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THE VISUAL CELLS OF LAMPREYS*

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Introduction

The question of the nature of the visual cells of these most primitive of living vertebrates has been controversial for decades. It is especially in need of a final answer because of its bearing upon the problem of the priority of origin of the rod and cone. The accepted theory in this latter connection, holding that the rod is the primitive visual cell and that the cone is a more complex derivative thereof, was put forward by Max Schultze (1866, 1867) and has served as the basis of Mrs. Ladd-Franklin's "genetic" theory of colour-vision and of Parsons' (1927) analysis of the visual sense into "dyscritic" and "epicritic" components.

Examined critically, Schultze's evidence is seen to be no evidence at all; for he mistakenly believed that not only the lampreys and elasmobranchs, but also the ganoids and primitive teleosts[1] have pure-rod retinae. Again, it is scarcely safe to assume with Schultze that the rod is physiologically the simpler element (and "therefore" the older), in spite of the apparent complexity of colour-vision; for not

* A contribution from the Departments of Zoology of the University of Michigan and the State University of Iowa.

[1] Here Schultze was following Haeckel's belief in the eels as the most primitive teleosts; but the eel has enormous cones (Garten 1907).

only may rod vision be quite as complex, but colour-vision may well have been invented long after the advent of the cone as a distinct, high-threshold, isolated-conduction element.[2]

Heinrich Müller (1856) first noted that in the European River Lamprey, *Lampetra fluviatilis,* the visual cells are of two types, long and short, in equal numbers (see Fig. 1). He termed them both cones, but later examined *Petromyzon marinus* in which he found the short elements predominating, and in a footnote to a paper on quite another subject (1862) he suggested that the short elements might be rods. Schultze (1866) first tentatively, later (1867) with more assurance pronounced the *L. fluviatilis* retina pure-rod. W. Krause (1868) insisted that both rods and cones were present, but failed to make clear which was which. Schultze now (1871*a*) about-faced, and termed *L. fluviatilis* pure-cone; but in the same year (1871*b*) once more returned to the pure-rod concept in a contribution to a reference work, practically the last of his many writings. Langerhans (1873, 1876) described the European Brook Lamprey, *L. planeri,* as having rods and cones, figuring the rod as the long cell and giving it a cylindrical outer segment.

Wilhelm Müller (1874) now gave descriptions of *L. fluviatilis, L. planeri,* and *P. marinus,* with figures of the two latter species. His drawing of "*P. marinus*" was certainly based upon an exceptionally large *L. fluviatilis,* however, for the two cell-types are shown in equal numbers as they are in *Lampetra.* Müller saw only conical outer segments and compared the long-cells with the rods of higher forms.

In Krause's later papers (1872, 1876*a,* 1876*b*) the duplex condition is again affirmed, and he definitely sides with Langerhans in identifying the long-cell as the rod.

Kühne (1878*a,* 1878*b*) reported rhodopsin in *L. fluviatilis,* and considered the small amount he found indicative either of a low concentration in all cells or of the presence of many rhodopsin-free elements (cones). From this point on it would seem that none could deny the presence of rods, but Kohl (1892*a,* 1892*b*), who made a maximum of errors in his histological observations, found only cones in *L. planeri,* and thought that the Müller fibres had been mistaken for rods by others. Only Kohl has claimed to find "vacuoles" (oil-droplets) in lamprey visual cells—which, if true, would, of course, be the best of evidence for considering them cones.

Greeff (1900) was impressed with the ambiguity of the genus *Lampetra,* but followed Langerhans and Krause in his identifications. His drawing, though labelled *L. planeri,* must have been made from a small *L. fluviatilis,* as it shows the single outer nuclear layer of the latter species. Pütter (1912) added nothing original, but did call attention to the obscure and neglected mention of *P. marinus* by H. Müller, whose identifications, it will be recalled, were the inverse of those of later workers. It is quite clear that W. Müller's erroneous figure of "*P. marinus*" has kept all subsequent workers from discovering that in this form the situation is not so ambiguous as in *Lampetra,* for they would naturally be inclined to accept W. Müller's drawing over H. Müller's statement that the short-cells were much the more numerous. Mozejko (1912, 1913) made use of Kohl's pure-cone concept in arguing for the "primitiveness" of the lamprey eye.

Tretjakoff (1916) made a very great advance by applying neurological methods to *L. fluviatilis,* and while he identified the long-cell as a rod and the short one as a cone purely upon the basis of size, he found the long-cell to have a dendritic foot-piece and that of the short-cell to be a smooth knob, which by analogy with other cases of such differentiation would tend rather to support Heinrich Müller.

R. Krause (1923) followed Langerhans, W. Krause, and Tretjakoff in his designations, but claimed that in *L. fluviatilis* the short-cells out-number the long to a "not inappreciable" extent. Plate (1924) now pronounced the elements "undifferentiated"—neither rods nor cones—and was followed by Dücker (1924), whose material he supplied.

The most recent writer on the subject, Franz (1932), has put forward a new form of Schultze's pure-rod idea (foreshadowed by the views of Vogt and Yung, 1894), for he considers that in *L. fluviatilis* there is actually but a single type of element present, the bacillary layer being passively pseudo-stratified because of the bulky

[2] Greef (1900) has advanced the only other bit of evidence for rod-primitiveness, in his suggestion that the dendritic foot-piece of the cone is an advance over the smooth, compact rod end-knob. But in view of the ependymal origin of the visual cells (Walls, 1934*b*) the dendritic terminus is seen to be the more primitive one, the compact rod ending being adaptive to the multiple connection of rods to a bipolar neuron.

