which made this study possible. My thanks are also due to Mr. Albert Goebel for constructing the optical apparatus.

REFERENCES

1. ADRIAN, E. D. Synchronized reactions in the optic ganglion of Dytiscus. *J. Physiol.*, 1937, *91:* 66–89.
2. ADRIAN, E. D. AND MATTHEWS, R. The action of light on the eye. Part I. The discharge of impulses in the optic nerve and its relation to the electric changes in the retina. *J. Physiol.*, 1926, *63:* 378–414.

3. ADRIAN, E. D. AND MATTHEWS, R. The action of light on the eye. Part III. The interaction of retinal neurones. *J. Physiol.*, 1928, *65:* 273–298.
4. BERNHARD, C. G. Contributions to the neurophysiology of the optic pathway. *Acta physiol. scand.*, 1940, *1:* Suppl. 1.
5. CAJAL, S. RAMÓN Y. La rétine des vertébrés. *Trab. Lab. Invest. biol. Madr.*, 1933, Suppl. *28.*
6. CHIEVITZ, J. H. Über das Vorkommen der Area centralis retinae in den vier höheren Wirbelthierklassen. *Anat. entw.-gesch. Monogr.*, 1891, pp. 311–334.
7. CRESCITELLI, F. AND JAHN, T. L. The effect of temperature on the electrical response of the grasshopper eye. *J. cell. comp. Physiol.*, 1939, *14:* 13–27.
8. DONNER, K. O. AND GRANIT, R. The effect of illumination upon the sensitivity of isolated retinal elements to polarization. *Acta physiol. scand.*, 1949, *18:* 113–120.
9. DONNER, K. O. AND WILLMER, E. N. An analysis of the response from single visual-purple-dependent elements in the retina of the cat. *J. Physiol.*, 1950, *111:* 160–173.
10. FRY, G. A. Depression of the activity aroused by a flash of light by applying a second flash immediately afterwards to adjacent areas of the retina. *Amer. J. Physiol.*, 1934, *108:* 701–707.
11. GRANIT, R. Comparative studies on the peripheral and central retina. I. On interaction between distant areas in the human eye. *Amer. J. Physiol.*, 1930, *94:* 41–50.
12. GRANIT, R. Some properties of post-excitatory inhibition studied in the optic nerve with micro-electrodes. *K. svenska VetenskAkad. Arkiv. Zool.*, 1945, *36:* 1–8.
13. GRANIT, R. *Sensory mechanisms of the retina.* London, Oxford Univ. Press, 1947, 412 pp.
14. GRANIT, R. Neural organization of the retinal elements, as revealed by polarization. *J. Neurophysiol.*, 1948, *11:* 239–253.
15. GRANIT, R. The organization of the vertebrate retinal elements. *Ergebn. Physiol.*, 1950, *46:* 31–70.
16. GRANIT, R. AND THERMAN, P. O. Excitation and inhibition in the retina and in the optic nerve. *J. Physiol.*, 1935, *83:* 359–381.
17. HARTLINE, H. K. The response of single optic nerve fibers of the vertebrate eye to illumination of the retina. *Amer. J. Physiol.*, 1938, *121:* 400–415.
18. HARTLINE, H. K. The receptive field of the optic nerve fibers. *Amer. J. Physiol.*, 1940, *130:* 690–699.
19. HARTLINE, H. K. The effects of spatial summation in the retina on the excitation of the fibers of the optic nerve. *Amer. J. Physiol.*, 1940, *130:* 700–711.
20. HARTLINE, H. K. The nerve messages in the fibers of the visual pathway. *J. opt. Soc. Amer.*, 1940, *30:* 239–247.
20a. HARTLINE, H. K. The neural mechanisms of vision. *Harvey Lect.*, 1941, *37:* 39–68.
21. HARTLINE, H. K. AND GRAHAM, C. H. Nerve impulses from single receptors in the eye. *J. cell. comp. Physiol.*, 1932, *1:* 277–295.
22. HARTLINE, H. K., WAGNER, H. G., AND MCNICHOL, E. C. The peripheral origin of nervous activity in the visual system. *Cold Spr. Harb. Symp. quant. Biol.*, 1952, *17.*
23. HODGKIN, L. A. The subthreshold potentials in a crustacean nerve fiber. *Proc. Roy. Soc.*, 1938, *B126:* 67–121.
24. HUNT, C. C. AND KUFFLER, S. W. Stretch receptor discharges during muscle contraction. *J. Physiol.*, 1951, *113:* 298–315.
25. KATZ, B. Experimental evidence for a non-conducted response of nerve to subthreshold stimulation. *Proc. Roy. Soc.*, 1937, *B124:* 244–276.
26. POLYAK, S. L. *The retina.* Chicago, Univ. of Chicago Press, 1941, 607 pp.
27. RENSHAW, B. Effects of presynaptic volleys on spread of impulses over the soma of the motoneuron. *J. Neurophysiol.*, 1942, *5:* 235–243.
28. RUSHTON, W. A. H. The structure responsible for action potential spikes in the cat's retina. *Nature*, 1949, *164:* 743–744.
29. SVAETICHIN, G. Analysis of action potentials from single spinal ganglion cells. *Acta physiol. scand.*, 1951, *24:* Suppl. 86.
30. TALBOT, S. A. (personal communication).
31. TALBOT, S. A. AND KUFFLER, S. W. A multibeam opthalmoscope for the study of retinal physiology. *J. opt. Soc. Amer.*, Dec. 1952 (in press).