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INTRARETINAL RECORDING WITH MICROPIPETTE ELECTRODES IN THE INTACT CAT EYE

By K. T. BROWN* and T. N. WIESEL†

From the Wilmer Institute, The Johns Hopkins Medical School, Baltimore 5, *Maryland, U.S.A.*

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The earliest recordings from single units of the mammalian retina were made by Granit (1941), who removed the cornea and lens and recorded the activity of single ganglion cells with a glass-insulated wire electrode on the retinal surface. Talbot & Kuffler (1952) developed methods for using wire electrodes in an intact mammalian eye. The normal optics made it possible to stimulate the retina with small, well-focused spots of light, and to view the retina directly for positioning the electrode and stimulus. With this technique Kuffler (1953) investigated receptive field organization of single ganglion cells.

Since wire electrodes record only from the retinal surface, intraretinal electrodes extend the problems which may be studied. Micropipette electrodes have now been used in the opened cat eye by Motokawa, Oikawa & Tasaki (1957) and Grüsser (1957). The present authors have given a preliminary report on intraretinal recording with micropipette electrodes in the intact cat eye (Brown & Wiesel, 1958), and the techniques will be described fully in this paper.

The main problems undertaken were as follows: (1) Determination of the sequence of electrical events during a retinal penetration, and development of physiological methods for locating the electrode tip. (2) Detection of a membrane with characteristics similar to the 'R membrane' described by Brindley (1956), and anatomical identification of that membrane. (3) Identification of single cells in the ganglionic and inner nuclear layers, and study of the discharge patterns and receptive field organization of these cells.

* Present address: Department of Physiology, University of California Medical Center, San Francisco 22, California, U.S.A.

† Present address: Harvard Medical School, 25 Shattuck Street, Boston 15, Mass., U.S.A.

(4) Isolation and study of single units which yield only graded slow potentials. The intraretinal e.r.g. has also been studied, but detailed results of that work will not be presented in this paper.

METHODS

Material. About 125 cats were used over a three-year period. Animals were first anaesthetized by an intraperitoneal injection of pentobarbital (20–30 mg/kg), followed within about 1 hr by dial-urethane (Ciba, 0·25 ml./kg). The injection of dial-urethane was repeated later as required. Stable relaxation of skeletal muscles was obtained by a continuous intravenous infusion of succinylcholine at about 10 mg/kg/hr. The succinylcholine was given in a solution of 2·5% dextrose and 3% dextran, in normal saline, injected at about 6–9 ml./hr. Normal rectal temperature was maintained by chemical heating pads. Experiments frequently lasted 16 hr, and on several occasions the preparation was in good condition for 2 days.

Eye operation. The head was held with two ear plugs and a screw on the back of the head. The head holder was mounted on a compound rest for aligning the eye with the beam of the ophthalmoscope. A device for holding the left eye and controlling the electrode was attached directly to the head holder; this device is illustrated in Pl. 1, fig. 1.

Atropine was used to dilate the pupil and relax accommodation. The temporal side of the eye was exposed, the membrana nictitans was removed, and a contact lens was applied to protect the cornea from drying. An incision was made in the conjunctival sac, nearly all the way around the eye, at about 8 mm from the limbus. The eye ring was then positioned and the limbal portion of the conjunctiva was brought up through the centre and spread outward. The conjunctiva was attached to the ring with haemostatic forceps, and after 10–15 min it adhered firmly to the ring by drying so that the forceps could be removed. The ring was used to lift the eye slightly from the orbit; otherwise the orbital blood vessels sometimes caused retinal movements. Since the eye was held firmly, care was required that the position of the eye was sufficiently normal not to interfere with the retinal circulation. Movement of blood could be seen in the smaller vessels of the cat retina, even when the eye was unmounted, by using a hand ophthalmoscope. Thus blood flow was observed directly to make sure it was normal after mounting the eye.

Contact lens technique. Plano lenses 16 mm in diameter were used. Two lenses with internal radii of curvature 9·0 and 9·5 mm respectively were sufficient to fit adult cats. Glass lenses were required, since the front surface was used dry and had to be free from scratches (lenses made by Parsons Optical Laboratories, San Francisco). A buffered contact-lens solution was used to prevent loss in transparency of the corneal epithelium. With 0·9% saline the corneal epithelium usually becomes cloudy within a few hours, presumably because CO_2 from corneal metabolism causes a decrease in pH of the solution (Hind & Goyan, 1950). The buffered solution in this work was Barnes–Hind No. 502, which was changed about every 6 hr, and which maintained transparency of the cornea even during the longest experiments (obtained from Barnes–Hind Pharmacy, San Francisco).

Electrode insertion and control. The device for holding and advancing the electrode was supported by a ball joint, the ball of which was against the sclera just below the limbus (see Pl. 1, fig. 1). A No. 18 steel needle with a solid steel core was inserted into a passage through this ball, and then pushed through the outer coats of the eye into the fundus. The needle did not penetrate into the vitreous body because the hyaloid membrane enveloping the vitreous is exceedingly tough and elastic. The most satisfactory tip shape was like that of a spinal Quincke needle, but the length of the tapered portion was increased about 50% and the tip was sharpened by hand to a very fine edge. The taper of the core was also made blunter than that of the tube, to eliminate edges which tend to catch membranes. Such a needle readily penetrates the outer coats of the eye, and any retinal or choroidal detachments which occur do not spread beyond the immediate locus of penetration. The fundus was observed during penetration, and the needle was inserted by hand only far enough to penetrate the outer coats of the eye. Then the needle was withdrawn

until it began to move through the tissue, thus re-establishing the normal shape of the eyeball at the point of insertion, and the outer tube was clamped in this position. The core was then drawn straight out, leaving over the end of the tube a single layer of hyaloid membrane which could be penetrated by the micro-electrode. As the core was withdrawn, the outer end of the tube was bathed in saline so that saline, instead of air bubbles, was drawn into the tube as the core was removed. The core was immediately replaced by an electrode which fitted the tube closely enough to prevent any appreciable fluid loss. The electrode was pushed by hand to within 1 or 2 mm from the retina, and then clamped to the advancer. The electrode was aimed by means of the ball joint, and the range of movement included the nasal retina, optic disk and area centralis.

The electrode advancer consisted of a glass syringe at the electrode end and a metal bellows at the other end, connected by a polyethylene tube. The system was filled with mineral oil, and a micrometer screw controlled the length of the metal bellows. The electrode could be moved over a range of 5 mm by steps as small as about 1·0 μ. If a given micrometer setting was made repeatedly, always moving in the same direction, the electrode varied only ± 1 μ from the mean position; backlash was 10 μ.

Micro-electrodes. The electrodes were pulled from lime glass tubing with an outside diameter of 0·8 mm (Schaar and Co., No. G 3620). Tips were formed by a two-stage puller of the type designed by Alexander & Nastuk (1953) and made by Industrial Science Associates, Ridgewood, N.Y. The distance from the tip to the end of the taper was 4–6 mm. The tip diameter was less than 0·5 μ, the taper was approximately straight during the first 300 μ, and at 300 μ from the tip the outside diameter was about 15 μ. When filled with 3 M-KCl, d.c. resistances were 15–30 MΩ; d.c. resistance was determined by a matching method, with the electrode in the eye, using a current through the electrode not exceeding 10^{-9} A.

Electrodes were filled by a modification of the alcohol method described by Tasaki, Polley & Orrego (1954). After filling with absolute methyl alcohol by gentle boiling, a slender tube was inserted into the electrode shaft to fill it first with distilled water and then with 3 M-KCl. Next the electrode was placed in 3 M-KCl, and the d.c. resistance dropped to its final value within about 1 hr. Thus each electrode could be stored in alcohol until the day it was used; this minimized damage to the tips by KCl.