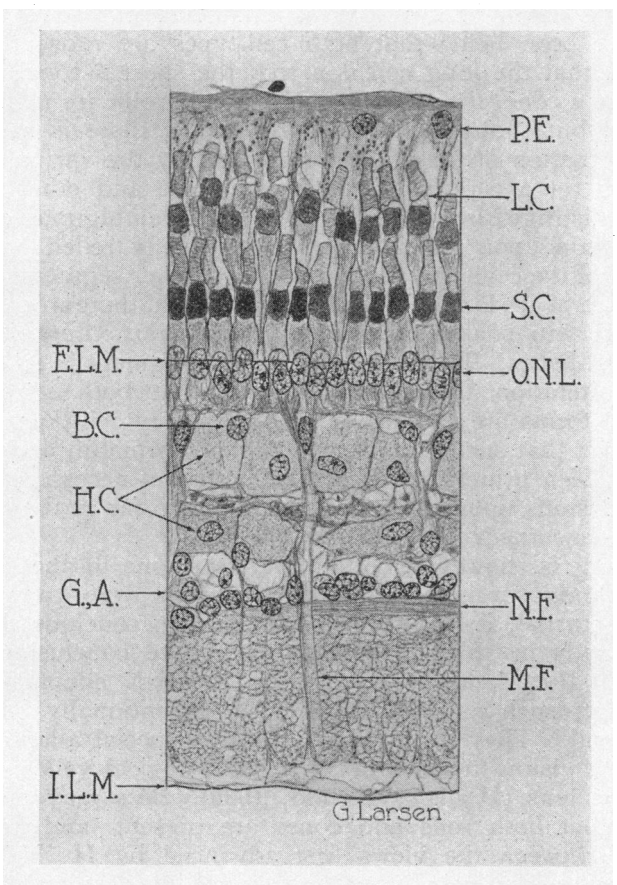


FIG. 1.

Retina of European River Lamprey, *Lampetra fluviatilis*. Kolmer's fluid; × 600.

P.E —pigment epithelium; L.C.—long visual cells (cones); S.C.—short visual cells (rods); E.L.M.—external limiting membrane; O.N.L.—outer nuclear layer; B.C.—bipolar cell; H.C.—horizontal cells; G.A.—ganglion and amacrine cells; N.F.—bundle of optic nerve fibres; M.F.—Müller fibre; I.L.M.—internal limiting membrane.

ellipsoids which interfere with close congregation.[3] He bases his case upon the cylindrical form of all outer segments as seen in his formalin and Bouin fixations—both most unsuitable for visual cells—upon the presence of myeloidal spirals in all outer segments, a rod-characteristic in his present opinion, though he himself admits that they have often been seen in cones (*cf.*. Franz, 1913); and upon an alleged nocturnality of lampreys, of which more later.

[3] Franz was, of course, unaware that there are species in which one type of cell so greatly outnumbers the other as to render this view untenable.

From the above brief review it will be seen that all possible views have been held; that both cell-types are rods; that both are cones; that the long cell is a rod, the short a cone; that the long cell is a cone, the short a rod; that the cells are neither rods nor cones, but "undifferentiated." Clearly, there is need for a close examination of the criteria employed by the various investigators and for a careful evaluation of these and other possible means of distinguishing rods from cones in ambiguous cases.

The criteria upon which others have chiefly relied have been the length of the cells and the form of their outer segments. While rods in general are longer than cones in general there are exceptions such as certain snakes (*Dasypeltis, Tarbophis, Leptodira*) and most teleost fishes. The form of the outer segment is so prone to artificial alteration that the mere fact that both conical and cylindrical forms are claimed for each lamprey cell-type, coupled with the fact that such fixatives as alcohol, formalin, and Bouin's fluid have been principally employed, makes it necessary to withhold conclusions upon this important point so far as the literature to date is concerned.

One thing is, however, certain: at least one of the cells is a rod, for rhodopsin is present. If lampreys were known to be strictly nocturnal, it would be almost safe to conclude that both cells are rods in spite of Kühne's tentative conclusion to the contrary. But lampreys have no special adaptations for nocturnality, such as a tapetum or an exceptionally large lens or/and pupil. They do have at least one special adaptation for bright-light vision in the form of a physiological yellow colouration of the lens (Walls and Judd, 1933). We may tentatively conclude that both rods and cones are present, and have then to decide between the views first advanced by H. Müller and Langerhans respectively.

In re-examining the species of the genus *Lampetra* and in surveying other genera as yet untouched or but superficially studied by others, we may look for the following features with some hope of finding a meaningful differentiation of the two types of cells.

Form and size of the outer segment.—Herein lies the most characteristic visible difference between rods and cones. In a given retina, the rod ordinarily has the more massive outer segment and this is almost invariably perfectly cylindrical. The cone outer segment is smaller in sympathy with its higher threshold, and thus is conical unless exceptionally slender (see Rochon-Duvigneaud, 1917; Walls, 1934*a*). There are cases, however, where the rod and cone outer segments are about alike in size and shape, *e.g.*, certain urodeles, where they are bluntly conical, and the Macaque, where they are cylindrical and of equal

length (Garten, 1907). The absence of a differentiation is of no fundamental significance, however.

