Ultrastructure within the Lateral Plexus of the *Limulus* Eye

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ABSTRACT The ultrastructure of the lateral plexus in the compound eye of *Limulus* is investigated by serial section technique. "Cores" of tissue containing the axons, lateral plexus, and neuropile associated with one sensory ommatidium show the following features: (*a*) collateral branches from retinular cells do not contribute to the lateral plexus proper, but do form "retinular neuropile" by contacting collaterals of a self-contained cluster of retinular axons; (*b*) collateral branches from eccentric cell axons always branch repeatedly upon leaving the parent axon, and compose the bulk of the lateral plexus; (*c*) the most distal collateral branches from an eccentric cell axon appear to form neuropile and synaptic contacts with each other, whereas more proximal branches form synaptic contacts with collaterals from eccentric cell axons of neighboring ommatidia. We conclude that the ribbon synapses and associated transmitter substance in eccentric cell collaterals must be inhibitory, and that two pathways for self-inhibition may exist. We suggest, as a working hypothesis for the structure of the lateral plexus, a branching pattern with depth that mirrors the horizontal spread of lateral inhibition measured physiologically.

INTRODUCTION

Function of the lateral plexus of the *Limulus* eye has been studied extensively (for recent reviews see Hartline, 1969 and Knight et al., 1970), but little direct information is available on the fine structure of the plexus that underlies its function. Miller's excellent light microscopy (in Hartline et al., 1956 and Ratliff et al., 1958) reveals extensive branching between ommatidia, but the thickness of the sections and the nonselectivity of the silver stain make difficult an interpretation of relative contributions made by retinular and eccentric cells to the plexus. Moreover, Miller's electron micrographs (in Ratliff et al., 1958; Hartline et al., 1961; and Ratliff et al., 1963) show that the dense pattern of branching within the lateral plexus, and more particularly within the neuropile areas where synaptic contacts have been described

(Whitehead and Purple, 1970), are beyond the resolving power of the light microscope.

The lateral plexus is "large",[1] but a limited, ultrastructural, serial section study of the lateral plexus appears worthwhile because of the importance of the lateral eye in neurophysiology and because of the potential help this study could bring to understanding the neuropile in general; meaningful questions of neuronal specificity and genetic determination of branching patterns are more easily formulated and answered if the normal end product is known.

Our approach has been to study "cores" of tissue each containing at least a single ommatidium, its axons, and associated plexus and neuropile. The cores are approximately 150 μ in diameter and extend some 300–500 μ proximal from the eccentric cell body. In addition to studying adult animals (15 cm prosomal width), young, intermolt animals and developing eyes of embryos are being studied, although this report will deal almost exclusively with adults.[2]

METHODS

1. *Material* Adult, intermolt animals of *Limulus polyphemus* from Cortez, Florida, were maintained in "Instant Ocean" aquaria (Aquarium Systems, Inc., Eastlake, Ohio), exposed to a 12 hr light, 12 hr dark regime. Methods of fixation and embedding have been published (Whitehead and Purple, 1970). Although good fixation has been obtained using a number of different combinations of buffered glutaraldehyde and postfixing in osmium, virtually all methods employed (see, for instance, Fahrenbach, 1969) suffer from "spottiness." Even within the same block of tissue some ommatidia fix well while neighboring ommatidia show poor fixation. Subsequent to most of the work reported here, the general take of the fixative was much improved by killing and fixing immediately upon receipt of the animals, thus indicating that storage, even in well-maintained aquaria, may work physical changes on *Limulus*.

2. *Procedure* To obtain cross-sections perpendicular to the cornea, blocks of tissue (about 1 mm square) were oriented and sectioned from the cornea proximally to the level of the eccentric cell body. The tissue blocks were then trimmed to about 0.7 × 0.6 mm, oriented a final time, and serial sectioning was begun. Serial sections averaged 90.0 nm (900 A) in thickness and the number of sections multiplied by the average thickness served as the primary measure of distance. The sections were floated onto Formvar-coated (Belden Mfg. Co., Chicago, Ill.), carbon-stabilized

[1] In adult animals, the lateral plexus is a sheet of branching axon collaterals some 500 μ thick and greater than 1 cm^2 in area. Within the lateral plexus are regions of neuropile that surround eccentric cell axons, extending out as much as 30 μ and ranging in depth along the axons from 20 to 300 μ.

[2] Patten (1912) and Waterman (1954) have suggested that the lateral eye of the adult *Limulus*, which has received the brunt of neurophysiological studies on the species, may be a degenerating organ. To correlate structure and function, the larger animals have thus been studied, although it enhances the difficulties of the serial section approach. Reports on the younger animals and on embryological material will follow later.

grids, and were observed and photographed using a Hitachi HU-11 or an RCA-EMU3G electron microscope.

A typical core of tissue included the axons from the primary ommatidium in addition to parts of the neighboring ommatidia on each side. Each core required some 2000–3000 sections to follow the axons of an ommatidium through most of the plexus area. The diameter of the ommatidia studied ranged between 150–200 μ at the distal portion, and 60–100 μ proximal to the sensory portion of the ommatidium in the region of the lateral plexus. About five to seven picutres at a magnification of 3000 were required to construct a montage covering the whole central region of a section. Where fine details of collateral branches or of synaptic regions were desired, additional pictures of magnification 15,000 or greater were obtained. Because economy of time and resources dictated that photographs and montages of each section could not be made on adult tissue, we resorted to the following procedure. Nearly 100% of sections available were observed and charted using the electron microscope. The quality (see next Section) of each section was estimated as well as the possible useable area if photographs were desired later. The quality of the sections, and the extent of gross changes observed, dictated the spacing of photographs for careful evaluation. Photography was often done in a second round of section scanning.

3. *Quality Control* The percentage of "publication perfect" photographs obtained in a study such as this is small compared to the number of sections and photographs that, while technically defective, may still be used for tracing and reconstruction. As often as not, an important change, worthy of illustrating the nature of the branching pattern, will occur in a section whose photographic quality is not good. As a guide to our terminology, we indicate for each of the figures illustrated the quality of the section, using the terms good, fair, and useable. These terms indicate the general condition of the section for tracing purposes.