Stimulus system. The multibeam ophthalmoscope designed by Talbot & Kuffler (1952) and slightly modified by Barlow, Fitzhugh & Kuffler (1957) was used for stimulating and viewing the retina. As used in this work, the instrument provided one background and one stimulus beam. A tungsten light source in the background beam gave a uniformly illuminated circular area about 3·58 mm in diameter on the cat's retina, with a maximum retinal illuminance of 2·34 log m.c. A glow modulator tube in the stimulus beam gave square light pulses of variable duration and repetition frequency, with a maximum retinal illuminance of 1·85 log m.c. The procedure for measuring retinal illuminance has been described previously (Barlow *et al.* 1957). The intensities of both beams were controlled by neutral density filters. A set of field stops in the stimulus beam provided stimuli with diameters varying from about 0·125 to 3·00 mm on the retina. Central portions of the larger apertures could be occluded to produce annular stimuli. A movable aperture was also provided to position a small stimulus spot anywhere within a circular retinal area about 3·00 mm in diameter. Thus receptive fields could be explored while holding the background beam in a constant position. A telescopic viewing beam was used at a magnification of about 40 ×. The background, stimulus, and viewing beams were combined by semi-reflecting mirrors so that they were concentric with one another and all moved together wth the head of the ophthalmoscope. The beams could be directed toward any part of the retina within about 40° from the optical axis of the eye. Stimuli were focused upon the retina by observing the retinal stimulus spot directly through the viewing beam. Spherical lenses in 0·25 dioptre steps were used for refraction.

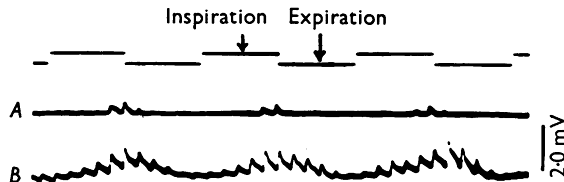
Recording system. One chlorided silver wire made contact with the KCl in the micro-electrode, and another was used subcutaneously on the back of the head as an indifferent electrode. A negative capacitance pre-amplifier was used, of the type developed by MacNichol & Wagner (1954),

with a grid current of about 5×10^{-13} A. The pre-amplifier was fed into a wide-band a.c.–d.c. amplifier and then to a loudspeaker and one beam of a dual-beam oscilloscope. The second beam recorded duration of stimulus.

Stabilization of retinal tissue

Small retinal movements are a greater problem with micropipette electrodes than with wire surface electrodes, since micropipettes move readily through the retina. Thus the methods of Kuffler (1953) for controlling retinal movements with surface electrodes were inadequate with micropipettes.

Mechanical disturbances were minimized by mounting the preparation and ophthalmoscope at two separate points on a heavy Kemrock table top. Succinylcholine seemed superior to other muscle relaxants tested (tubocurarine, gallamine triethiodide, decamethonium bromide) for maintaining the general condition of the animal during long experiments. There is also evidence that the other relaxants affect central nervous activity, but little or no effect has been found with succinylcholine (Purpura & Grundfest, 1956; Chennells, 1957; Mountcastle, Davies & Berman, 1957). After a single injection of succinylcholine the extraocular muscles undergo a strong contracture which rotates the eye into a fixed position and raises intraocular pressure (Lincoff, Breinin & DeVoe, 1957; Macri & Grimes, 1957). With continuous intravenous infusion these phenomena disappeared within 10–40 min, after which there were no serious difficulties from muscle movements.



Text-fig. 1. Recording of pulse and respiratory movements at retinal surface, using the transducer property of a micropipette electrode. In A the electrode made contact with the retina only during late inspiration and early expiration, as is indicated by recording pulse movements. In B the electrode was advanced another 6 μ, which was just enough to establish continuous retinal contact, as is indicated by continuous recording of pulse movements. Artificial respiration at 2·7 sec per cycle, closed chest cavity, and low intraocular pressure. Negative is upward in this record and all other records in this paper, except for intracellular recordings which are shown with positive upward.

Pulse pressure, respiration, and slow changes in fluid balance within the eye all created movement problems. Any retinal movement which deformed the tip of the electrode produced a transducer effect and was recorded as a voltage signal. In the vitreous humour the recording base line was steady, but when contact was made with the retina the pulse beat of the surface blood vessels appeared in the record. This index of retinal contact was used to detect larger types of movement. Text-figure 1 illustrates retinal respiratory movement under conditions of a closed chest cavity, and unusually low intraocular pressure, so that the movement was especially distinct. The respiratory excursion of the retina was measured by how far the electrode had to be advanced to pass from the point of minimal intermittent contact (Text-fig. 1A) to the point where continuous contact with the retina was established (Text-fig. 1B). The respiratory excursion was only 6 μ; thus the intermittent contact in Text-fig. 1A illustrates that the pulse beat is a sensitive and unequivocal index of retinal contact.

Respiratory movement of the retina was found to be due primarily to changes of intrathoracic pressure, rather than movements of the chest wall. This was determined by using both natural and artificial respiration and noting that the retina always moved forward when intrathoracic pressure

increased, regardless of the accompanying direction of movement of the chest wall. Intrathoracic pressure is known to affect intracranial pressure through the circulatory system (Livingston, 1955), and intracranial pressure influences venous return from the retina through the central retinal vein (Adler, 1953). This is probably the major route whereby respiration produces retinal movements. Unilateral pneumothorax greatly reduced respiratory movement, providing that the chest cavity was kept large in relation to the lung expansion. A blunt hook was inserted to lift the chest wall, maximally expanding the chest cavity. Artificial respiration was carried out with room air at about 18 c/min, and the lungs were minimally inflated consistent with adequate ventilation. Some respiratory movement may also be transmitted to the retina mechanically; precautions against such movements included severing the trachea above the cannulation and clamping the jaws together.

The pulse pressure movements seem due primarily to pulse pressure in the retinal and choroidal vessels, since pulse recording during a retinal penetration was greatly reduced or absent in avascular layers of the retina. Pulse movements involving the entire retina were negligible unless the eye was allowed to rest in the orbit, presumably pressing against orbital blood vessels.

Since pulse and respiratory movements seem to be due to changes of blood pressure within the eye, these movements are influenced by intraocular pressure. Both types of movement are greatly enhanced by lowering intraocular pressure and clearly damped by increasing the intraocular pressure toward normal. Thus the intact eye serves the same purpose for stable retinal recording that the closed chamber developed by Davies (1956) does for cortical recording.

If a retinal contact was made immediately after inserting an electrode, the contact was soon lost. A further advance of the electrode re-established contact, after which the process was repeated. Thus the retina drifted slowly away from the electrode. Some fluid was lost from the eye while inserting an electrode, which produced a distinct fall of intraocular pressure, and the slow retinal drift was undoubtedly due to an increase of intraocular pressure toward normal as new aqueous humour was formed. These movements always disappeared slowly and were usually undetectable after 10–15 min.

When movements were handled as described, the retina was very stable. Respiratory movement was so small that an intermittent contact could not be made, and pulse movements were too small to measure. Thus stable extracellular recordings from single cells were maintained up to 4 hr, and 1 hr was not unusual.

Transducer properties of micropipette electrodes

Mechano-electric transducer actions of micropipette electrodes were found very useful in this work. Since small tissue movements were recorded with great reliability, it seemed likely that the transducer action depended only upon deformation of the electrode tip. This hypothesis was tested by advancing electrodes against a smooth glass surface, at angles of 15–30°, while observing under water immersion at 990 times magnification. When the tip made contact with the glass, the electrode became highly microphonic. Scratching the bottom of the heavy table top produced signals of 1 or 2 mV, although the vibration of the electrode shaft was barely visible under the microscope. After advancing another 1–3 μ, changes in d.c. level and increases of electrode resistance were clearly detected. These observations were also made by advancing the electrode against polished platinum. Thus deformation of the electrode tip produces a voltage signal, and the transducer action is extremely sensitive.

The electrode could sometimes be advanced a considerable distance against glass without damage. In a typical case an advancement of 15 μ beyond the contact point produced a positive d.c. shift of 15 mV while the electrode resistance increased from 12 to 40 MΩ, the electrode remaining microphonic all the time. The tip then passed over a small pit in the glass face. In such cases as soon as the tip visibly lost contact with the glass, the microphonicity disappeared and both the electrode resistance and d.c. level returned to the values obtained before advancing against the glass. Since the portion of the electrode behind the tip was still in contact with the glass, and distinctly bent, all effects of advancing against glass were due to changes occurring

within a few micra from the ultimate tip. The effects of pressing against glass also disappeared if the tip was broken to a diameter of about 1 μ.