Presence or absence of rhodopsin in certain cells.—Visual cells which contain rhodopsin when dark-adapted are unquestionably rods, and very few cases are known of functional rods lacking this sensitizing pigment—such rods are among those which have originated secondarily from cones (certain nocturnal snakes, the night lizards, *Sphenodon,* etc.; Walls, 1934*a*). Cones never contain macroscopic amounts of rhodopsin and probably do not contain even a trace.

Relative extent of summation of the cell-types in bipolar and ganglion cells.—This criterion has been used by Woollard (1927) and by the writer (Walls, 1934*a*) as an index of sensitivity; for rods are always synapsed in multiple to bipolars while cones have more isolated conduction to the brain. If Cell "A" out-numbers Cell "B" more greatly in species "X" than in species "Y," and species "X" has much the higher visual cell—ganglion cell ratio, then Cell "A" is the rod and Cell "B" the cone.

Relative numbers of the cell-types in relation to pelagic vs *benthic, and diurnal* vs *nocturnal habits.*—This is a direct corollary of the Duplicity Theory: if Cell "A" out-numbers Cell "B" more greatly in species "X" than in species "Y," and species "X" is the more strongly benthic or/and nocturnal, then Cell "A" is the rod and Cell "B" the cone.

Relative numbers of the cell-types in the fundus as compared with the periphery.—In duplex retinae, cones are always concentrated in the fundus and more sparsely set in the periphery. If Cell "A" out-numbers Cell "B" much more greatly in the periphery than in the fundus, then Cell "A" is the rod and Cell "B" the cone.

Direction of migration in light and darkness.—This criterion was made use of by Laurens and Detwiler (1921) on the alligator, and depends upon the rule that if the visual cells of a given retina migrate at all, they move in opposite directions. If Cell "A" elongates in light and contracts in darkness while Cell "B" elongates in darkness and contracts in light, then Cell "A" is the rod and Cell "B" the cone.

Differentiation of the nuclei.—Cone nuclei tend to be larger, more ovoid, and nearer the limitans; rod nuclei smaller, more spherical, and in contact with the limitans only between cone nuclei. Cone nuclei tend to have small chromatin granules and linin; rod nuclei, large masses of chromatin and no linin (Menner, 1929). In amphibians, however, the nuclei are all of the "cone" type as here described, and the rod myoids being much the heavier (a unique situation) the result is that the *rod* nuclei are the nearer to the limitans.

Differentiation of the foot-pieces.—Cone foot-pieces are always heavy and dendritic, while rod fibres are often slender and terminate in a smooth knob. This difference holds only for forms whose rods very greatly out-number their cones. In amphibians, all foot-pieces are of the "cone" type.

We may now proceed to apply each of these criteria in turn to the eight species of lampreys studied by the writer: *Icthyomyzon concolor, I. unicolor, Petromyzon marinus unicolor, Entosphenus tridentatus, E. appendix, Lampetra fluviatilis, L. planeri, and L. lamottenii.*

I wish, first, to express my gratitude to the many persons who, with advice and labour, furthered the solution of the problem discussed in this paper. The accumulation of material of several of the species, preserved by the special methods necessary for the study of the retina, was made possible only by enlisting the co-operation of a number of conveniently located biologists. My thanks are due especially to Dr. C. U. Ariens Kappers of Amsterdam, Mr. Leonard P. Schultz of the University of Washington, Dr. David H. Thompson of the Illinois State Natural History Survey, and Dr. Albert M. Reese of the University of West Virginia. Facilities and supplies were kindly furnished by the Department of Zoology of the University of Michigan and the Faculty Research Fund of that institution.

Special mention must be made of the valuable advice and assistance received from Dr. Carl L. Hubbs of the Museum of Zoology of the University of Michigan and Dr. Simon H. Gage of Cornell University. The latter placed his Ithaca laboratory at the disposal of the writer during the breeding season of *P. m. unicolor* in two successive years.

The research was supervised by Dr. John F. Shepard of the Department of Psychology of the University of Michigan, and the writer is heavily indebted to him for excellent advice and keen criticism. He is also grateful to Miss Gladys Larsen for her careful work on the drawings.

Observations

Form and size of the outer segment.—In *I. concolor* (Wittmaack's fluid) and *I. unicolor* (Birch-Hirschfeld's modification of Zenker's fluid) the short-cell outer segments are relatively long and are sub-cylindrical, while the long-cell outer segments taper more sharply to a blunt point (Fig. 2). In *P. marinus* (Kolmer's, Birch-Hirschfeld's, Wittmaack's fluids) the short-cell outer segment is almost cylindrical and the long-cell member is very stubby and rounded on the end (Fig. 3). In *E. tridentatus*

(Birch-Hirschfeld) and *E. appendix* (Birch-Hirschfeld, Kolmer) the short-cell outer segment is a long, perfect cylinder, but that of the long-cell is a small cone (Fig. 4; cf. Fig. 2 in Walls, 1928*a*), Myeloidal spirals are especially evident in the short cells of *E. tridentatus*. In *L. fluviatilis* (Birch-Hirschfeld, Kolmer), *L. planeri* (Birch-Hirschfeld) and *L. lamottenii* (Zenker) the short-cell member is cylindrical, the long-cell outer segment smaller and

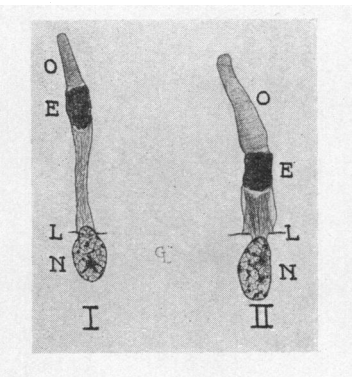


FIG. 2.

