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CONDUCTION VELOCITIES IN RABBIT'S OPTIC NERVE*

AND SOME OBSERVATIONS ON ANTIDROMIC RETINAL SPIKES

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Interest in the problem of conduction velocities in the optic nerve from other than a purely descriptive point of view arose when Chang and Kaada (1950) in the cat found three spike groups of different latencies to which Chang (1952) appointed a role in color reception. He maintained that they conducted specific sensitivities to red, green, and blue in decreasing order of velocity. Later Chang (1956) also found histologically three main groups of fiber diameter with peak sizes of nine to 10, four to five, and one to two μ , respectively. Conduction velocities were given as 70, 30, and 17 m./sec., respectively. P. O. Bishop, Jeremy, and Lance (1953) found two waves only with maximum velocity of 70 and 23 m./sec., respectively.

It is not our intention to pursue this problem and review the subsequent discussion pro and contra the conclusions of Chang. But two recent separate and distinct lines of ap-

proach have made us feel that Chang's suggestions should not be discarded too hastily. Lennox (1957) found by microelectrode recording within the optic tract in cats that individual spikes with fast conduction velocities arose from elements with greater sensitivity to red than to other colors. Similarly the spikes that had slow conduction velocity were found to be relatively more sensitive to blue. Recently Ingvar (1956) from a vast amount of data from *cerveau isolé* cats has demonstrated prominent narrow bands of modulator type—thus restricted to narrow regions of the spectrum—projected onto the cat's visual cortex.

Preliminary to work on the rabbit's visual pathway it was decided to combine some measurements on conduction velocities with microelectrode recordings from the retina of the opened bulb as done in the cat's eye by Granit (1955a, b) and Dodt (1956a). Stimulation in such work is antidromic, to the upper portion of the optic tract, and the discharge is identified as a wave in the optic nerve or as spikes in the retina. Compared with the cat, the rabbit as a preparation has

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the disadvantage that the optic nerve fibers, instead of suddenly losing their myelin sheath at the blindspot, turn nasally and temporally in two thick bundles. Within these the fibers lose their myelin irregularly forming a feather edge terminating in ganglion cells at unpredictable distances from the blindspot. The nervehead lies quite superiorly and is highly excavated. As one proceeds downward with a microelectrode—as we shall show—almost any latent period may be presented at any distance from it. It is impossible to know in casu by what route the antidromic impulse has arrived from the blindspot to the recording point. All the more important it is therefore to possess some measurements of conduction velocities in the optic nerve as a guide to the retinal findings. The two types of data supplement each other.

G. H. Bishop (1933) measured conduction velocities in the rabbit's optic nerve having made very extensive operations to lay it bare. He found two waves and the first one varied in conduction velocity as much as from 50 to 20 m./sec. There was no definite slow wave comparable to the one he had seen in the bullfrog. Bishop himself did not seem satisfied with this result. Theoretically his technique of setting up the optic nerve as a peripheral nerve should be set up for measurements of conduction velocities seems correct and ideal, but for biologic reasons we have preferred to use the stereotaxic Horsley-Clarke method of stimulation.

PROCEDURE

Rabbits were given urethane or prepared by the *encéphale isolé* method of Bremer (1936). The head was placed in the Horsley-Clarke stereotaxic apparatus and the roof of the skull opened. A drop of tetracaine solution was instilled into the right eye. A cannula was inserted into the femoral vein for later injections of the curarizing substance Flaxedil to prevent eye movements.

Stimulating electrodes were concentric needles inserted into the lateral geniculate

body or upper portion of the optic tract. For measurements of conduction velocities, one silver pin was stuck into the optic nerve through a retrobulbar cut, another inserted in the soft tissue covering the bulbar cavity. On the bulbar side the nerve was crushed. Both electrodes were conducting only at the tips. Retinal spikes were recorded by micropipettes filled with three molar sodium chloride solution and kept at resistances around a few megohms (Fatt, 1957). A circuit for checking electrode resistance from time to time was introduced. Cathode follower, amplifiers, and oscillograph were employed in the customary way.