4. *Lost Sections* The number of unuseable sections and of sections destroyed in handling before electron microscope inspection were recorded along with the useable sections. For tracing purposes, 6–10 consecutive sections can be lost without interrupting the ability to follow the course of the larger collaterals, or of a major structural change in the neuropile. Conversely, the loss of just one or two sections makes it impossible to trace perfectly the very fine collaterals. This latter problem makes particularly frustrating the task of producing a detailed, ultrastructural reconstruction of the core tissue.

5. *Nomenclature* Distal refers to the direction towards the eccentric cell body and the periphery. Proximal denotes away from the eccentric cell body and towards the optic nerve proper. Table I summarizes the essential material data at hand from the core of adult tissue presented in detail in this report. The data are typical for each of the three cores studied in most detail.

RESULTS

Three cores of adult material were studied in some detail. Areas from eight other cores were studied in less detail, including areas from ommatidia which

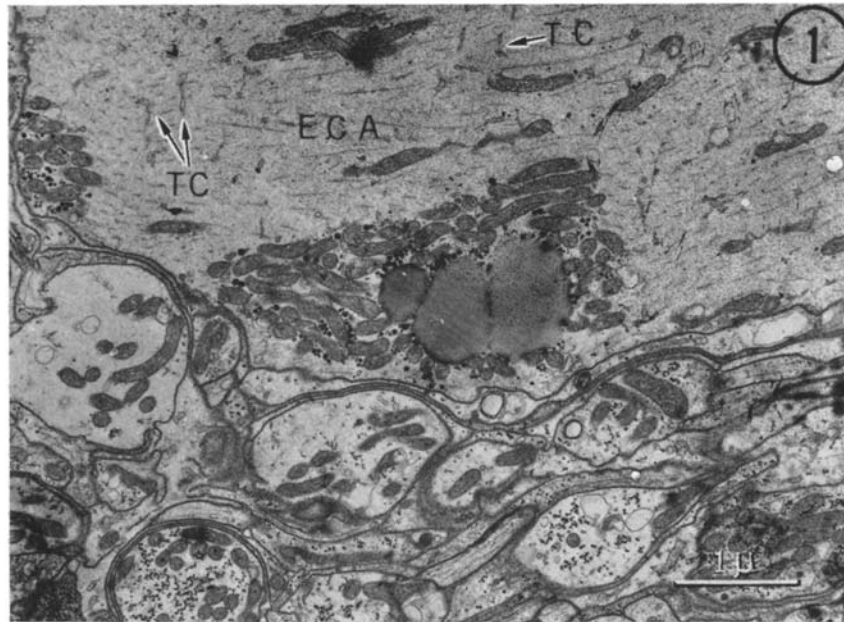
surrounded the primary ommatidium. Orientation of the sections presented no difficulties, since the eccentric cell axon (ECA) of each ommatidium has a more regular shape, less electron-opaque cytoplasm, and fewer cisternae than retinular cell axons (RTA). The ECA therefore provided an identifiable landmark to be used to insure against section reversal and improper orientation relative to other sections in the series (see Figs. 1 and 2).

TABLE I

MATERIAL DATA FROM CORE OF ADULT TISSUE

Section No.	Distance from cell body	Lost sections	Largest No. of consecutively lost sections	Sections photographed and montaged
	μ			
1–100	0–9	37	6	4
100–200	9–18	27	6	3
200–300	18–27	26	4	19
300–400	27–36	26	6	13
400–500	36–45	20	4	16
500–600	45–54	20	3	13
600–700	54–63	14	2	5
700–800	63–72	8	5	4
800–900	72–81	14	3	4
900–1000	81–90	15	3	5
1000–1100	90–99	10	3	1
1100–1200	99–108	13	4	11
1200–1300	108–117	7	2	3
1300–1400	117–126	34	5	3
1400–1500	126–135	20	5	2
1500–1600	135–144	31	3	1
1600–1700	144–153	29	4	3
1700–1800	153–162	18	13	1
1800–1900	162–171	35	15	1
1900–2000	171–180	20	7	1
2000–2100	180–189	22	17	1
2100–2200	189–198	0	0	0
2200–2300	198–207	63	20	3
2300–2400	207–216	25	10	1
2400–2500	216–225	5	5	0
2500–2600	225–234	20	10	2
Total		559		116

No attempt has been made to produce a pictorial, three-dimensional reconstruction of the cores studied. Rather, we present below a depth profile of one of the most extensively studied cores, and follow this with a comparison of similarities and differences seen in the other cores studied, along with more detailed observations we have made.



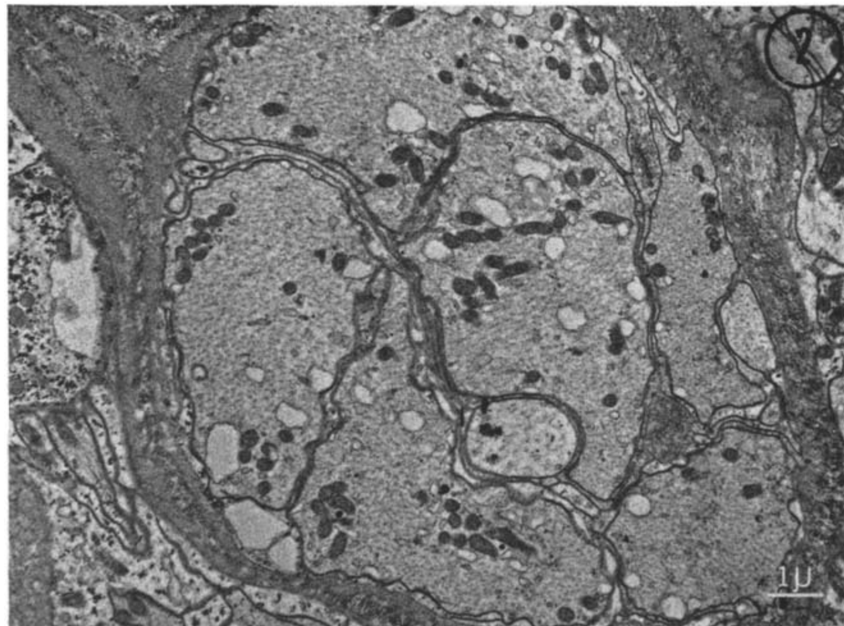


FIGURE 1. Longitudinal section of a portion of an eccentric cell axon (*ECA*) and associated axon collaterals. Transverse cisternae (*TC*) are well organized in compact layers lying perpendicular to neurotubules. × 16,000.