Tip deformation always increased electrode resistance. Changes in d.c. level were either positive or negative, however, so the voltage signals were not due to changes of electrode resistance in association with grid current. These voltages were probably due to changes in tip potentials. Thus it seems likely that changes of tip potentials, as well as changes of electrode resistance, can both be produced by the common factor of deformation of the electrode orifice. Tip potentials were not measured in the present study, but unpredictable changes of tip potentials have been reported by del Castillo & Katz (1955) and Adrian (1956). These authors attributed the changes to plugging of the electrode by tissue, and present evidence suggests that tip deformation may also have been involved.

Since tip deformation always increased electrode resistance, measurements of electrode resistance provided a sensitive and reliable indication of whether the electrode was advancing smoothly through tissue or pressing against some tough structure. If the electrode pressed against mechanically resistant tissue, such as the membranes at the retinal surface, advancing the electrode only 2 or 3 μ produced a discernible increase of electrode resistance. Appreciable increases of electrode resistance occurred only at certain retinal levels, primarily those levels where membranes are known to exist. Such increases of electrode resistance were usually reversible upon penetrating the membrane, or upon pulling back slightly. Occasionally an increase of electrode resistance was maintained even after withdrawing from the retina; these cases were attributed to persistent plugging.

RESULTS

The sequence of events during a retinal penetration

The retina was always approached from the vitreous humour, and several thousand penetrations were made. Different retinal areas yielded comparable qualitative results; the measured depth of a given event varied between retinal areas, however, because of differences in retinal thickness and different angles of approach. Most penetrations were done in the part of the nasal periphery shown in Pl. 1, fig. 2, where the electrode approached as perpendicularly as possible, and all electrode depth values in this paper will be characteristic of that part of the retina.

The stratification of retinal blood vessels provides three major landmarks when penetrating the retina. Surface vessels of the cat retina, like those of man, extend only from the internal limiting membrane to the outer margin of the inner nuclear layer (Michaelson, 1954). Deeper retinal layers are avascular, and the choroidal circulation extends from the outer margin of Bruch's membrane to the sclera. A marked pulse beat was recorded from the retinal surface to about 40% of the total retinal depth, where it was sharply reduced in magnitude. The pulse beat was small or absent for the remainder of the retinal depth; then it returned strongly and remained strong during further advance through the choroid. Thus pulse recording by electrode transducer properties permitted identification of the retinal surface, outer margin of the inner nuclear layer, and Bruch's membrane.

The complete sequence of events during a retinal penetration will now be

described. The changes in pulse recording and other striking electrical events are summarized in Pl. 1, fig. 2. Earliest appearance of the pulse beat signalled retinal contact and gave the zero point for depth measurements. During a further advance of 15–30 μ, electrode resistance increased as much as 20–30 MΩ. Then a fast positive–negative electrical transient occurred, after which electrode resistance returned to its original value. The diphasic transient was a reliable sign of penetrating surface membranes, which consist of the hyaloid membrane overlying the internal limiting membrane.

Single unit discharges were sometimes seen immediately after penetrating the retina, but a slight additional advancement or withdrawal was usually required. These units were identified as ganglion cells because of their proximity to the retinal surface. Beyond the first layer of impulse activity was a narrow zone, presumably corresponding to the inner plexiform layer, in which no stable single unit activity was recorded. At measured depths of 40–100 μ another layer of single unit impulse activity was often found. These units were identified in the inner nuclear layer by criteria described later in the present paper.

On most penetrations some type of mechanical barrier was encountered at the outer margin of the second layer of impulse activity. Electrode resistance usually increased at that point; then a transient occurred, after which electrode resistance was back to normal. The intraretinal b- and c-waves increased sharply in amplitude when the barrier was passed. Single unit impulse activity was sometimes recorded just before penetrating the barrier; these impulses always disappeared at the moment of penetration, and impulse activity at further depths was never found. The sharp reduction of pulse recording, mentioned earlier, occurred when the barrier was passed. Thus the barrier seemed to be at the outer margin of the inner nuclear layer. No definite membrane is known in this region, but the barrier may result from the high density of structures forming the sharp line of synapses between receptors and second-order neurones.

Within a narrow zone of about 30 μ, which seemed to be just beyond the inner nuclear layer, recordings were occasionally obtained from single units which responded to light only with graded slow potentials. Detailed findings from these units are reported later in this paper.

On rare occasions a sharp transient was noted at measured distances of 75–100 μ beyond the inner nuclear layer. No d.c. shift or distinct change in the e.r.g. was associated with this transient. After the transient was a zone of 30–50 μ, within which pulse and respiratory movements were almost undetectable. This zone of movement-free recording was found consistently, whether preceded by the transient or not. The rare transient is believed to be due to the electrode occasionally catching upon a filament of the external limiting membrane, and the zone of the movement-free recording seems to

correspond to the layer of inner and outer receptor segments. Further details supporting these identifications will be presented later in this paper.

At the outer margin of the movement-free zone, and at a total measured depth of 165–250 μ, a strong pulse suddenly reappeared and electrode resistance increased. Then a negative d.c. shift of 30–60 mV occurred; after this the electrode resistance was back to normal but the strong pulse remained. After the d.c. shift the entire e.r.g. was greatly reduced in amplitude. This complex of events occurred with great reliability and was identified with Bruch's membrane.

Beyond Bruch's membrane the only electrical events were frequent and randomly occurring transients, d.c. shifts, and changes of electrode resistance. Pulse recording remained strong, and there were no further changes in the e.r.g. These results are predictable in a vascular, essentially equipotential medium like the choroid. The electrode always broke within about 100 μ beyond Bruch's membrane, presumably against a blood vessel or the sclera.

During withdrawal any given point was found at a measured depth of about 50 μ less than on the way in, owing primarily to tissue lag. Distances between events were generally the same as during penetration. Changes in pulse recording and the e.r.g. were faithfully reversed during withdrawal, and some remainder of the d.c. shift across Bruch's membrane was usually found, but single cell activity was seldom recorded. Neither changes in electrode resistance nor electrical transients were seen on the way out, indicating further that these events during penetration were due to mechanical barriers.

Location of electrode within retina

Since electrical events during a retinal penetration may be correlated with definite structures in the histologically stratified pattern, the electrical events may be used to locate the electrode. This method of location has been used to determine the significance of electrode depth measurements in this preparation and to study how the electrode moves through the tissue.

MacNichol & Svaetichin (1958) found measured depths were systematically too low in isolated retinas. The possible sources of this type of error were considered and avoided in present work on the intact cat eye; thus it seems safe to assume that measurements of electrode depth in this work were greater, if anything, than the actual depth. Of course tissues cling to the shaft of a micro-electrode, and hence become somewhat distorted. This effect, commonly called 'tissue lag', was evaluated at both the retinal surface and Bruch's membrane. At the surface the electrode was advanced to retinal contact, then the withdrawal distance to lose contact was measured, then the electrode advance required to re-establish contact was measured. Ten measurements were thus obtained for each direction of movement, and the procedure at Bruch's membrane was identical. These measurements were corrected for

backlash in the electrode advancer. The resulting values for tissue lag varied considerably between preparations, probably owing to different electrodes, and ranged from about 20 to 80 μ. In a typical case the average surface values were 51 μ going in and 51 μ coming out, while values at Bruch's membrane were 53 and 54 μ. The reliability of these measurements is indicated by close agreement for the two directions of movement. These measurements also illustrate the general rule that tissue lag was almost identical at the retinal surface and Bruch's membrane; thus when the electrode is moving in a given direction all retinal layers must be distorted to about the same extent, and depth measurements should not be in error due to different amounts of distortion at different levels. It also follows that measurements during penetration and withdrawal should agree, as has been observed, and this could not be predicted if greater distortion occurred within the retina than at the retinal surface. Therefore tissue lag, which Tomita & Torihama (1956) found to produce spuriously high depth values in the frog retina, seems to produce no significant error in the intact cat eye. Apparently this is because the vitreous humour and retina both cling to the electrode with about equal force.

The above account of tissue lag, due to clinging of tissue to the shaft of the electrode, has ignored the separate problem of specific membranes. When a membrane is encountered, of course a local distortion of tissue must occur until the membrane is penetrated. Such distortions are readily detected and presumably transient. Also they can be avoided by using measurements during withdrawal. The electrode seems to move very smoothly through the tissue during withdrawal, and sometimes also during penetration, as indicated by gradual transitions in magnitude of the intraretinal e.r.g.