Visual cell types of *Icthyomyzon unicolor*. Birch-Hirschfeld's fluid; ×1000. I.—long cell (cone); II.—short cell (rod); O.—outer segment; E.—ellipsoid; L.—limitans; N.—nucleus.

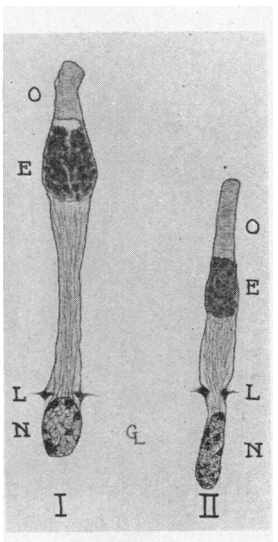


FIG. 3.

Visual cell types of landlocked Atlantic Lamprey, *Petromyzon marinus unicolor*. Wittmaack's fluid; ×1000. I.—long cell (cone); II.—short cell (rod); O.—outer segment; E.—ellipsoid; L.—limitans; N.—nucleus.

bluntly conical (Fig. 5). In the huge elements of *L. fluviatilis*, spiral threads are visible in both cases.

Thus wherever there is a clear-cut structural differentiation in this important feature, it is the short-cell outer segment which is large and cylindrical, whereas the long-cell member is smaller and more conical, though never sharply so as in many vertebrates. A staining differentiation is also usual, the short-cell outer segment taking the Orange G and the long-cell organelle the Aniline Blue in Mallory's triple stain, while in haematoxylin preparations the long-cell structure shows the greater affinity for the dye.

The differentiation of the outer segment is perhaps most sharp in *E. tridentatus*, but in all except the two species of the primitive

genus *Icthyomyzon* the evidence clearly supports Heinrich Müller's view.

Presence or absence of rhodopsin in certain cells.—The presence of rhodopsin in lampreys was confirmed on *P. marinus* by placing the excised retinae of five specimens, which had been in darkness overnight, in a vial of normal saline solution by ruby light. Removed to diffuse daylight and exposed before witnesses, the

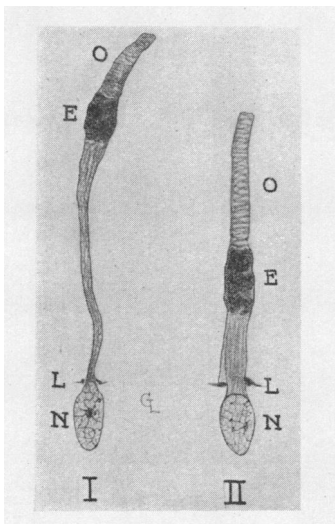


FIG. 4.

Visual cell types of Pacific Lamprey, *Entosphenus tridentatus*. Birch-Hirschfeld's fluid; ×1000. I.—long cell (cone); II.—short cell (rod); O.—outer segment; E.—ellipsoid; L.—limitans; N.—nucleus.

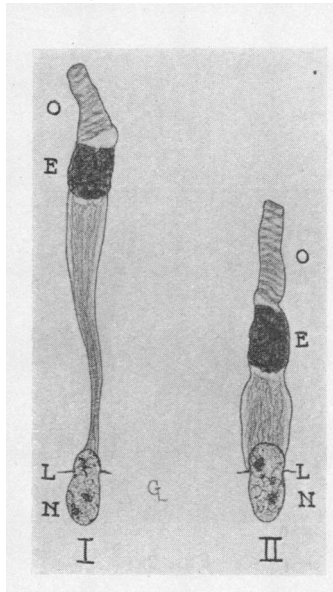


FIG. 5.

Visual cell types of *Lampetra fluviatilis*. Kolmer's fluid; ×1000. I.—long cell (cone); II.—short cell (rod); O.—outer segment; E.—ellipsoid; L.—limitans; N.—nucleus.

mass of retinae was seen to be truly purple (rhodopsin is usually red in colour), the colour fading in two seconds when placed in the light from a south window.

Attempts to determine which type of cell contained the rhodopsin, by the use of Stern's (1905) platinic chloride technique, were all failures. The method was tried on *I. concolor, I. unicolor, P. marinus,* and *E. appendix.* In all of these a yellow colouration was seen in sections of *both* dark- and light-adapted retinae, the colour not restricted to the outer segments, but being deepest in the ellipsoids. A proper Stern's test should show the outer

segments of only the rhodopsin-bearing type of cell stained yellow, in only dark-adapted material. The test is for some reason inapplicable to lamprey tissue or lamprey rhodopsin.[4]

At the suggestion of Dr. Selig Hecht an attempt was then made on *P. marinus* material to see directly, under the microscope, which cell-type contained rhodopsin. A Wratten "70" red filter, which passes only wave-lengths which do not bleach rhodopsin, was fitted to a substage lamp which was then used to focus upon a fresh dark adapted retina. The filter was then removed and the focus quickly improved, but no colouration of either type of cell was visible under the magnification needed to distinguish them.

Although direct methods failed, the writer was led to believe that it is the short-cell which contains the pigment. In *P. marinus* the amount of purple colour seen appeared far too much to have been located in the very small outer segments of the relatively scanty long-cells.