FIGURE 2. Cross-section of a cluster of retinular axons bounded by glial cells and basement lamina. × 7500.

1. *Depth Profile* Table I is a tabulation of sections from this core.

Distance from cell body	*Comments*
0–18 μ	No significant changes in the size of the ECA and no collateral branching are observed. RTAs tend to aggregate in pairs, threes, and fours, each cluster surrounded by glial cells in turn surrounded by basement lamina[3] (Figs. 2 and 3).
19–23 μ	A collateral (1–2 μ in diameter) branches from the ECA. It produces several large branches almost immediately upon leaving the ECA. All main collaterals branch from one pole of the axon and each succeeding one tends to displace the preceding branches away from the main stem of the axon. The collaterals remain oriented along one side of the ECA.
23–25 μ	The original collaterals branch profusely, the offshoots undergoing much additional branching. Basement lamina now surrounds the ECA and associated collaterals. The pattern of branching is from the larger collaterals (which form a semicircle) inward towards the center, collateral branches becoming smaller as the center of the mass is reached. Synaptic vesicles can be observed in many of the branches and the area may be properly termed a "neuropile." No branches are observed in the clusters of RTAs. All the branching collaterals in the ECA neuropile belong to that ECA.
25–33 μ	The neuropile area of the ECA increases in size, but keeps the same topographical relationship to the ECA. Synaptic ribbons (Whitehead and Purple, 1970) can be observed (Fig. 7). All collateral branches come from the ECA itself. No change is observed in the RTAs except for a small decrease in size. At about 30 μ proximal to the cell body, the neuropile area separates from the parent ECA such that basement lamina now can be seen completely surrounding the neuropile area.
33–37 μ	The ECA neuropile becomes completely separated from the ECA by basement lamina. No collateral branches appear to be formed by the ECA. Branches can be observed in the clusters of RTAs at this level (Fig. 3), forming what we term "retinular neuropile" (to be discussed later).
37–50 μ	One RTA, separated from a cluster, becomes partially enclosed by the basement membrane surrounding the ECA neuropile. A branch from this RTA might have entered the ECA neuropile, although no evidence for this is seen in any of the useable sections. RTAs shrink in diameter, but the ECA and ECA neuropile remain constant in size.
50–68 μ	The ECA neuropile area decreases in diameter. The surrounding ECA collaterals are devoid of synaptic vesicles. New collaterals begin to branch from the ECA, which is still separated from the first neuropile by basement lamina. The collateral formation is similar to that observed distally. The beginnings are seen of a second ECA neuropile with a profusion of collateral offshoots containing synaptic vesicles and synaptic ribbons. The whole region of the ECA, including the two neuropile regions, remain surrounded and isolated by basement membrane. No connections to RTAs or to other ommatidia are observed. At this level, all RTA neuropiles but one have terminated. The RTA neuropiles never extended over more than a few microns of depth, and each remained with the cluster of RTAs from which it originated.

[3] Glial cells generally invest all axons of the lateral plexus and many of the fine collaterals within the neuropile. Since they have been described previously (Whitehead and Purple, 1970), no further comment will be made on them in this report.