The only significant depth error known in this preparation is due to deviation of the electrode from a perpendicular course through the retina. Since minimal electrode depths should be most significant, a series of penetrations was made with special precautions for obtaining accurate minimal depth measurements for Bruch's membrane. In these penetrations 165 μ proved to be a reliable minimal value. Near the nasal border of the tapetum lucidum, where the electrode depth measurements were made, the histologically measured depth of Bruch's membrane was 168–176 μ. Since the electrode depths were not larger than the histological depth, there was no indication that the electrode did not penetrate perpendicularly in these cases. There was likewise no indication that a tissue shrinkage allowance was necessary, so none is made in Pl. 1, fig. 2. The small discrepancy between the two kinds of measurement is probably because histological sections were not quite perpendicular to the retinal surface, as is indicated by failure to obtain sharply aligned inner and outer receptor segments. The agreement seems satisfactory, considering the problems involved. This finding further supports our

identification of Bruch's membrane and indicates that minimal electrode depth measurements can be reasonably accurate.

The range of depth values for Bruch's membrane was about 165–250 μ, however, and absolute depth of any given event in the retina varied greatly between penetrations. Thus a perpendicular approach to the retina did not assure a perpendicular passage through the retina, and absolute depth values were often too great. The percentage of the total depth at which a given event occurred was much more constant, and hence more meaningful for electrode location. The use of electrical signs appears inherently more accurate, however, than any method depending upon depth measurements.

Conditions of single cell recording

It was not uncommon to miss impulse activity entirely on a given penetration, but when a single unit was detected it was almost always recorded in complete isolation from other units. Since it proved possible to record impulse activity from single units of the inner nuclear layer, these results pertain even to that densely packed layer of cells. Thus extracellular recordings appear to have been made only with the electrode very close to the cell membrane. Extracellular impulses were typically positive–negative in shape, and previous authors have concluded that such impulses are characteristic of recording close to the cell membrane (Li, 1955; Mountcastle *et al.* 1957).

Stable recordings were frequently obtained for over an hour with no indication of cell damage. Small pulse movements were often noted in the d.c. record with no accompanying effect upon either impulse size or frequency. In some cases impulse size varied, and in other cases impulse size and frequency both followed the pulse movements. When studying impulse patterns, pulse movement artifacts were minimized by positioning the electrode only close enough to the cell to obtain clear recordings. Small variations of impulse size were accepted, but recordings were rejected if impulse frequency varied with the pulse beat.

Organization of receptive fields of single ganglion cells

Kuffler (1953) found that receptive fields of ganglion cells in the light-adapted state are organized so that either an 'on' discharge is produced by stimulation of the centre and an 'off' discharge by stimulating the periphery of the field, or vice versa. He also demonstrated that mutual inhibition occurs between central and peripheral zones, and that the response type of the central zone dominates the response to large stimuli. He points out that the wire electrodes in his work probably recorded selectively from the larger ganglion cells, which have diameters as great as about 30 μ, and evidence for this has been presented by Rushton (1949). This left open the possibility that the receptive field organization found by Kuffler was characteristic

only of the larger ganglion cells. The smallest ganglion cells are 4–8 μ in diameter, and the cells of the inner nuclear layer are about the same size. Since single unit recordings were obtained in the present study from cells of the inner nuclear layer, it appears that micropipette electrodes record from ganglion cells of all sizes.

In the peripheral cat retina the only cell bodies at histological depths of less than 40 μ are ganglion cells. Recorded depth of a single unit was measured as the distance from retinal contact to where impulses were first clearly detected, and any unit with a recorded depth of 30 μ or less was identified in the ganglionic layer. Known errors of depth recording would not produce false identification, and 10 μ was allowed for unknown factors. In the area centralis, where the inner margin of the inner nuclear layer is at 50 μ, the criterion for ganglion cells was a recorded depth of 40 μ or less.

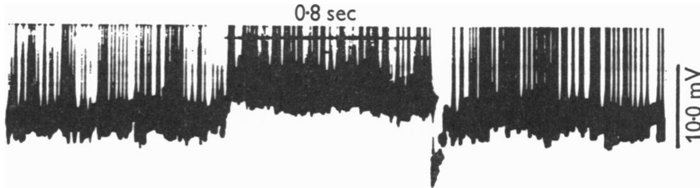
In some cases the activity of a ganglion cell was recorded from its axon in the optic fibre layer, rather than from the cell soma. Recordings were clearly from optic nerve fibres whenever the receptive field was located some distance peripherally on the retina from the electrode (see Kuffler, 1953). In these cases the nerve impulses had a shorter time course, and gave a distinctly higher pitched sound from the loudspeaker, than when the receptive field was centred around the electrode. The sound of the impulses was so different in the two cases that recordings from nerve fibres and cell somata could be distinguished by this method whenever the recording site could not be identified positively by location of the receptive field relative to the electrode.

For each single unit the centre of the receptive field was found by Kuffler's method of using a small stimulus spot to locate the most sensitive part of the receptive field. Background retinal illuminance was about 0·34 log m.c., and stimulus intensities were near threshold. The centre response was tested with a centred stimulus spot, and the peripheral response was tested by an annulus with inside diameter equal to the size of the centred spot. Mutual inhibition was then tested by combining the two stimuli into a single large spot. About 100 ganglion cells were tested, and receptive fields of all cells were functionally organized as described by Kuffler. Thus the same general principles of receptive field organization seem to hold for ganglion cells of all sizes. Ganglion-cell receptive fields exhibit variations in size and detailed organization, however, which have been reported by Wiesel & Brown (1958).

Intracellular recordings from ganglion cells

Intracellular recordings were occasionally obtained from ganglion cells. Penetrated cells deteriorated quickly and impulse activity usually failed within 10–15 min. Stable response patterns were established in a few units, and a response from one of these is shown in Text-fig. 2. A high-frequency 'on' discharge was elicited, and throughout the stimulus the discharge frequency

was higher than before stimulation. This increased discharge rate was accompanied by a maintained depolarization of the cell membrane. After the stimulus a brief increase of membrane potential was accompanied by inhibition of impulses. Intracellular recordings showed a consistent linkage of increased discharge frequencies with depolarization and inhibition of impulses with increased membrane potentials. The well maintained depolarization in Text-fig. 2 is of special interest. Similarly maintained slow potentials have also been obtained from spinal motoneurones of the cat during natural activation (Kolmodin & Skoglund, 1958). Our intracellular observations from ganglion cells have recently been confirmed in further work by Wiesel (1959).



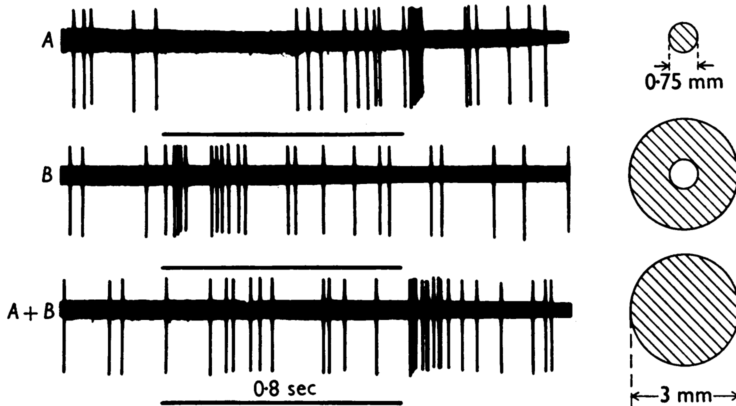
Text-fig. 2. Intracellular recording from ganglion cell of the area centralis. Membrane potential = 60·0 mV. Background retinal illuminance = 0·34 log m.c. Retinal illuminance of stimulus = 1·85 log m.c. Stimulus was a 3·0 mm spot, centred approximately on the electrode and repeated at 5 sec intervals; d.c. recording. Impulses were cut off at the top and impulse size was not measured.