Relative extent of summation of the cell-types in bipolar and ganglion cells.—The fact that relatively few bipolar cells are present in lampreys was first noted by Greeff. Considerable summation must then occur, indicating that functional rods are surely present. Tretjakoff was unfortunately unable to determine, in neurological preparations, which type of visual cell is connected in multiple to the greater extent, so that the indirect method resorted to by Woollard on Primates and by Walls (1934*a*) on reptiles must also be employed here.

The discrimination of bipolar nuclei being difficult, and the certain identification of ganglion-cell and amacrine nuclei impossible in the writer's preparations, the extent of summation was roughly determined by comparing the number of visual-cell nuclei with the total of all other nuclei in the retina exclusive of the easily identified horizontal-cell and Müller-fibre nuclei, which must be excluded as they belong to non-conductive elements. The procedure, deemed adequate for the purpose—an exact determination being impossible and unnecessary—was to estimate the number of rows which would be formed by the projective nuclei if they could be rearranged into definite, compact rows (*cf.* Fig. 1) and then to compare this number with the number of rows in the outer nuclear layer.

One might suppose that this procedure would be vitiated unless the amacrine cells were somehow identified and excluded; but these elements are present in greatest numbers in those retinae in which summation is at a minimum and cone-to-rod ratios (and visual

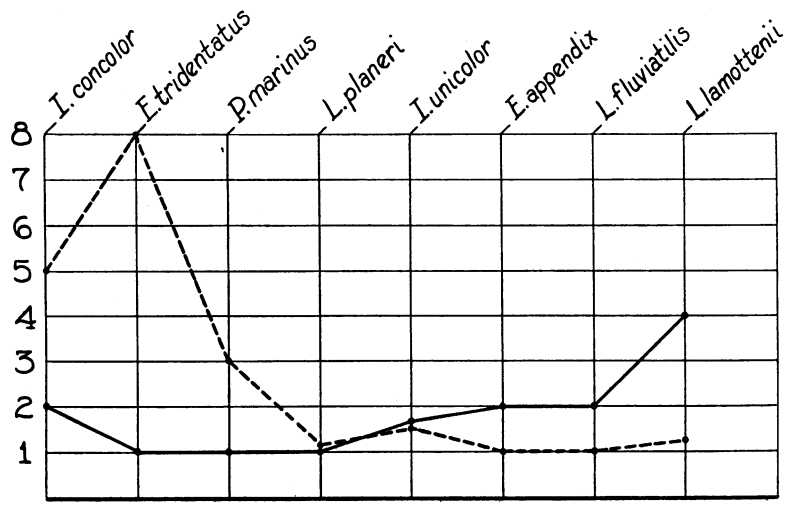
[4] Dr. Katherine Tansley (personal communication) states: "Stern's method gives a positive result only when there is a comparatively large amount of rhodopsin present. I have had negative results by this method in Vitamin A deficient rats when the retinae were distinctly, though only faintly, pink to the eye."

acuity) at a maximum (*e.g.*, birds), so that inasmuch as they are relatively abundant when bipolars, etc., are numerous, and scanty when much summation exists, their inclusion in the present determination cannot result in a masking of the true situation. The reason for these "comings and goings" of amacrines along with the straightforward elements of the visual pathway is, of course, wholly mysterious, as is the functional significance of the amacrine element in the first place.

The situation in the various species, determined as above, is as follows:—

	Rows of visual-cell nuclei:	Rows of projective (and amacrine) nuclei:
I. concolor	1	2
I. unicolor	1½	2½
P. marinus	2	2
E. tridentatus	2	2
E. appendix	2	4
L. fluviatilis	1	2
L. planeri	3	3
L. lamottenii	1	4

These ratios, reduced to a basis of one row of visual-cell nuclei, are shown on the accompanying graph along with the relative numbers of short-cells to long-cells (v.i.). It will be seen that



Solid line: no. rows neuron cell-bodies per row visual-cell nuclei.
Broken line: no. short visual cells per long visual cell.

there is a decided tendency for high numbers of short visual cells to go with low numbers of projective cells—that is, the species with the most short-cells have the most summation, and those with the most long-cells have the most isolated conduction.

This can only mean that the long-cell is the cone, the short-cell the rod.

Relative numbers of the cell-types in relation to pelagic vs *benthic, and diurnal* vs *nocturnal habits*.—The relative numbers of the cell-types were determined by counting the cells in a microscope field 290μ in diameter when the bacillary layer lay along the diameter of the field. The ratio in the fundus was found to be as follows for each species:—

I. concolor	...	...	5	short to	1	long.
I. unicolor	...	...	3	,,	2	,,
P. marinus	...	...	3	,,	1	,,
E. tridentatus	...	..	8	,,	1	,,
E. appendix	...	...	1	,,	1	,,
L. fluviatilis	...	...	1	,,	1	,,
L. planeri...	...	...	8	,	7	,,
L. lamottenii	...	...	5	,,	4	,,

Thus, in the species which live in the shallowest water, *L. fluviatilis* and the four brook lampreys *I. unicolor, E. appendix, L. planeri and L. lamottenii,* the ratio of short-cells to long is lowest—1 : 1, or nearly so. *I. concolor,* until the recent invasion by the land-locked marine lamprey, the characteristic lamprey of the Great Lakes, and the two marine forms *P. marinus* and *E. tridentatus* have at least the greatest depths available in which to swim, though it is not known what depths any of these species prefer.[5] In these three species the short-cells greatly predominate, so that if there is indeed a relation of cell numbers to depth of habitat, the short-cell is inevitably indicated as the rod.