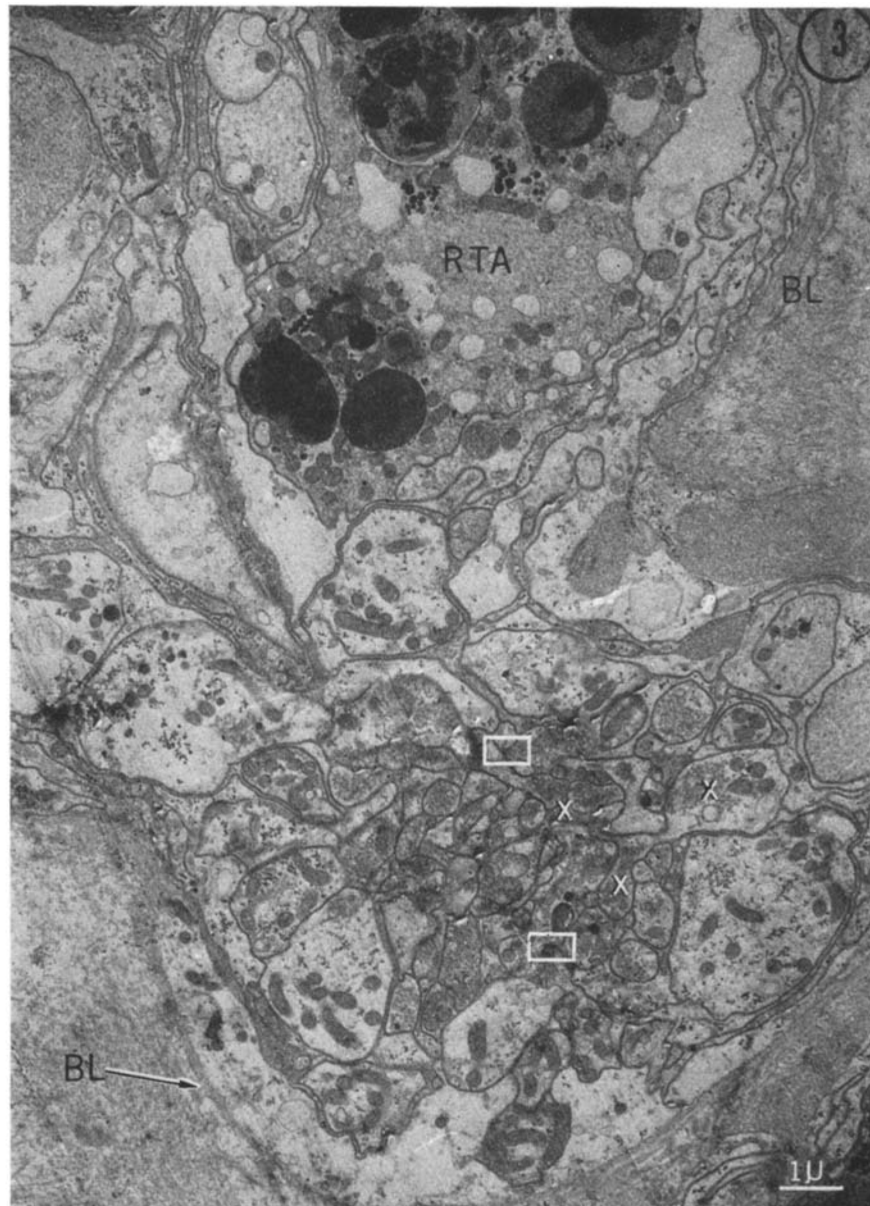


FIGURE 3. Useable micrograph of a retinular cell axon (*RTA*) and associated retinular neuropile from a section that included four other RTAs in the cluster. Boxes mark areas of synaptic contacts within the neuropile. *x*'s indicate portions of the same collateral branch that can be identified from this and adjacent serial sections. *BL* marks basement lamina. Retinular cytoplasm is typical for sections taken in the lateral plexus area. Note that this section illustrates the most extensive retinular neuropile we observed. × 7000.

68–73 μ	The first ECA neuropile area terminates in this portion of the plexus without connecting to any other collateral branches; i.e., it proves to be a self-contained area of neuropile contributed to only by the first ECA collaterals observed. The second ECA neuropile increases in area; the last RTA neuropile terminates in a fashion similar to the others.
73–100 μ	The second ECA neuropile reaches its maximum area (Figs. 4–6) at 90 μ proximal to the cell body. RTAs continue to decrease in diameter.
100–140 μ	The second ECA neuropile area gradually recedes in size, and becomes separated from the ECA. Due to loss of sections it is not possible to confirm that some of the untraceable, large collaterals in this relatively large area of neuropile are not contributed from below by axons of neighboring ommatidia. No RTA collateral branches are observed, and the RTAs continue to descend in clusters isolated by basement lamina. Occasionally an RTA is seen to shift from one cluster to another when two clusters come into relatively close apposition.
140–180 μ	The second ECA neuropile decreases. Fewer vesiculated collaterals can be observed in the center of the neuropile. The periphery becomes composed of large (3–5 μ), unvesiculated collaterals, whose origins could not be traced.
180–200 μ	The diameter of the ECA decreases somewhat. More 1–2 μ collaterals branch off, and other collaterals, which could not be traced to their origin, are seen.
200 μ and below	The process of ECA collateral and neuropile formation continues much as described above, although relatively more collateral contributions, which appear to come from neighboring ommatidia, are seen at this level. Retinular axons continue almost unchanged in appearance; no sign of branching from them is observed.

2. *Differences and Similarities between the Above and Other Cores* In the core cited, two major areas of ECA neuropile were clearly differentiated. The first was self-contained and received no input from other ommatidia or retinular cells (with the remote possibility of one RTA collateral). Collaterals which appeared to come from other ommatidia entered the second neuropile only at about 200 μ below the cell body.

In each of the other cores studied in detail, the division of the ECA neuropile into two distinct sections was not observed. The ECA neuropile typically appears to be continuous starting 10–20 μ below the cell body and lasting throughout the thickness of the lateral plexus, although the volume occupied by the neuropile waxes and wanes, generally reaching a maximum some 150–300 μ below the cell body. The division of the neuropile in the case illustrated may thus be fortuitous, but it serves to emphasize a common finding in all cores: the distal regions (regions close to the eccentric cell body) of the lateral plexus neuropile are formed almost exclusively of multibranching axon collaterals from the single eccentric cell of each ommatidium. Elements from other ommatidia contribute heavily at more proximal regions of the plexus.

3. *Retinular Cell Collaterals* In all cores studies, there was no significant (if any at all) contribution of retinular cell collaterals to the lateral plexus. Eccentric cell collateral branches compose virtually all of the lateral plexus.