Single cells of the inner nuclear layer

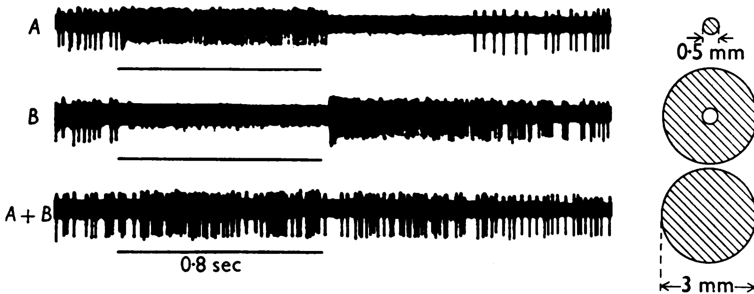
In the periphery of the cat retina single units of the inner nuclear layer were identified by the following four criteria. (1) The ganglion cells constitute a single layer of scattered cells (see Pl. 1, fig. 2); thus if two cell somata which discharged impulses were recorded in sequence on a given penetration, the second cell was almost certainly in the inner nuclear layer. (2) The b-wave of the e.r.g. does not invert polarity until the region of the inner nuclear layer (Brown & Wiesel, 1958), so a negative b-wave was required at the recording site. (3) The histologically measured thickness of the inner nuclear layer is about 20 μ; thus the distance the electrode traversed after leaving the cell, in order to pass the outer margin of the inner nuclear layer, was required to be 20 μ or less. Known errors of depth recording make this criterion conservative. (4) Measured depth of the cell beyond retinal contact was required to exceed 40 μ. In certain cases a single cell was identified in the inner nuclear layer by all four criteria, and in these cases the identification seemed positive.

Cells of the inner nuclear layer showed impulse activity in complete darkness and also discharged impulses in response to light stimuli. No single units were identified in this layer which responded to light with slow potentials only, as was found from slightly deeper units. Thus impulse activity is a general, if not infallible, rule for cells of the inner nuclear layer.

Receptive fields were studied by the same methods used for ganglion cells. Results from two units are shown in Text-figs. 3 and 4. Both units were identified by all four criteria; the negative b-wave does not appear in the figures, however, because the illustrated recordings were made with a short time constant. The receptive field in Text-fig. 3 had an 'off-centre' and 'on-periphery'. When central and peripheral zones were stimulated simultaneously, the 'off' response was weak and the 'on' response was almost absent. Thus mutual inhibition occurred between central and peripheral



Text-fig. 3. Functional organization of receptive field of a cell in the inner nuclear layer. Cell identified in that layer on the basis of all four criteria described in text. Background retinal illuminance = 0·34 log m.c. Retinal illuminance of stimuli = 1·74 log m.c. Stimulus pattern producing each response shown at right of figure. In *A* a small stimulus spot was located in the centre of the receptive field. In *B* the stimulus was an annulus around the spot stimulated in *A*. In *A* + *B* the two stimuli were combined by using a single large stimulus spot. Stimulus repeated every 10 sec; a.c. recording; impulses 8·0 mV peak-to-peak, retouched.



Text-fig. 4. Functional organization of receptive field of another cell in the inner nuclear layer; this cell also identified in that layer on the basis of all four criteria. Background retinal illuminance = 0·34 log m.c. Retinal illuminance of stimuli = 0·74 log m.c. Stimulus pattern for each response shown at right of figure, and procedure identical to that for Text-fig. 3. Stimulus repeated every 10 sec, a.c. recording, impulses 1·1 mV peak-to-peak, retouched.

zones, and the central zone dominated the response to a large stimulus. The receptive field of the cell in Text-fig. 4 was functionally organized like that of Text-fig 3, except for having an 'on-centre' and 'off-periphery'. Receptive fields of all cells identified in the inner nuclear layer have been organized as shown in Text-figs. 3 and 4. Thus receptive fields of these cells seem to be functionally organized like those of ganglion cells.

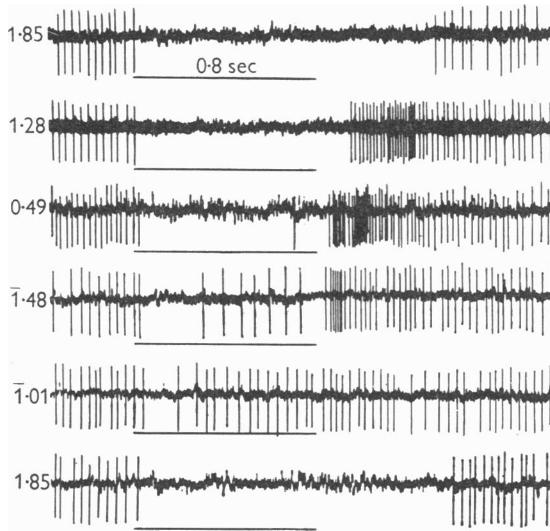
Inhibitory responses

Previous studies of ganglion cell discharges have shown that 'on' discharges are frequently followed by post-stimulus inhibition, whereas 'off' discharges are often preceded by inhibition during the stimulus (Granit, 1950; Kuffler, 1953). Under special conditions responses in this study revealed only inhibition, with no discharge at either 'on' or 'off'. Such responses are illustrated in Text-figs. 5 (top and bottom records) and 6, and they will be called pure inhibitory responses. This term does not imply that the light stimulus produces only inhibitory effects upon the cell, since excitatory effects may be present but masked. The term does designate a response in which inhibition is the only *observed* result of light stimulation.

This type of response was found from certain cells of both the ganglionic and inner nuclear layers. Under light-adapted conditions it was obtained only with a small stimulus spot, not exceeding 0·25 mm in diameter, accurately located in the centre of the receptive field. These responses were also obtained only at high and low stimulus intensities, whereas intermediate intensities evoked 'off' discharges. This is illustrated in Text-fig. 5. Peripheral portions of these receptive fields gave 'on' discharges. Thus these units may be classified as 'off-centre' units which yield pure inhibitory responses at certain stimulus intensities. In 'off-centre' units which gave 'off' responses at all intensities above threshold, it was a general rule that the strength of the 'off' discharge was maximal at intermediate intensities. Hartline (1940) found a similar relation between strength of 'off' discharge and stimulus intensity in the frog. Thus the general rule seems to be well illustrated by units which yield pure inhibitory responses, since the 'off' discharge is completely absent at high and low stimulus intensities.

The inhibitory effects of light stimuli were graded with stimulus intensity, as is shown in Text-fig. 5. With increasing intensity the latency for inhibition of impulses decreased. The duration of complete inhibition of impulses during the stimulus increased, as did the duration of complete inhibition following the stimulus. The number and frequency of impulses during the stimulus both decreased. Thus by all five of these criteria the inhibitory effects increased in a graded manner with stimulus intensity. Inhibitory effects were also controlled by several other factors. The inhibitory effect of a given stimulus was increased by either reducing the intensity of background illumination or

by dark-adapting the eye. If a small stimulus was moved away from the centre of the receptive field, inhibitory effects gradually disappeared and were finally replaced by an 'on' discharge, characteristic of the peripheral portion of the receptive field. When the size of a centred stimulus spot was gradually increased, there was first an increased inhibitory effect and later a decrease.



Text-fig. 5. Effect of stimulus intensity upon the response of a ganglion cell in the area centralis which was capable of yielding a pure inhibitory response. Background retinal illuminance = 2·34 log m.c., and retinal illuminance of each stimulus given in log m.c. at left of figure. Responses recorded in sequence from top to bottom. Stimulus was 0·25 mm in diameter, in the centre of the receptive field, and repeated every 10 sec; a.c. recording, impulses 1·7 mV peak-to-peak, retouched.

Text-fig. 6. Inhibition at both 'on' and 'off', with no sign of excitation, recorded from a ganglion cell on the border of the area centralis; no background illumination. Retinal illuminance of stimulus = 1·85 log m.c. Stimulus was 2·0 mm in diameter, in the centre of the receptive field, and repeated every 5 sec; d.c. recording, impulses 1·2 mV peak-to-peak, retouched.

Thus inhibitory effects seemed to sum over the central portion of the receptive field, but with large stimuli competitive effects from the peripheral zone appeared.