RESULTS

1. OPTIC NERVE AND TRACT

In Figure 1 record A1 is exceptional in that in this case four entomologic pins, insulated except at the tip, were thrust into the optic nerve at interelectrode distances of 1.5 mm. Stimulation took place between pins 1 and 2, recording between pins 3 and 4. The picture is superimposed DC records at high sweep speed. The shock artefact is seen to end on the rising phase of the response.

Records A2 and 3, at different sweep speeds, show the characteristic shape of the wavelets obtained for full conduction distance (table 1). Nearly always the first big wave is split up into three humps. If these are not seen from the beginning slight adjustment of the stimulating electrodes generally suffices to make them visible. With stronger stimuli, such as used in the instances illustrated, a delayed, flattened wave follows the early better synchronized responses. At the slower sweep speed in A3 the delayed wave is better set off against the baseline. This record was taken at high sensitivity of the amplifier and was one of the many fruitless attempts to find later waves than the ones seen. When such later waves occasionally were noticed they did not appear in superimposed records and hence were due to casual variations. All records A are taken with DC amplification.

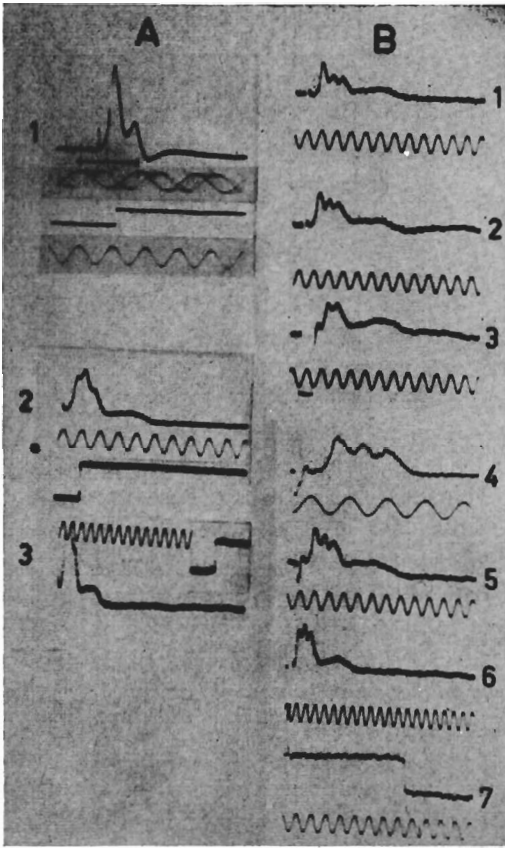


Fig. 1 (Granit and Marg). (A) DC records. (1) Four insulated steel pins with free tips in a row, 1.5 mm. interelectrode distance, stuck into optic nerve between chiasma and foramen opticum. Stimulus between 1 and 2, recording between 3 and 4. (2) Standard leads from retrobulbar end of optic nerve, concentric needle electrode placed by Horsley-Clarke instrument in lateral geniculate body or upper end of optic tract for delivery of single shock initiating sweep. DC calibration to 300 μ V. (3) Same but sweep speed reduced and amplification increased (calibration to 100 μ V in inset) in order to look for later waves. None visible. (B) AC records with standard leads and slow condensers, as shown by calibration to 100 V in 7. Shock shifted inward on sweep. (1) Brief shock. (2 and 3) Stimulating electrode withdrawn 0.5 mm. Longer shock duration in 3 emphasizes late wave. (4 to 6) Another experiment shown on three sweep speeds. This experiment ended with section of optic nerve by a thin scalpel cutting through chiasma. The wavelets disappeared. All times in msec.

The records B were obtained with large condensers, as shown by calibration in B7. B1 differs from B2 and 3 merely by a slight vertical shift of the stimulus electrode. B2

and 3 compare a short with a long stimulus duration. The latter emphasizes the delayed wave. The first wavelet is distorted by the stimulus artefact. Records B4 to 6, from another experiment, show the same response at three different sweep speeds. The fast wavelets have also been seen by Noell (1953).