Of previous investigators, only Franz (1932) has sought to apply the duplicity theory as a criterion. He states that *L. fluviatilis* becomes more active at nightfall, and that only at this hour does it come forth from places of concealment. Franz does not say whether this observation was made in the laboratory or in the field, on vegetating specimens or on those excited by the breeding season. Gage has recorded in several places that *P. marinus* moves only at night on its way upstream to breeding grounds. This is, however, in the same category with the nocturnal migrational

[5] A.U.S. National Museum specimen designated as the type of the genus *Bathymyzon*, which Creaser and Hubbs (1922) have pronounced a *P. marinus*, was taken at a depth of 547 fathoms. The chorioids of the two marine lampreys are remarkably thick, that of *E. tridentatus* particularly. This is presumably adaptive to maintaining the circulation under the pressure of considerable depths.

flights of otherwise strictly diurnal birds; and photographs have often been made, in daylight, showing *E. tridentatus* leaping riffles on its way to spawn, much as does the salmon. All lampreys carry on their breeding activities in bright light, and no one has ever found them on the nests at night or even early in the morning. Professor T. L. Hankinson has told the writer that the easiest way he found to collect *P. marinus* in Oneida Lake in New York State was to go about for awhile in a white-bottomed motorboat and scoop the lampreys from the bottom of the boat with a dipnet as soon as the boat was stopped. Curiously, the following summer a red-bottomed boat was used and though the lampreys were as numerous as ever they were no longer attracted to the moving boat. Lampreys have given much inconvenience to swimmers during "marathon" swims (in daylight, of course) in Lake Ontario (see also Dymond *et al.*, 1929; Creaser, 1932).

Captive, breeding lampreys may be *photophobic*, as the observations of Reighard and Cummins (1916) and Lubosch (1902) attest, but the undisturbed lamprey is apparently diurnal in its breeding and at least not nocturnal in its feeding activities. Moreover, there is the yellow lens, a positive adaptation to diurnality (known elsewhere only in diurnal snakes, diurnal squirrels, the diurnal tree-shrew *Tupaia* and the diurnal gecko *Lygodactylus*) to account for.

Franz regards the sparse retinal pigment as an indication of nocturnality, but this—to anticipate—has the same significance as the similarly scanty pigmentation in the human; the pigment does not migrate, and there is, therefore, no need for much of it. Franz also makes the surprising statement that the "moderate size of the eye" predisposes to nocturnality, for the eye is "much larger in indubitably diurnal fishes, *e.g.*, *Esox*." Apart from the impropriety of comparing such diverse forms as *Lampetra* and *Esox*, it is common knowledge that *large* eyes are characteristic of nocturnal and crepuscular vertebrates; and this is no better seen anywhere than among the fishes.

There are no direct or indirect reasons for considering the lampreys nocturnal and hence pure-rod. The fairest possible estimate we can make of them at present is to say that they may be indifferent to night and day—for which behaviour only the duplex retina lays a basis. The writer would suggest that non-breeding *E. tridentatus* may well be quite nocturnal and that this species may prove to have a very pale yellow or even colourless lens.

The evidence from the habits of lampreys supports the conclusion that they have both rods and cones, but is too incomplete for particular species of diverse long-to-short-cell ratios to shed light upon the identity of the two cell-types.

Relative numbers of the cell-types in the fundus as compared with the periphery.—Cell counts were made in the periphery as described above for the fundus.[6] In the small eyes of *I. concolor*, *I. unicolor*, and *E. appendix* and in the case also of *L. fluviatilis* no difference was found, the same ratio obtaining throughout the retina. In the remaining four species the situation in the two retinal regions was found to differ as follows:—

	Fundus:	Periphery:
P. marinus ...	3 short to 1 long.	4 short to 1 long.
E. tridentatus ...	8 ,, 1 ,,	15 ,, 1 ,,
L. planeri ...	8 ,, 7 ,,	1 ,, 1 ,,
L. lamottenii ...	5 ,, 4 ,,	4 ,, 3 ,,

It will be seen that there is a concentration of long-cells in the fundus, marked in *P. marinus* and especially so in *E. tridentatus*, insignificant in the degenerate *L. lamottenii;* and that only in *L. planeri* is there a reversed situation, the long-cells being slightly more frequent in the periphery.

Such evidence as there is here—and it is again excellent for the marine species—supports Heinrich Müller's view that the short-cell is the rod, the long-cell the cone.

Direction of migration in light and darkness.—There has been no certain demonstration by previous investigators of a migration of either type of visual cell. Kohl and Franz have thought that photomechanical changes might occur in lampreys, and Tretjakoff describes an extensive migration of the retinal pigment in *L. fluviatilis*[7] accompanied by a 5 per cent. shortening of the short-cell myoid in light. This latter observation, if verified, would support Langerhans and Krause; but Tretjakoff fails to mention duration of exposures, water temperatures, number of animals, number of cells measured, etc.

The writer's material of the various species, light- and dark-adapted by himself or others, received varying treatment with respect to light strength, water temperature, etc., to be mentioned in connection with the respective species.

All material received similar treatment in preparation and study, however; the excised eyes of the larger species and the entire heads of the brook types were embedded by the hot celloidin method (Walls, 1932) and the eyes sectioned at 10μ in their median vertical planes. Mallory's triple stain was found especially valuable for

[6] These ratios as seen in sagittal sections are, of course, not the true ratios which would be seen in tangential views of the mosaic, except in the case of the 1:1 proportion.