The possibility that with a repetitive light stimulus the increased stimulus intensity produced its effect by altering state of adaptation, instead of by stimulus intensity *per se*, was checked by using single-stimulus flashes. Both

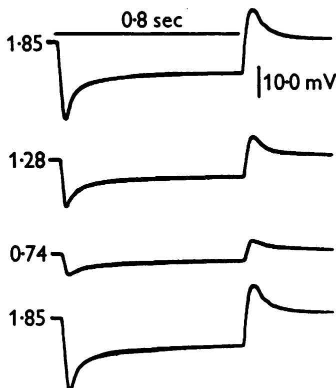
methods produced comparable results. Thus the background illumination seemed to be effective in preventing the stimulus from producing disturbing adaptive effects. When observing the effects of stimulus location and stimulus spot size, the effects of stray light were also minimized by background illumination.

When impulse activity was not absent throughout the stimulus, the form of the inhibitory response during the stimulus was as illustrated in Text-figs. 5 (fourth and fifth records from the top) and 6. Shortly after onset of the stimulus impulses were suddenly and completely abolished; then impulses began again and became stabilized rather quickly at a lower frequency than that of the pre-stimulus maintained discharge. Thus inhibitory effects decreased during a steady stimulus, indicating an adaptation of inhibition. This form of response has also been observed in *Limulus* during lateral inhibition (Hartline, Wagner & Ratliff, 1956).

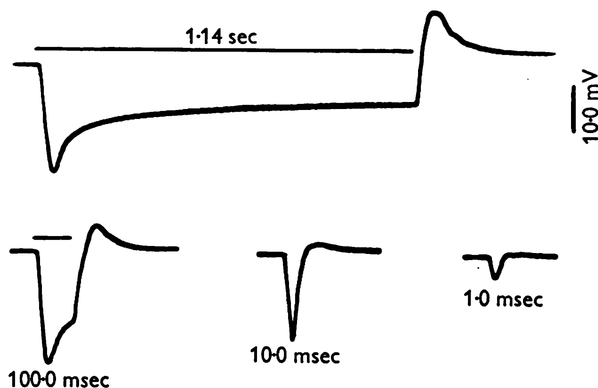
A renewal of strong inhibition was often noted immediately after the stimulus, as shown in Text-fig. 6. Thus inhibition sometimes occurs at both 'on' and 'off', and this type of response may be regarded as the inhibitory analogue of the 'on-off' discharge. The discharge pattern in Text-fig. 6 is similar to that found by Jung, Creutzfeldt & Grüsser (1957) from certain cells of the cat's optic cortex. Such cells were designated Type C neurones, which were not considered to have retinal counterparts; but it appears that the discharge pattern of Type C neurones can also be found from certain retinal cells. When this type of response was seen in extreme form, impulses were completely inhibited throughout the stimulus and for as long as 7 sec after the stimulus was turned off. Thus inhibitory effects in the retina can be quite long-lasting.

Single units responding only with slow potentials

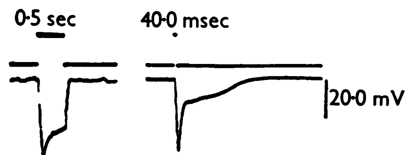
No impulse activity was detected deeper than the inner nuclear layer. When the electrode was located just beyond the inner nuclear layer, in a zone about 30 μ thick, single units were sometimes found which responded to light only with graded slow potentials. These units were rarely obtained and difficult to maintain, by comparison with single cells which discharged impulses. Both intra- and extracellular recordings appear to have been obtained. Certain units showed a resting potential of 50–60 mV, upon which was superimposed a negative response to light of 25–40 mV, and such recordings were considered intracellular. The resting potential in these cases always appeared suddenly and was usually lost immediately or declined rapidly, presumably owing to injury. A few recordings, however, showed stable resting potentials and responses for up to 30 min. Typical intracellular responses to light stimuli are shown in Text-fig. 7. An initial rapid negative potential declined quickly to a lower level, which was well maintained during the remainder of the



Text-fig. 7. Effect of stimulus intensity on a unit which responded to light only with slow potentials. Unit slightly deeper than the inner nuclear layer, at a measured electrode depth of 135 μ in the area centralis. Background retinal illuminance = 0·34 log m.c. Retinal illuminance of each stimulus given in log m.c. at left of figure. Stimulus spot was 3·0 mm in diameter, centred on the electrode and repeated every 10 sec. Responses recorded in sequence from top to bottom; intracellular d.c. recording; membrane potential remained stable at 50 mV for about 30 min.



Text-fig. 8. Effect of stimulus duration on same unit as in Text-fig. 7. Background retinal illuminance = 0·34 log m.c. Stimulus was 3·0 mm spot, with a retinal illuminance of 1·85 log m.c., centred on the electrode and repeated every 10 sec; intracellular d.c. recording.



Text-fig. 9. Effect of stimulus duration upon responses from another unit which responded to light stimuli only with slow potentials. Unit was in nasal periphery and was found during electr de withdrawal at 90 μ from Bruch's membrane, just before the electrode seemed to enter the inner nuclear layer. No background illumination. Stimulus was a spot 3·0 mm in diameter, centred on the electrode and repeated at 5 sec intervals. Retinal illuminance of stimulus = 1·85 log m.c. Intracellular d.c. recording. Membrane potential was stable at 60·0 mV during recording, but this unit was lost after a few minutes.

stimulus. This relatively steady potential declined only slightly during stimuli lasting up to several minutes, indicating very slow adaptation during this part of the response. When the stimulus was turned off a brief positive potential occurred. Text-fig. 7 also shows that the response magnitude was graded with stimulus intensity, while the general response form remained constant.

Text-figure 8 shows the effect of duration of stimulus on the same unit. As the duration of the stimulus was shortened, the duration of the relatively steady potential was first shortened and then the magnitude of the initial rapid response was decreased. Text-figure 9 shows the effect of duration of stimulus in another intracellular recording. The response to a 0·5 sec stimulus was similar to responses of the first unit to long stimuli, except that no positive potential followed the stimulus. With a 40 msec stimulus the response of this unit broke into distinct fast and slow components, and the slow component outlasted the stimulus by about 1·5 sec. Such a long response to a brief stimulus is surprising, especially since the total response was about three times longer than produced by a 0·5 sec stimulus. This phenomenon was not an isolated observation, since it was also seen in other units with extracellular leads. It should be noted that the unit in Text-fig. 8 was light-adapted with the background beam, whereas the unit in Text-fig. 9 had no background illumination. Thus the difference in response patterns may be due to a difference in state of adaptation, rather than a difference between units. Analysis of responses from these units proved difficult because the responses were highly localized and stable recordings were seldom obtained. Responses tended to be lost whenever the background illumination was turned on or off, suggesting some type of photomechanical movement.

About thirty to forty units gave responses similar in form and polarity to those illustrated, but these units showed no membrane potential. Also in these cases the initial rapid potential in response to light was only 5–15 mV in magnitude, compared to 25–40 mV when a membrane potential was recorded. Thus these appeared to be extracellular responses from the same units that yielded intracellular recordings. In the absence of impulse discharges, of course, the usual criteria for single unit extracellular recording could not be applied. These responses were easily distinguished from slow potentials of the intraretinal e.r.g., however, since the single unit responses were sharply localized and much larger in magnitude.

It is puzzling that responses were the same form and polarity with apparently intra- and extracellular leads. Tomita (1957) also found in fish retinas that responses with or without what appeared to be resting potentials were similar in form and polarity, although markedly different in magnitude (see especially Tomita's Fig. 2*b*, *c* and Fig. 4*a*, *b*, *c*). Other investigators, however, have recorded a reversal of response polarity when these units were penetrated in fish (Motokawa *et al.* 1957; MacNichol & Svaetichin, 1958). Intracellular

responses to white light have been found negative by all investigators in both fish and cat; thus the difference in results appears to be in the extracellular polarity.

DISCUSSION

Identification of the 'R membrane'

Brindley (1956, 1958) describes a membrane in frog and rat retinas which has a high radial resistance relative to the resistance of the rest of the retina and choroid. He calls this the 'R membrane', to designate a membrane of high electrical resistance without identifying it with a specific structure. He detected the 'R membrane' by passing current pulses between a micropipette electrode in the retina and an electrode on the back of the eye and measuring resistance between the electrodes. The micropipette entered the retina from the optic fibre side, and when it penetrated beyond a certain point there was a sudden decrease of resistance. At the same time the e.r.g. decreased greatly in magnitude and a negative d.c. shift of 10–30 mV was recorded. Brindley provisionally identified the 'R membrane' with the external limiting membrane.