The delayed wave, as stated, required stronger shocks than the early, well synchronized portion of the response and experiments with double shocks showed it to have a longer refractory period than the others. In one experiment we successfully injected a drop of two-percent procaine solution into the chiasma and found the slow wave to disappear before the other ones. In another experiment a thin scalpel was used to cut the chiasm from above. All waves then disappeared instantaneously. Results of this type have been grouped together in Table 1 for which the conduction distances were measured immediately after the experiment. For this purpose the optic foramen was opened and the brain tissue cautiously aspirated without moving the electrodes so that the optic tract and nerve lay bare for inspection and measurement. Latency was measured to the beginning of each early wavelet and the delayed wave.

Conduction velocity of the fastest spike varied between 49 and 65 m./sec. with an average value of 56 m./sec. The fastest wave is thus slower than in the cat (see above; 70 m./sec.). Very regular was also a wavelet with conduction velocities varying between 21 and 24 m./sec., average 23 m./sec. Equally regular was the delayed wave with a conduction velocity from 9.4 to 11 m./sec., average 10 m./sec. The third fast wavelet mostly appeared at 16 m./sec., but in two cases, when this was absent, the third fast wavelet occurred at respectively 32 and 38 m./sec. All these are maximum values.

The stimulating needle will, of course, sample bundles selectively (Noell, 1953; Chang, 1956) but there can be no doubt about the existence of groups of different conduction velocities in the fast range nor is

TABLE 1

Conduction Distance (mm.)	Velocity (m./sec.)	Velocity (m./sec.)	Velocity (m./sec.)	Velocity (m./sec.)	Velocity (m./sec.)
20	57		23	16	11
31	52	38	23		9.9
32	49		22	15	9.4
32	56	32	21		10
26	65		24	16	10
AVERAGE	56	32-38	23	16	10

the existence of the delayed wave in the least doubtful. It was seen in every case and the variation in conduction velocity was small. Once, a still later wave conducted at 7.6 m./sec. was seen to rise on top of the slow wave. The most regular faster wavelets are apparently represented by bundles of considerable size containing many fibers of approximately the same diameter.

2. RETINAL RECORDS

In spite of the difficulties of determining intraretinal conduction distance - it - was deemed necessary to make an attempt at verifying the existence of spike groups of different conduction velocities also by recording with microelectrodes from retinal ganglions. While in the cat a very precise value can be obtained at the blind spot (Granit, 1955a) and the spikes immediately slow down outside it owing to demyelination (see the later measurements of intraretinal conduction velocities by Dodt, 1956b), it is not feasible in the rabbit to make similar measurements. Firstly, the blind spot does not seem to respond. Secondly, demyelination is irregular.

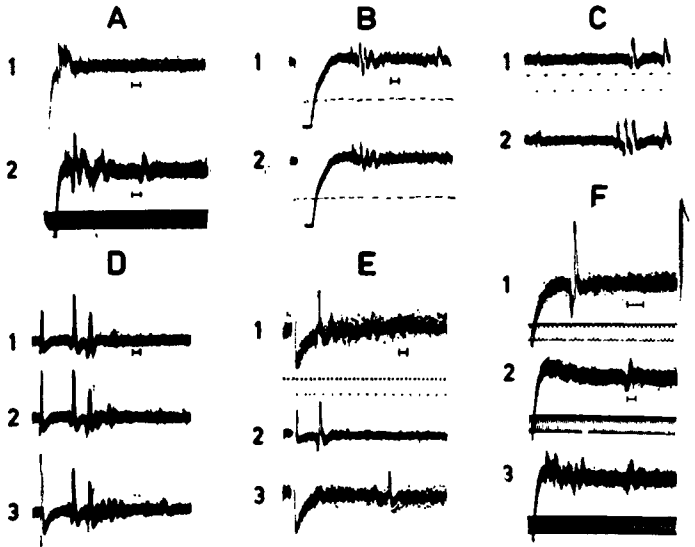
In some measurements we found that the conduction distance from the retrobulbar tip to the blind spot was about 6.0 mm. (the nerve runs for a brief distance alongside the bulb and the tip of the retrobulbar electrode cannot be placed immediately opposite the blindspot). At rate 56 m./sec. and 6.0 mm. between the blindspot and the retrobulbar electrode the earliest spikes should be recordable at the blindspot at a latency of about one-half msec. (table 1). Actually, however, our shortest values have been 1.7 msec. and

the commonest ones around 2.0 msec. Thus the micropipette has not recorded any spike from the blindspot itself.