Collateral branches from retinular cells form what we have termed

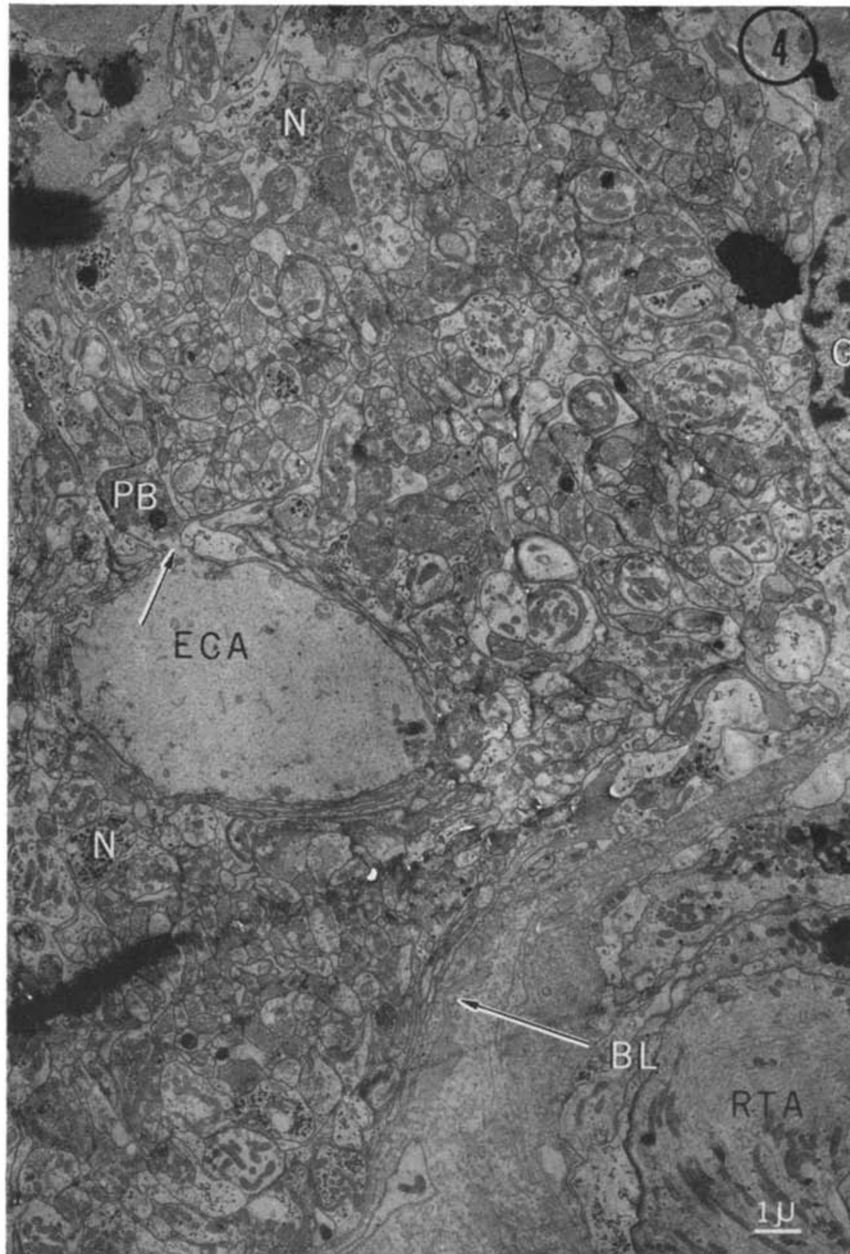


FIGURE 4. Fair micrograph from the core of tissue documented in Table I. The eccentric cell axon (*ECA*) is surrounded by neuropile (*N*) at approximately 90 μ below the cell body. *BL* marks basement lamina lying between the ECA and an adjacent cluster of retinular cell axons (*RTA*). *PB* labels a primary branch from the ECA. *G* labels a glial cell nucleus. Note that the figure shows only two-thirds of the neuropile area surrounding the ECA. At least 50% of this neuropile is composed of collaterals from this ECA. $\times$ 6300.

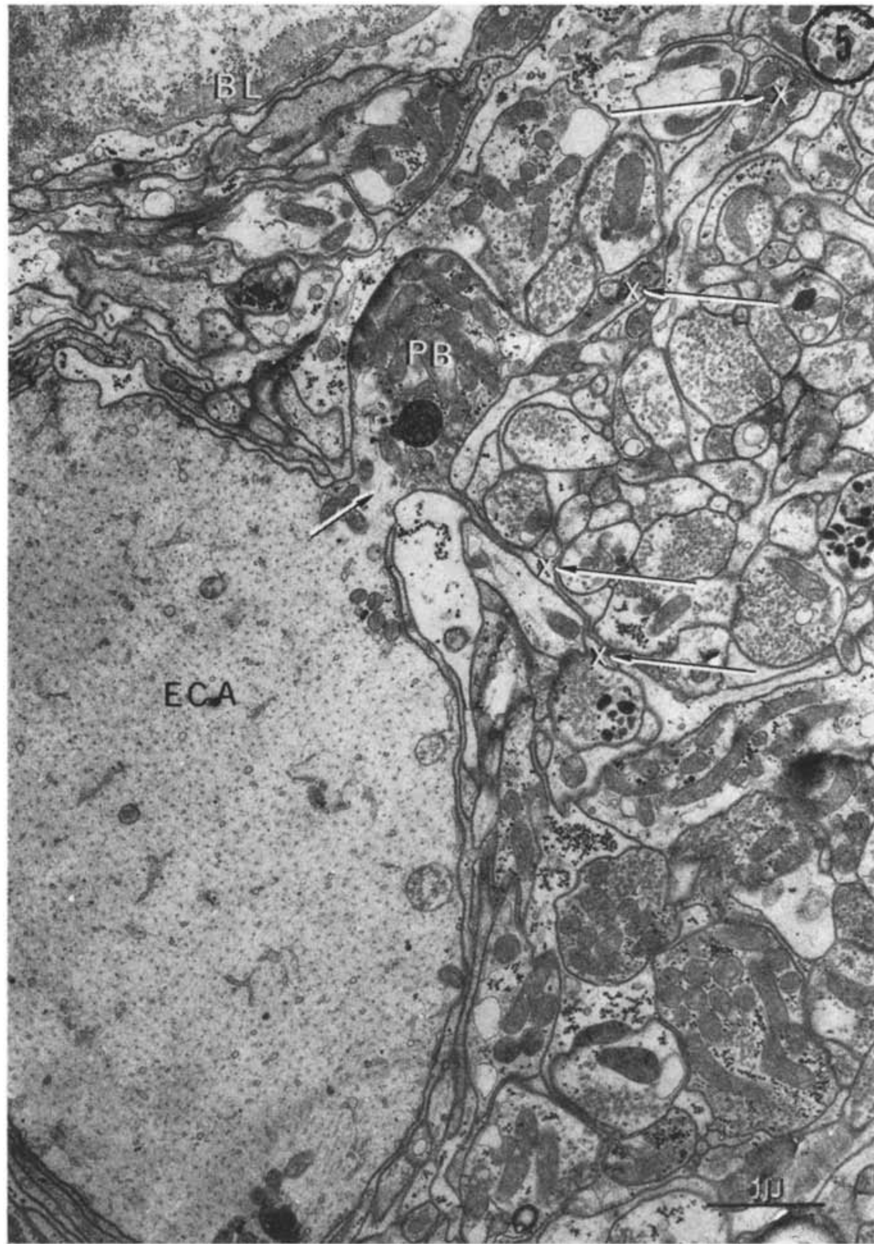


FIGURE 5. Enlargement from Fig. 4 of an area surrounding primary branch (*PB* and short arrow). Note the accumulation of mitochondria within the primary branch. Longer arrows and *x*'s mark collateral branches that can be traced directly to the PB in this section. *ECA*, eccentric cell axon; *BL*, basement lamina. × 16,000.