In this study most of the e.r.g. likewise appeared across a single membrane, which supported a d.c. potential of 30–50 mV with the choroidal side negative; thus an 'R membrane' is also found in the cat. This 'R membrane' was identified with Bruch's membrane for the following reasons. (1) Light micrographs of monkey retinas by Wislocki & Sidman (1954) show Bruch's membrane as a continuous line, while the external limiting membrane looks clearly discontinuous, appearing to consist only of dense rings of material around the bases of rods and cones. These findings are supported and amplified by electron microscopy. Sjöstrand (1953) finds that the rings of material around the bases of guinea-pig rods are not interconnected and sometimes exhibit a fibrous or lamellated structure. E. de Robertis (personal communication) states that 'the external limiting membrane is made of fine interlacing expansions of the Müller cells' and does not consider that it would impede the advance of a micropipette electrode. On the other hand, Bernstein & Pease (1959) show no clear discontinuities of Bruch's membrane in electron micrographs from cat retinas, and L. K. Garron and M. L. Feeney (personal communication) report similar results from human material. Thus it seems reasonable that Bruch's membrane, but not the external limiting membrane, could have a high electrical resistance. (2) Electrical signs were found in this work which permitted provisional identification of the external limiting membrane, and clear identification of Bruch's membrane. The external limiting membrane was detected only on rare occasions, as one would predict from its structure, and it showed no sign of electrical resistance. The 'R membrane' appeared on every penetration, at a deeper level than the external

limiting membrane, and appeared to be identical with Bruch's membrane. (3) Whenever an electrical transient was noted at the external limiting membrane, pulse recording was clearly reduced immediately afterward. The inner and outer receptor segments are highly oriented, and the axis of orientation coincides approximately with the axis of the electrode. Small pulse movements of this part of the retina probably occur, but the failure to detect them is readily understood since the electrode would have nothing to press against. A strong pulse reappeared upon approaching the 'R membrane' and remained after the membrane was penetrated. These results of pulse recording seem predictable only if the 'R membrane' is identified with Bruch's membrane. (4) After penetrating the 'R membrane', no other membranes were detected during deeper penetration. If one identified the 'R membrane' with the external limiting membrane, the failure to detect Bruch's membrane at a deeper level would be difficult to explain.

Brindley's provisional identification of the 'R membrane' was based on two kinds of evidence. (1) When electrical resistance was measured between the front of the retina and the back of the sclera, a large part of the resistance disappeared when the retina was stripped off (Brindley, 1956). This was assumed to be due to removal of the external limiting membrane, but of course Bruch's membrane may have been damaged. The latter assumption seems supported by the more recent finding that Brindley (1958) could not detect an 'R membrane' in frog retinas which had been stripped from the underlying layers. He maintained identification of the 'R membrane' with the external limiting membrane, but his resolution of the contradictory results was based upon unsupported assumptions concerning histology of the external limiting membrane. (2) The 'R membrane' was found at an electrode depth corresponding approximately to *corrected* histologically measured depth of the external limiting membrane (Brindley, 1956). It was observed that electrode depth measurements of retinal thickness were 33% greater than uncorrected histological measurements, and on this basis a 33% shrinkage correction was applied to all histological measurements. Since the observed discrepancy is readily explained by electrode bending, the shrinkage allowance which was applied is arbitrary. A shrinkage allowance of about 10% would have favoured identifying the 'R membrane' with Bruch's membrane. Thus Brindley's evidence seems compatible with ours, although his interpretation is not. Species differences may be involved, but that seems unlikely in this case. Identification of the 'R membrane' is of special interest since it is a significant structure in recording the intraretinal e.r.g.

Impulse activity in the inner nuclear layer

Since impulse activity of the inner nuclear layer has been difficult to demonstrate in retinas of cold-blooded animals, there has been speculation

that ganglion cells are excited only by graded slow potentials from the bipolar cells. In this study many units were identified in the inner nuclear layer, and all such units discharged impulses. Thus evidence of this study would support the view that bipolar cells discharge impulses. The methods of electrode location, however, did not permit identification of the types of cells within the inner nuclear layer from which impulses were recorded. Thus there is a possibility that all impulses from the inner nuclear layer were from horizontal and/or amacrine cells. Definite proof of impulse activity from bipolar cells can probably be obtained only by single-unit marking methods.

It is particularly interesting that receptive fields of cells in the inner nuclear layer were functionally organized like those of ganglion cells. Cells at both levels seem to be similarly controlled by a combination of excitatory and inhibitory influences.

Spontaneous impulse activity, defined as activity in the absence of light stimulation, has been well demonstrated in ganglion cells of the mammalian retina (Kuffler, 1953; Granit, 1955, p. 84). Impulse activity in complete darkness seems not to be unique to ganglion cells, since it was also noted in this study from cells of the inner nuclear layer. Thus spontaneous activity of ganglion cells is probably due, at least partly, to spontaneous firing at a more distal level. On the basis of present evidence spontaneous activity may even originate in the receptors themselves. Although it seems unlikely, the possibility cannot yet be excluded that spontaneous activity of single cells in the inner nuclear layer was an artifact due to electrode pressure. This type of artifact is readily excluded in the case of ganglion cells by recording from their axons (Kuffler, 1953), but no well identified recordings have yet been made from axons of cells in the inner nuclear layer.

Response patterns of single units which discharge impulses

An examination of single unit responses reveals that inhibitory and excitatory effects of light stimuli have many common characteristics, as follows: (1) adaptation to the stimulus; (2) increased effects with stimulus intensity; (3) area summation within certain limits; (4) increased effects by reducing background illumination or by dark-adapting the eye; (5) analogous effects when the stimulus is moved away from the centre of the receptive field, regardless of whether a centred stimulus produces excitation or inhibition. Also (6) excitatory responses are usually classified as 'on', 'on–off', and 'off', depending upon when the discharge occurs in relation to the stimulus; inhibitory responses also fall into these three categories, and pure inhibitory responses of the first two types have been observed.

Although ganglion cell responses have been classified primarily in terms of excitatory effects, this emphasis may be questioned. Since cells of the ganglionic and inner nuclear layers exhibit maintained discharges, the rate of

firing may be increased or decreased by excitatory or inhibitory influences. Changes of discharge rate in both directions probably contribute to the information transmitted. Thus the question arises of how responses should be classified. The response pattern of a single cell can be varied so greatly by manipulating stimulus intensity, size, and location that a complete classification of responses would become very complex. Perhaps it is more important at this time to learn the mechanisms which make such great variability possible, and the factors which determine the response in a given case.

Although the mechanism is not known whereby 'off' discharges are generated, one hypothesis is that they are due to a release from inhibition (Granit, 1955, p. 72). Certain observations of this study bear upon that hypothesis. The pure inhibitory response shows that release from inhibition does not necessarily produce an 'off' discharge. Of course the possibility remains that when 'off' discharges occur they are due, at least in certain cases, to release from inhibition. In Text-fig. 5 'off' discharges were produced only by intermediate stimulus intensities which produced intermediate amounts of inhibition during the stimulus. If these 'off' discharges were due to a release from inhibition, the results suggest that there is an optimal amount of inhibition during the stimulus for production of a maximal 'off' discharge, with no such discharge occurring at the extremes when the inhibition is too weak or too strong. It seems very likely, however, that at least some 'off' responses are not due to release from inhibition. Since cells of the inner nuclear layer give 'off' discharges, such discharges over excitatory pathways would produce 'off' responses in ganglion cells. Further information on this problem may be expected from intracellular recording.

Units responding to light only with slow potentials

Intracellular recordings from single units which respond to light only with slow potentials have been described previously in fish and cat retinas (Svaetichin, 1953, 1956; Grüsser, 1957; Motokawa *et al.* 1957; Tomita, 1957; Tomita, Tosaka, Watanabe & Sato, 1958; MacNichol & Svaetichin, 1958; Oikawa, Ogawa & Motokawa, 1959). In this study the intracellular response to white light was always an increase of membrane potential, and the response was graded with stimulus intensity. These findings, as well as the general form of the response and the absence of impulse activity, agree with previous results from fish and cat retinas. By comparison with fish, intracellular recordings from these units in the cat were rarely obtained and more difficult to keep stable; thus the units which produce these responses are probably smaller in the cat. The polarity of the response from certain cells in fish has been shown to vary with stimulus wave-length (Svaetichin, 1956; Motokawa *et al.* 1957; MacNichol & Svaetichin, 1958). Similar work has not yet been done in the cat because of difficulty in obtaining stable intracellular recordings. Responses

like those of Text-fig. 9, which break with short stimuli into distinct fast and slow components, have not been reported in fish, but certain responses recorded by Svaetichin (1953) appear somewhat comparable (see especially his Fig. 16*c*).