Samples of microelectrode records are shown in Figure 2. Only records from the two ends, superior or near blindspot, and inferior or as far away from it as practicable (about 8.0 mm.), have been selected for publication because these best illustrate the salient point, namely that distance from the blindspot is less significant than the joint effect of unknown demyelination and similarly unknown circuitous route of individual fibers as they emerge from the feather edge of the nasal and temporal bands of massed fibers. However, nerve spikes, which are of brief duration compared with ganglion spikes, are oftener seen near the blind spot than elsewhere. Samples are shown in A1 and E1. Because of the large gaps between the ganglion cells the thin microcapillaries sometimes pass through the ganglionic layer without recording spikes. If advanced too far, the electrode may break its tip against the sclera. It was therefore found advantageous to keep a continuous record of the DC level as well as of the response caused by a flickering light stimulus. The spikes, elicited by this light, signal the ganglionic layer by on/off-discharges heard in the loudspeaker; the DC control gives a big shift of potential somewhere inside the retina, possibly Brindley's (1956) so-called R-membrane. When this happens, electrode has been advanced too far.

The characteristic picture obtained when no attempt is made to localize individual ganglion cells is shown in A2 and F3. There is an early grouped response, a silent period

Fig. 2 (Granit and Marg). Six experiments—A-F. Microelectrode records from rabbit's retina responding to antidromic stimulation of optic tract. Superimposed sweeps. Time in msec., marked by vertical lines, except in C where time marker shows up despite superposition. (A) (1) Superior, briefest latency 1.7 msec. Strength 6.4 V; (2) inferior, early spike at 2.4, late at 10.6 msec. Strength 20 V. (B) Inferior, strength 6.4 V; (1) early spikes at 2.5, late spike at 16 msec.; (2) same after withdrawing electrode 30 μ upward. (C) Superior (1) latency 5.5 msec., strength 1.6 V; (2) latency 4.6 msec., strength 2 V. (D) Inferior (1) latency 3.6 msec., strength 0.26 V; (2) same, 0.4 V; (3) same at 1.2 V, latency of brief, earliest deflexion 3.1 msec. (E) Superior (1) at threshold 0.4 V (note fast nerve spike), latency 2.8 msec., high gain; (2) slight movement to isolate cell response, decrease of gain and increase of strength to 2 V; (3) adjacent place, strength 2 V, late spike at 11.0 msec. latency. (F) Inferior (1) latency 2.7 msec., strength 3.2 V; (2) slight lateral shift of microelectrode, latency 11.0 msec., strength 16 V; (3) increased gain, strength 5 V. Big spike in F1 is 380 μ V, small spike in F2 140 μ V. B, C and D are *encéphale isolé* preparations.



and a delayed spike which always is small. Series D is taken at increasing stimulus strength to show that the delayed small spike as a rule requires stronger shocks. The microelectrode is located between two fairly well isolated cells. With stronger stimuli more distant cells give responses filling out the gaps between the better isolated ones. The latency of the delayed wave is 13.6 msec.

Such findings raise the question of whether or not the delayed response is due to repetitive firing. Two lines of evidence show that it really is an individual spike responding once. In B1 it is present, in B2 absent. These records merely differ by a shift upward of 30 μ on the vertical scale of the micromanipulator. The small delayed spike

was seen at the greater depth. In the records F a large early spike was first isolated. Then the delayed spike was found by a small lateral shift of the microelectrode. (Note change of time base.) By moving the stimulating electrode and adjusting strength of the shock it proved possible to obtain the delayed wave in perfect isolation (F2). Another similar experiment is illustrated in series E. In this case, exceptionally, the small delayed spike was found in the superior portion of the retina (E3). More often the small late spikes are located to the middle or inferior part of the eye. Thus the small delayed spike is an individual late response from a small cell activated by high threshold fibers and not a repetitive dis-

charge of cells at some distance from the electrode.