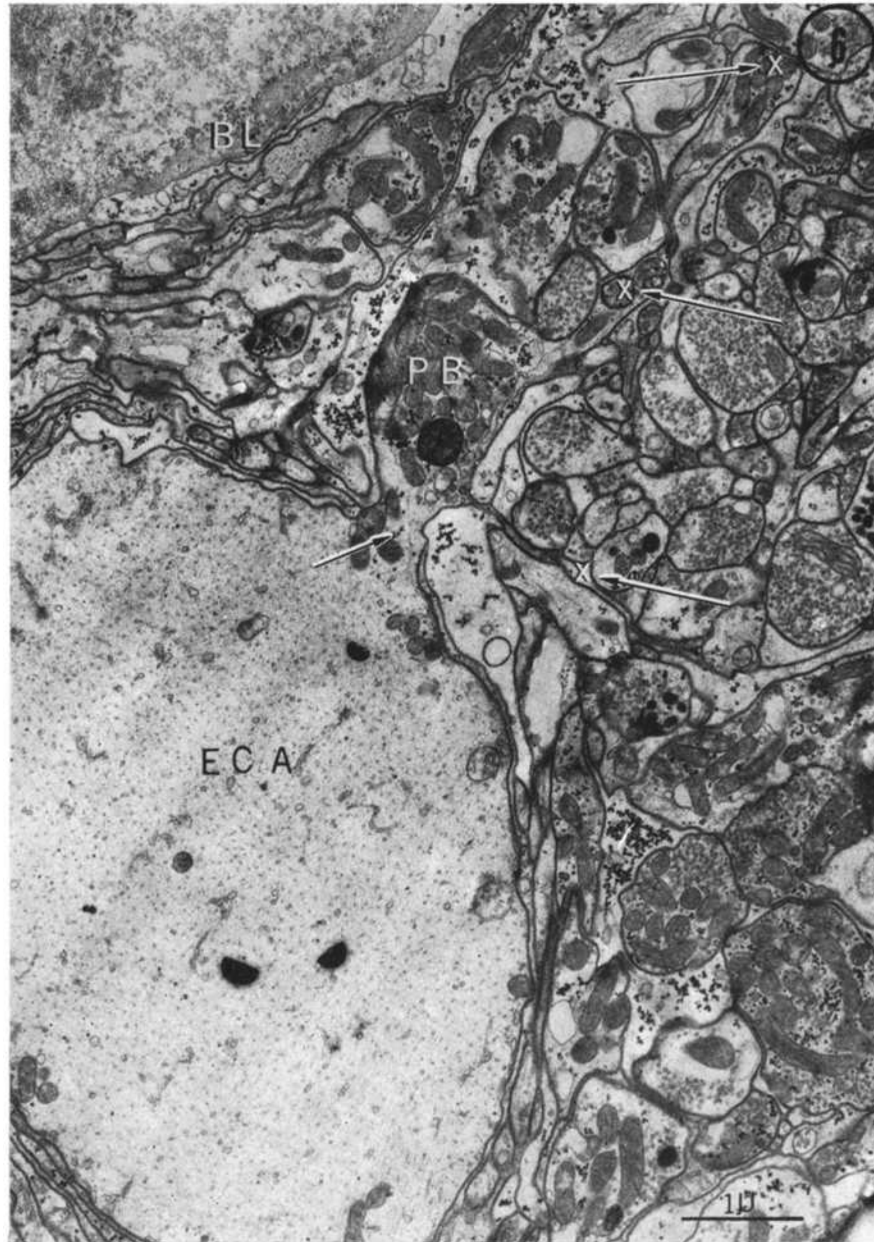


FIGURE 6. Enlargement from next consecutive section below that illustrated in Fig. 5. Long arrows and *x*'s mark the same collateral branches identified in Fig. 5. Figs. 5 and 6 illustrate changes in ultrastructure that occur from one 90 nm section to the next which make complete tracing of collateral branches difficult. Both micrographs are of good quality for tracing. *ECA*, eccentric cell axon; *BL*, basement lamina; *PB*, primary branch. × 16,000.

"retinular neuropile" (see Fig. 3). Retinular neuropile is composed exclusively of branches from retinular cells of a cluster, which after traversing distances of less than 50 μ terminate without making contact with structures other than collaterals from the same cluster of retinular cells. Retinular neuropile is small in cross-sectional area, short, and lacks the very fine branching pattern seen in eccentric cell neuropile. The retinular collaterals do contain some synaptic vesicles and synaptic ribbons which appear similar to those of eccentric cell collaterals (see Whitehead and Purple, 1970).

4. *Collateral Branching Pattern and Structure of the ECA Neuropile* The most distal ECA collaterals terminate after giving rise to the initial neuropile. In all cores studied, each ECA collateral repeatedly sends out clusters of smaller branches within 2–3 μ of leaving the ECA. The neuropile near the ECA is therefore composed of at least 50% by volume of its own collateral branches. Close to the cell body, this figure approaches 100%. Our observations appear to rule out the possibility that any significant number of ECA collaterals proceed directly without branching to neighboring ommatidia. Figs. 5–8 illustrate the above points.