In fish retinas the units giving only slow potentials were first identified with the myoid portion of the cone inner segment, and responses from these units were called 'cone action potentials' (Svaetichin, 1953). MacNichol & Svaetichin (1958) showed later by electrophoretic staining that these units are located at two different retinal levels. One type of unit responded with an increased membrane potential to light of all wave-lengths, and this was called the 'luminosity response'. Such responses were tentatively identified with the very large horizontal cells of the fish retina. Other units yielded responses which changed polarity with stimulus wave-length; these units were located slightly more proximally and were tentatively attributed to bipolar cell somata. Oikawa *et al.* (1959) also conclude from electrophoretic staining in fish that the single units which are readily recorded by relatively large micropipettes are probably the large horizontal cells.

In the cat retina Motokawa *et al.* (1957) and Grüsser (1957) have recorded from units which yield only slow potentials. These responses have been called 'receptor potentials', referring to Svaetichin's early work on fish. Physiological methods of location in this study show that these responses are obtained slightly beyond the outer margin of the inner nuclear layer, in the region of the external plexiform layer. This location agrees closely with that of the 'luminosity responses' in fish retinas by electrophoretic staining. The present authors first considered somata of receptor cells the most likely sources of these potentials. In the peripheral cat retina, however, the outer nuclear layer is 60 μ thick; this layer is about 15 μ from the inner nuclear layer and 35 μ from Bruch's membrane. If the single units yielding only slow potentials were receptor cell somata, they should be found at all levels of the outer nuclear layer. Actually no units have been found far enough beyond the inner nuclear layer, or close enough to Bruch's membrane, to be in the outer portion of the outer nuclear layer. Thus these potentials are probably not recorded from the somata of receptor cells, but there are at least two other possibilities: (1) receptor terminals, namely, rod spherules and/or cone pedicles; (2) horizontal cells. Physiological methods of electrode location cannot distinguish between these possibilities, and single-unit-marking methods seem required.

SUMMARY

1. Techniques are described for intraretinal recording with micropipette electrodes in the intact cat eye. Physiological criteria are used for locating the electrode tip within the retina. Major landmarks which can be identified

on each penetration include the retinal surface, outer margin of the inner nuclear layer, and Bruch's membrane. Other structures identified on certain penetrations include the external limiting membrane and cell somata within the ganglionic and inner nuclear layers.

2. A membrane of high electrical resistance, similar to Brindley's 'R membrane' in the frog, is described in the cat and identified with Bruch's membrane.

3. Receptive fields of all ganglion cells studied in the light-adapted state had either an 'on-centre' and 'off-periphery', or vice versa, and mutual inhibition occurred between the central and peripheral zones. This finding with micropipette electrodes makes it likely that the receptive field organization described by Kuffler applies to ganglion cells of all sizes.

4. Intracellular recordings from ganglion cells showed increased discharge frequencies accompanied by depolarization of the cell membrane, whereas inhibition of impulses was linked with an increased membrane potential. Maintained depolarizations were noted during stimuli as long as 0·8 sec.

5. Single cells were identified within the inner nuclear layer and all these discharged impulses. Receptive fields of these cells in the light-adapted state were functionally organized like those of ganglion cells. Impulse activity was also recorded in the absence of light stimulation.

6. Some responses to light showed inhibition of the maintained discharge, with no burst of impulses at either 'on' or 'off', and hence are called pure inhibitory responses. Such responses were obtained under specified stimulus conditions from certain 'off-centre' units of both the ganglionic and inner nuclear layers. Inhibitory effects increased with stimulus intensity, showed area summation, and also responded to several other stimulus variables in a similar manner as excitatory effects. Adaptation of inhibition was observed, and inhibition sometimes outlasted the stimulus as long as 7 sec.

7. No impulse activity was found deeper than the inner nuclear layer. In a narrow zone of about 30 μ, which seemed to be just beyond the inner nuclear layer, single units were occasionally found which responded to light stimuli only with slow potentials. With intracellular leads the response to white light was always an increase of membrane potential. These potentials were well maintained during the stimulus and graded with stimulus intensity. In certain cases the response to short stimuli showed distinct fast and slow components, with the slow component outlasting the stimulus by 1·5 sec.

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EXPLANATION OF PLATE

Fig. 1. Device for holding the eye, introducing a micropipette electrode into the eye, and controlling the electrode position; see text.

Fig. 2. Photomicrograph of peripheral retina of a normal adult cat. From a histological section prepared and kindly lent by K. L. Chow, Department of Physiology, The University of Chicago. Eye was fixed in Susa's fluid and doubly embedded in celloidin and paraffin. Sections were 10 μ thick, approximately perpendicular to the retinal surface, and stained with haematoxylin and eosin. Part of retina illustrated is the nasal periphery about 1 cm centrally from the border of the tapetum lucidum. Approximate thickness of each retinal layer given in micra at left; total thickness of retina was 168 μ; no shrinkage allowance made, for reasons given in text. On right are shown symbolically the approximate relative amplitudes of pulse recording during a retinal penetration; pulse recordings shown only at retinal levels where reliable changes in pulse amplitudes occurred. At extreme right other striking electrical events are indicated at retinal levels where they were located.

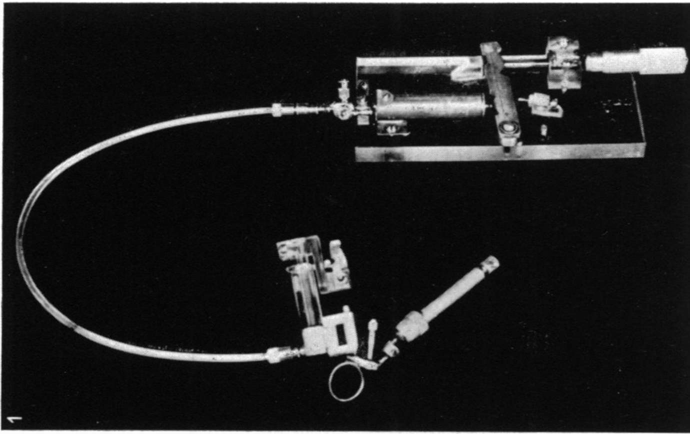

Fig. 1

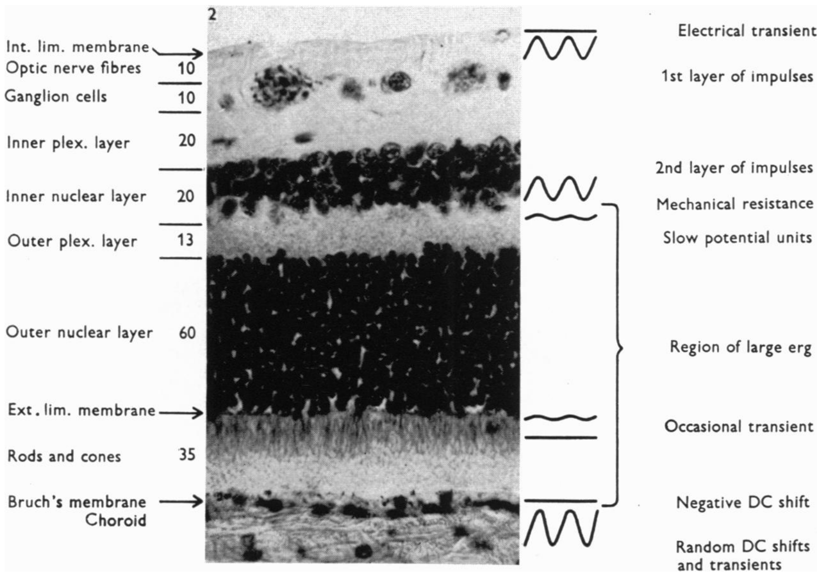

Fig. 2

(*Facing p.* 562)