Finally records C show with large spikes that near the blind spot latencies as long as 4.6 and 5.5 msec. can be obtained. The latencies in the records D (inferior) were by comparison 3.1 to 3.6 msec. for the first spike, varying with stimulus strength. The early big spike occurs at constant latency (3.6 msec.) but with the strong stimulus it is preceded by a small—apparently distant—spike of latent period 3.1 msec.

Ganglion cells in the rabbit retina have been studied by Noell (1953). In his pictures there is a range of variation in diameter of 1:3. Some of our Bodian stains, made for other purposes, are shown in Figure 3. The range of variation in a count of some 350

cells was from 4.0 to 12 μ with a broad peak around 7.0 to 8.0 μ . Great accuracy cannot be claimed for these figures as the nuclei could not be seen in the thick Bodian sections and the amount of shrinkage is unknown. It was also noted that ganglion cells occurred at various retinal depths (Polyak, 1941). It is, of course, not possible in physiologic experiments to deduce the actual depth of the cell from readings on the micromanipulator, the distortion by pressure being unknown. The histologic observations were made merely in order to ascertain that large variations in the size of the ganglion cells actually do occur.

In view of the fact that Dodt (1956a) has found delayed small spikes in the rabbit's retina which he by various criteria held to be centrifugal we occasionally tried two of those

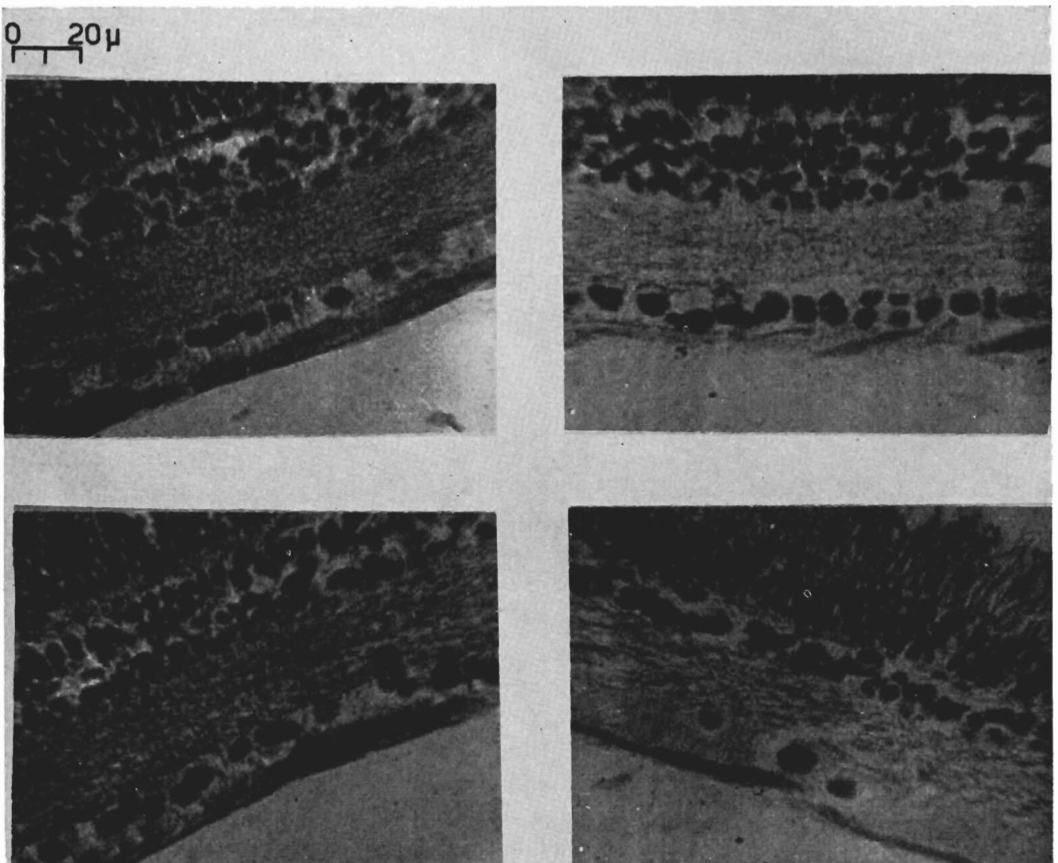


Fig. 3 (Grant and Marg). Four microphotographs from different parts of rabbit's retina. Bodian stains 15 μ thick. Layer of ganglion cells downward in all pictures. ($\times 500$.)