The large collaterals remain grouped about the periphery of the neuropile, and the pattern of branching is from the outside in. In the neuropile there is a wide spectrum of collateral diameters. The smallest, usually located in the center of the mass, are less than 0.3 μ, while the larger collaterals at the periphery range up to 5 μ in diameter. The rate of change with distance in neuropile structure appears to depend upon fiber size. As noted in the Methods section, a major collateral and its large subbranches leave the ECA (what we call a major structural change), over 10–15 μ of the axon length (approximately 100–150 thin sections). Thus, in large collaterals, a loss of 6–10 consecutive sections did not interrupt our ability to trace these collaterals or to note major branching from them. A landmark which aided in tracing the branching pattern was the clustering of mitochondria at branch points. Within the ECA, that portion, or "pole," of the axon where branches left was always marked by an accumulation of mitochondria around the branch area (Fig. 5).

The finer collaterals within the neuropile have very tortuous courses, branch repeatedly, and are almost impossible to trace for distances of more than a few microns. The loss of one thin section makes certain identification and tracing of the fine collaterals a tenuous thing indeed. This difficulty in tracing was due as much to the relatively abrupt changes in the ultrastructure of the collaterals as to their abrupt changes in course. Portions of the fine collaterals would be packed with synaptic vesicles, other portions relatively devoid of all electron-opaque material except for neurotubules (Figs. 5–8). Synaptic ribbons appeared scattered along the course of the collaterals with no hint of placement according to any ordered pattern, except for their

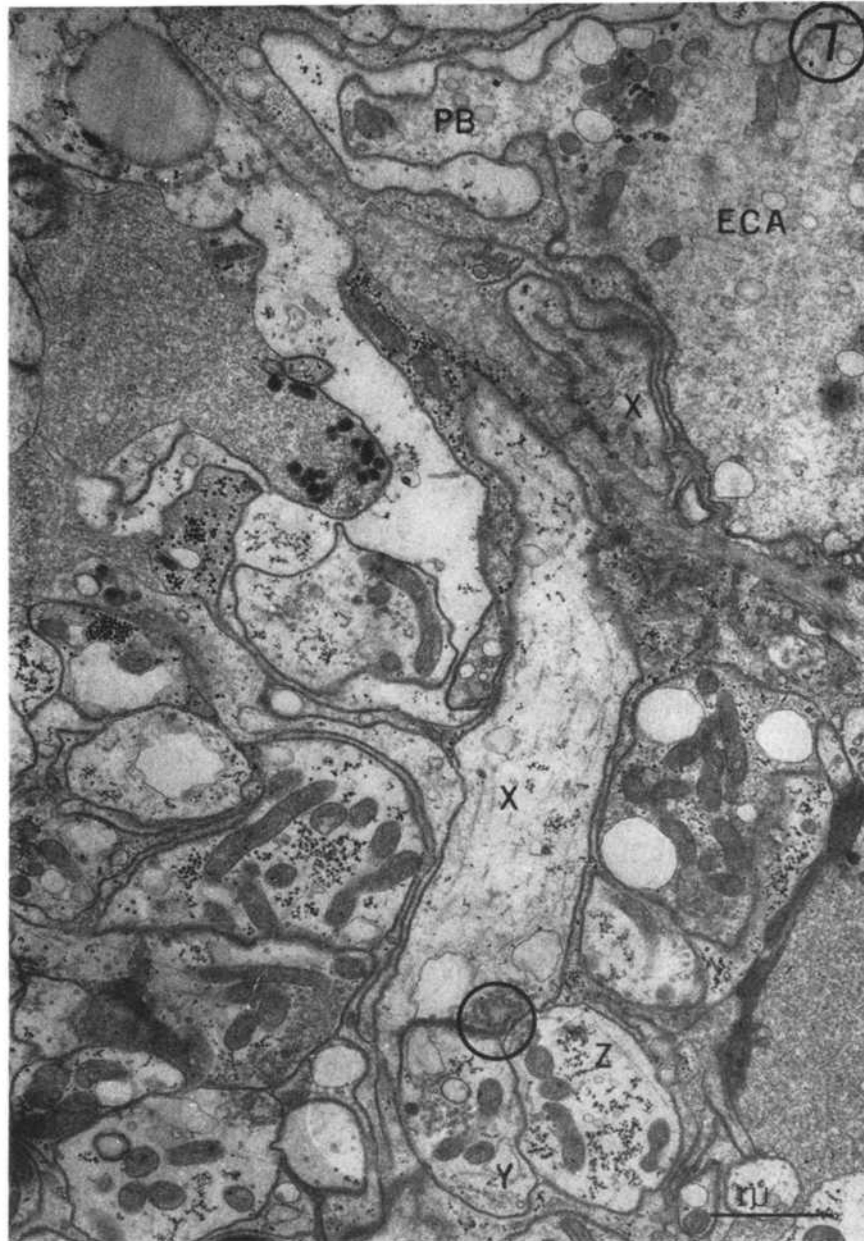


FIGURE 7. Good section of *ECA* in distal neuropile (approx. 30 μ below cell body). *PB* is part of a primary branch and *X*'s are a major collateral from that branch. *Y* and *Z* are collateral branches from the same ECA. The circle marks a ribbon synapse, cut in a plane just above the electron-opaque ribbon (see Whitehead and Purple, 1970). $\times$ 16,000.