[7] On the other hand Gage (1911—unpublished) found no pigment migration in *P. marinus*.

differentiating clearly the parts to be measured, and some sections were mounted unstained to afford a clear view of the retinal pigment. A portion of the fundus was chosen in which the retina showed no distortion and the full length of the ocular-micrometer line was placed at a level representing the average position of many pigment-process tips, ellipsoids, etc. In this way the average length of a 100 or more short- or long-cell myoids, pigment streamers, etc., was quickly obtained as accurately as could have been done by any other method. The following measurements were made on each eye:—

(*a*) The distance from the limitans to the short-cell ellipsoids.

(*b*) The distance from the limitans to the long-cell ellipsoids.

(*c*) The thickness of the mass of retinal pigment.

(*d*) The thickness of the retina. This last was taken to make possible an expression of the extent of migration in per cent. of retinal thickness, so as to average more fairly the individuals of a species; but as will be seen, there was no need to resort to this refinement.

All dark-adapted material was, of course, killed under ruby lights and the material allowed to fix in complete darkness. Light-adapted specimens were killed and fixed in the light. No adapted material of *I. concolor* or of *E. tridentatus* was obtainable. The data for the experimented species follow:—

I. unicolor

Twelve specimens were placed in diffuse daylight for three hours, then in a jar with a white background and substrate, surrounded closely by six 60-watt lamps. After three hours, six animals were killed. The room was darkened for three hours, when the remaining six were beheaded. The water temperature was kept between 18°C. and 22°C.

The long-cell myoids averaged 1·2μ longer, the short-cell myoids 0·35μ longer, in the light group. Here also the pigment band averaged 0·8μ thicker. These figures are of no consequence, and it is obvious that there are no photomechanical changes in this species.

P. marinus

The water temperature was constant at 17°C. in both light and darkness, and the animals stayed overnight at this temperature before adaptations were begun. Light exposures were made with the animals swimming in a white sink, in daylight supplemented by a 300-watt lamp in a mirror reflector.

The six animals in each group were exposed for one hour (one

specimen), two hours (one specimen), and four hours (four specimens). The dark group received a preliminary light-exposure of four hours.

The long-cell myoids averaged 1·47μ longer, the short-cell myoids 0·17μ shorter, in the light group. The pigment bands averaged 1·74μ thicker in the light group, but the retinae of these six animals averaged 11μ thicker. Such small differences were deemed negligible.

E. appendix

In an earlier paper (Walls, 1928*a*), the writer reported the absence of photomechanical changes in this species. Later experiments were made at a constant temperature of 15°C. Light adaptations were made in a white-lined box seven inches square, with a 60-watt lamp in a parabolic reflector pulled down over a four-inch hole in the lid, which could be covered for dark-adaptations. A light-tight air inlet insured sufficient oxygen.

Twelve specimens were placed in light for three hours; six were killed, and the remaining six dark-adapted for three hours. The long-cell myoids averaged 1·42μ longer, the short-cell myoids 1·03μ longer, in light. The pigment band was 1·03μ thicker in the light group.

In a second series, six animals were light-adapted for 24 hours; three were sacrificed and the other three beheaded after 24 hours in darkness. The long-cell myoids were 0·97μ longer, the short-cell myoids 0·5μ shorter, in light. The pigment band was 2·1μ thicker in the dark group. Obviously, there are no migrations in this form.

L. fluviatilis

The water temperature was not recorded. Ten animals were left in a dark-room overnight. They were then flooded with bright artificial light and killed in twos and threes at intervals of one-half, one, two, and three hours. Nine other specimens were left in the dark-room, flooded with light, overnight. The lights were then turned off and fixations made after one-half, one, two, and three hours as before. The purpose of these various lengths of exposure was, of course, to determine the minimum times of the adaptations, if any.

The long-cell myoids averaged 0·88μ shorter, the short-cell myoids 0·68μ longer, in light. The pigment band was 3·64μ wider in the dark group. The insignificance of these differences is emphasized by the fact that they seem to show the short-cell elongating by about the same amount which Tretjakoff claimed it to shorten, while the pigment "migration" is in the wrong direction! There are no photomechanical changes in *L. fluviatilis*.

L. planeri

Water temperature was maintained practically constant at 20°C. Twelve animals were used in two equal groups, the exposures to each situation varying from one to twelve hours; all were given a preliminary 12 hours' exposure to darkness. The adaptations were made in a dark-room, three 100-watt lamps furnishing the light.

The long-cell myoids averaged 0·39μ longer, the short-cell myoids 0·8μ shorter, in the light group. The pigment band averaged 0·59μ thicker in the light-adapted specimens. Here, again, the figures are meaningless.

L. lamottenii

Water temperature was not kept constant. Exposures were made in a dark-room, the animals being in a jar which rested on white paper and was surrounded by four 60-watt lamps at six inches distance. Eight animals were placed in darkness and pairs of them killed each hour for three hours. The remaining pair was illuminated for one hour and sacrificed.

The long-cell myoids averaged 0·7μ longer, the short-cell myoids 0·9μ shorter, in the light animals. The pigment band averaged 5·3μ thicker in the light specimens. Once more, there is no evidence of photomechanical changes.

The total absence, in lampreys, of these phenomena seems surprising in view of the fact that they are more and more conspicuous as one goes down the vertebrate scale toward the teleost fishes, while the pupil reaction (of similar dazzle-preventive function) shows the opposite trend, being absent in the teleosts and reaching its peak in the mammals (Walls, 1928*b*).