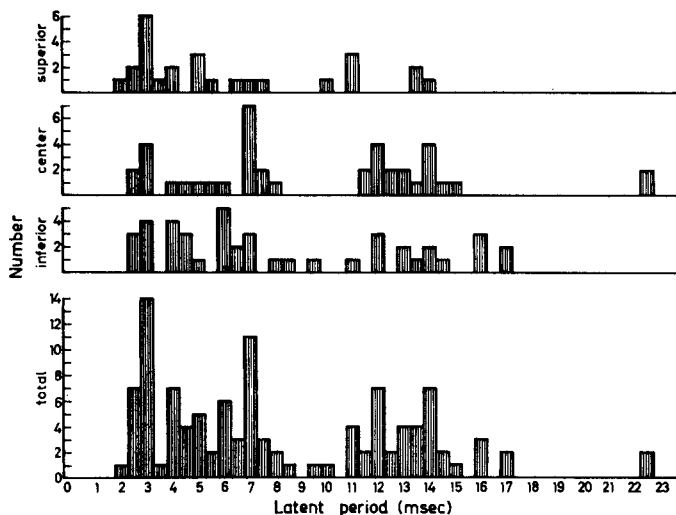


Fig. 4 (Granit and Marg). Histogram of the latency distribution of antidromic retinal spike groups from stimulation of the lateral geniculate body in the rabbit. Measurement was made to the beginning of the initial negative wave in each group of retinal spikes. Single repeatable spikes were measured when they were separate and distinct even though they were not part of a group. Hence this histogram tends to minimize the peaks of the distribution, yet it clearly shows a polymodal character. Latencies measured from the superior, central, and inferior retina indicate an increasing latency which is probably because of the longer, slow neural path as one goes down the retina from the optic nervehead. However the effect is not clear cut in the rabbit because the nerves are irregularly myelinated for some millimeters across the fundus.

criteria, light adaptation and stimulation at faster frequencies, to find out if the delayed spikes then disappeared as did Dodt's centrifugal spike. Every small and late spike was not studied in this manner but we proved to our satisfaction that some, at least, neither were influenced by light adaptation nor by increasing rate of stimulus repetition up to frequencies considerably beyond the values found by Dodt to block his centrifugal spikes. As a whole, then, our evidence goes to show that most of the delayed small spikes are due to ordinary centripetal fibers of high threshold being stimulated antidromically. These fibers are therefore likely to take their origin in the small ganglion cells of the retina.

Our results are displayed in the histogram of Figure 4, fully explained in its legend. The distribution is clearly polymodal and the early spikes, too, show preferential distributions. In the group of delayed spikes there may well be some belonging to Dodt's category.

COMMENT

Our results clearly show that impulses in the rabbit's optic tract and nerve travel in fibers of characteristically grouped conduction velocities, thus introducing a time factor in the excitation of higher stations. Such findings necessarily raise questions of functional differentiation, both with regard to entrance and end stations. We have already mentioned as one possibility the recent work on color discrimination in terms of conduction velocities. Functional differentiation may, however, concern any other aspect of the complex visual message. Particularly interesting is the slowly conducting system from small ganglion cells, likely to possess narrow dendritic fields. These are likely to belong to cones (Ramon y Cajal, 1894; Polyak, 1941). Noell (1953) also points out that the predominance of small ganglion cells in the central areas of most species in combination with the fact that most optic nerve fibers are small is contradictory to a generalization of

Bishop and O'Leary (1938) according to which the small fibers only run to the superior colliculi. Experimental evidence against this view has been presented by Granit (1955b) and Chang (1956). The rabbit has some peripheral discrimination of wavelength; Dodt and Elenius (1956), and has 265,000 optic nerve fibers as against 119,000 in the cat (Bruesch and Arey, 1942). However, having no evidence ourselves to contribute to the question of the physiologic significance of the different groups of conduction velocities, we do not think further speculation justified.

SUMMARY

By the Horsley-Clarke stereotaxic technique stimuli have been applied to the rabbit's optic tract and the response to each shock recorded (1) at the crushed retrobulbar region of the optic nerve and (2) from retinal ganglion cells by NaCl-filled capillaries with tips of the order of 2.0 to 5.0 μ .

1. The optic path (tract and nerve) con-

ducts distinct wavelets of grouped action potentials at maximum velocity of 56, 35, 23, 16, and 10 m./sec., respectively. Sometimes either of the wavelets, conducted at 35 and 16 m./sec., respectively, are absent. The other components are always found and must therefore represent large relatively homogeneous bundles of fibers of approximately the same diameter.

2. The spikes from the retinal ganglion cells show a polymodal distribution of latent period. Very characteristic is a delayed, small spike (11 to 15 msec. latency) which is assumed to belong to the fiber group conducting with maximum velocity of 10 m./sec. It has, as a rule, a higher electric threshold than more rapidly conducted spikes. Small retinal ganglion cells have been found from which thin, slowly conducting fibers would be likely to arise.

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REFERENCES

- Bishop, G. H.: Fiber groups in the optic nerve. *Am. J. Physiol.*, **106**:460-474, 1933.
- Bishop, G. H., and O'Leary, J.: Potential records from the optic cortex of the cat. *J. Neurophysiol.*, **1**:391-404, 1938.
- Bishop, P. O., Jeremy, D., and Lance, J. W.: The optic nerve. Properties of a central tract. *J. Physiol.*, **121**:415-432, 1953.
- Bremer, F.: Nouvelles recherches sur le mécanisme du sommeil. *Compt. Rend. Soc. Biol.*, **122**:460-463, 1936.
- Brindley, G. S.: The passive electrical properties of the frog's retina, choroid, and sclera for radial fields and currents. *J. Physiol.*, **134**:339-352, 1956.
- Bruesch, S. R., and Arey, L. B.: The number of myelinated and unmyelinated fibers in the optic nerve of vertebrates. *J. comp. neurol.*, **77**:631-665, 1942.
- Chang, H. T.: Functional organization of central visual pathways. *Proc. A. Research Nerv. Ment. Dis.*, **30**:430-453, 1952.
- : Fiber groups in primary optic pathways of cat. *J. Neurophysiol.*, **19**:224-231, 1956.
- Chang, H. T., and Kaada, B.: An analysis of primary response of visual cortex to optic nerve stimulation in cats. *J. Neurophysiol.*, **13**:305-318, 1950.
- Dodt, E.: Centrifugal impulses in rabbit's retina. *J. Neurophysiol.*, **19**:301-307, 1956a.
- : Geschwindigkeit der Nervenleitung innerhalb der Netzhaut. *Experientia*, **12**:34, 1956b.
- Dodt, E., and Elenius, V.: Spektrale Sensitivität einzelner Elemente der Kaninchennetzhaut. *Pflüg. Arch. ges. Physiol.*, **262**:301-306, 1956.
- Fatt, P.: Electro potentials occurring around a neurone during its antidromic activation. *J. Neurophysiol.*, **20**:27-60, 1957.
- Granit, R.: Receptors and Sensory Perception: A discussion of aims, means, and results of electrophysiological research into the process of reception. New Haven, Yale Univ. Press, 1955a, pp. vii + 369.
- : Centrifugal and antidromic effects on ganglion cells of retina. *J. Neurophysiol.*, **18**:388-411, 1955b.
- Ingvar, D. H.: Comparison of spectral sensitivity curves from the retina and central visual pathways in the cat. *XX Internat. Physiol. Congr.*, Brussels, 1956, pp. 459-460.
- Lennox, M. A.: Responses in single optic tract fibers of cat to monochromatic light: Correlation with conduction velocity. *Acta physiol. scand.*, **42** (suppl. 145):94-95, 1957.

- Noell, W. K.: Studies on the electrophysiology and the metabolism of the retina. USAF School of Aviation Medicine, Project No. 21-1201-0004, report no. 1, 1953, p. xi + 122.
- Polyak, S.: The Retina. Chicago, Univ. Chicago Press, 1941, p. 607.
- Ramon y Cajal, S.: Die Retina der Wirbeltiere. Wiesbaden, Bergmann, 1894, p. 168.