Photomechanical changes are secondarily lacking in duplex ophidian retinae, some of which, as in *Tarbophis,* are strikingly like lamprey retinae in visual-cell pattern and are similarly "statically dark-adapted." They are lacking also in mammals; but in all of these cases the great mobility of the pupil is sufficient explanation.

One can only conclude that the lampreys, whose pupils are motionless, are simply too primitive to have evolved a pattern of photomechanical migrations.

Differentiation of the nuclei.—This matter was omitted entirely from the review of literature above, because of the many contradictions resulting from the crude technical methods of the earlier workers, which so shrank and distorted the tissue as to shift the nuclei from their normal locations and elongate them abnormally.

In *I. concolor* the outer nuclear layer is irregular, but fundamentally single. Either type of cell may have its nucleus against the limitans, or slightly distant from it. This is true also of *I. unicolor,* whose outer nuclear layer is more nearly double. The outer nuclear layer of *P. marinus* is definitely double and the long-cell nuclei always touch the membrane, while those of the short-cells may lie at any level in the layer. In *E. tridentatus,* on the other hand, it is the long-cell nuclei which are most irregularly distributed, though none of the nuclei of either type are normally in contact with the limitans. The outer nuclear layer of *E. appendix* is quite precisely organized, the long-cell nuclei invariably lying against the limitans or even protruding through it. The short-cell nuclei form a definite second layer.

The visual-cell nuclei of *L. fluviatilis* form a compact, single layer; for in this form the cells are not very closely congregated. In *L. planeri* the layer is triple[8] and precisely formed with the long-cell nucleus always against the limitans. The *L. lamottenii* material was not sufficiently well preserved (Zenker's fluid) to permit of rigid conclusions.

It is clear that wherever there is a well-marked differentiation of position, the long-cell nuclei always occupy the place of the cone nuclei of higher vertebrates. There is, however, no regular differentiation of shape or size of nuclei in any species, and the organization of the chromatin in all visual-cell nuclei of all species is that described by Menner for cone nuclei in general.[9]

There is thus little or no evidence from the nuclei pointing to a definite conclusion—but such as it is, it tends to indicate that the long-cell is a cone and the short-cell a rod. The basis of the nuclear differentiation in higher vertebrates, it should be noted, is entirely unknown and in any case is assuredly not fundamental to the physiological differences between rods and cones.

Differentiation of the foot-pieces.—Here we must rely solely upon Tretjakoff's demonstration of a dendritic terminus in the case of the long-cell and a smooth knob ending in the short element of *L. fluviatilis.* If this difference means anything whatever, it is that the long-cell is the cone, the short-cell the rod; for as Pütter

[8] Because the first specimens supplied by Mr. Schultz were small compared with the average of European material, it was thought that this might be a juvenile characteristic, and that the layer might thin out as the eye grew. Very large individuals showed the same situation, however. The triple condition has not been seen by any European observer. Perhaps a subspecific difference of the West Coast *L. planeri* population may lie here.

[9] There is, of course, no more reason for considering the lamprey retina pure-cone, on this basis, than for similarly terming the visual cells of amphibians all cones. The usefulness of Menner's criterion is decidedly limited. Menner unfortunately failed to include a Cyclostome in his extensive survey of outer-nuclear layers.

(1912) has emphasized, all vertebrates which exhibit any foot-piece differences have dendritic cone-feet and compact rod end-knobs.

Conclusions

From the above descriptions, it is obvious that in the lampreys we are not dealing with "undifferentiated" cells. Nor can the lamprey retina possibly be considered either pure-rod or pure-cone. It is certain that both rods and cones are present, and it is equally certain that Heinrich Müller alone has hitherto held the correct view—that the long cells are cones and the short cells rods. Though the differentiation is "perfect" only in *E. tridentatus* and (histologically) "poor" in the genus *Icthyomyzon,* there is surely no reason to suppose that these identifications do not hold for all Holarctic lampreys. The situation in *Geotria* and related genera is entirely unknown, though Plate (1924) has given observations on a macrophthalmia of *G. chilensis* which he himself says was "schlecht konserviertes."

The presence of both rods and cones in these, the most primitive vertebrates, makes it impossible to rely, as Schultze did, upon comparative adult histology to solve the problem of the order in which the rod and cone originally evolved. The writer hopes eventually to present an embryological attack upon this problem, for the comparative histogenesis of vertebrate retinae, structural and physiological, appears now to be our sole possible source of evidence.

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SOME NOTES ON THE TREATMENT OF STRABISMUS*

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IT is the object of these remarks to outline a routine of treatment which has proved, in my hands and those of the group working in our clinic, very satisfactory. Many will differ with me on the details of operative procedure, for there are a number of operations for strabismus, each of which will give good results in the hands of those familiar with it. On the other phases of treatment, however, it seems that we should agree fairly well. After our two years' experience with a special clinic for orthoptic training I think we may set down certain definite facts as to the possibilities and limitations of such training, and give it its definite place in the treatment of strabismus.

The first step in any case is, of course, a complete examination, including refraction under atropine in children under 10 years of age. In older children homatropine is usually as effective. At least our retinoscopy under homatropine will tell us whether cycloplegia is complete and in a few cases will indicate the need for atropine refraction.

In concomitant convergent squint with hyperopia or hyperopic astigmatism as much of the full correction as will be tolerated is

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