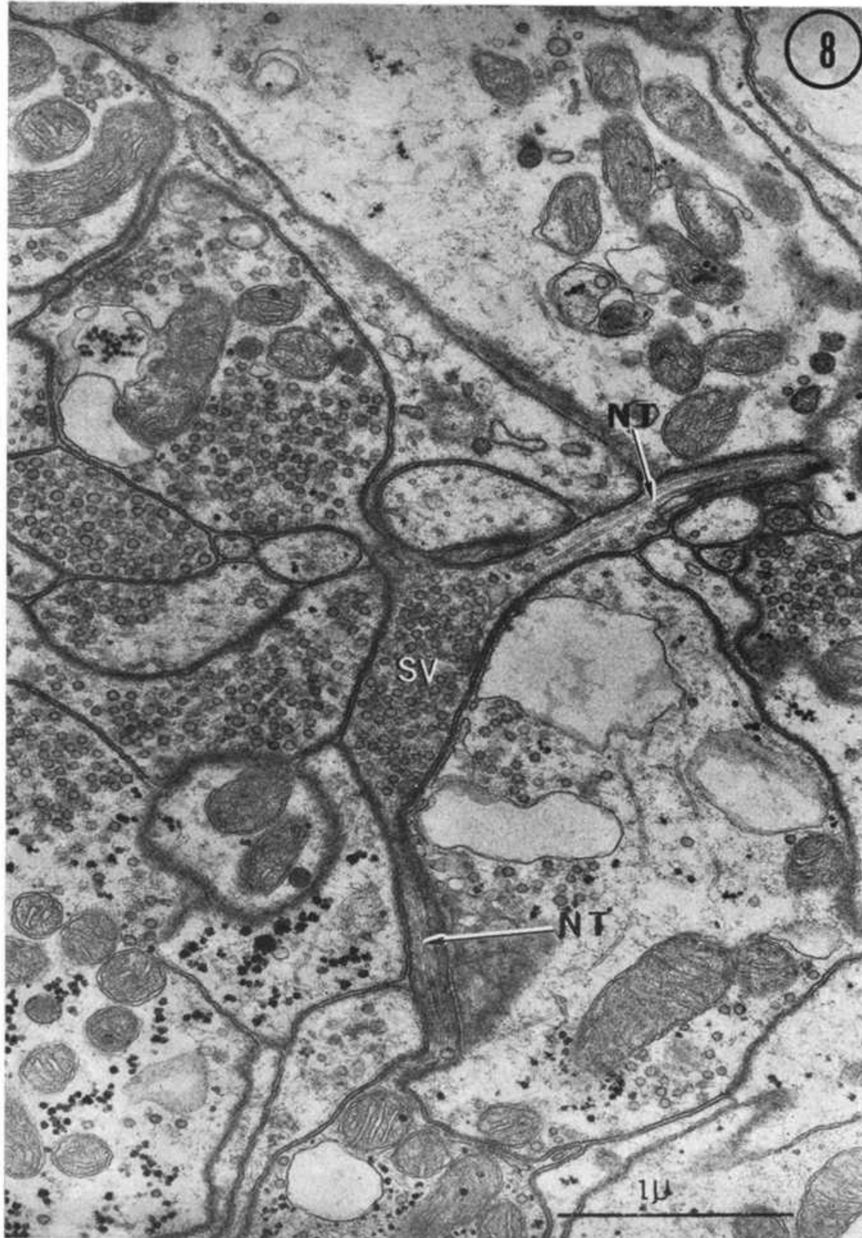


FIGURE 8. Region of ECA neuropile from a good section that illustrates the tortuosity of the branching pattern, and abrupt changes in both diameter and ultrastructure which occur within the branches. *NT* is neurotubules and *SV* identifies synaptic vesicles. × 31,000.

tendency to occur where three branches came into close apposition (Whitehead and Purple, 1970). While counts of synaptic ribbons tended to be higher in fine collateral branches (less than 1 μ in diameter), the ribbons occurred in larger collateral branches as well.

5. *Unidentified Collaterals* In each of the cores studied, the number of unidentified collaterals increased with distance from the eccentric cell body. We attribute most of these to input from neighboring ommatidia, although we could not trace the collaterals to their origins. In one core, we also noted two small axons that appeared at the level of the eccentric cell body where serial sectioning was commenced. It was possible to trace these axons to approximately 35 μ below the eccentric cell body, where their relative position at this level was displaced outside the section. These small axons did not branch, although they did run inside the basement lamina surrounding the distal neuropile for approximately 15 μ. We cannot say whether they are afferent or efferent, although they bore a close resemblance to axon structures in the *Limulus* lateral eye which Fahrenbach (1970) has labeled as neurosecretory efferents. We did not observe any other type of cell which could be labeled as an interneuron in the plexus, although pigment cell processes from cell bodies located in the ommatidia did accompany the axon bundles for several microns below the sensory portion of the ommatidia.

DISCUSSION

Our results are based mainly on serial section studies from three cores of adult material. Areas of eight other cores were studied in less detail. Conclusions drawn must be tempered by the relatively small sample size. We feel, however, that the sample is large enough to warrant a number of conclusions, inferences, and even frank speculations. From a macroscopic view, the lateral eye of *Limulus* is a homogeneous receptor complex and retina (Ratliff, 1965). Individual variations in ommatidial physiology have been noted often, however, and it appears reasonable to assume that some of these variations may have a structural basis that might correlate with differences noted in our sample.

1. *Retinular Cells and the Lateral Plexus* Collaterals from RTAs contribute little, if any, material to the lateral plexus proper. The one possible exception noted by us is listed as a possible exception because we could not trace completely the extent of that one RTA's penetration into the ECA neuropile. Of the 200 thin sections covering this region, 40 were lost or unusable, but the largest number of consecutive sections lost was only four (see Table I). We are relatively confident that, had the RTA collateral branched and contributed significantly to the ECA neuropile, we would have detected it.

Otherwise, the organization of RTA collaterals is so distinctly different

from that of ECA collaterals that we ascribe the term "retinular neuropile" to the organization of the RTA clusters and their collaterals. It remains to be shown: (*a*) if the RTA neuropile in *Limulus* is of functional significance and (*b*) if in other species (or in immature *Limulus*) the terminology can be useful. From a comparative viewpoint, the retinular neuropile in *Limulus* could be either a primitive or a degenerate anlage.

2. *Eccentric Cells and the Lateral Plexus* Four points will be discussed, followed by a "working hypothesis" for the structure of the lateral plexus. The first point is that the neuronal elements of the lateral plexus proper are almost exclusively eccentric cells. We conclude that synaptic ribbons observed previously (Whitehead and Purple, 1970), including ribbon synapses opposite each other, were eccentric cell in origin. Heretofore, two hypotheses existed to account for the spread of lateral inhibition (see, for example, Barlow, 1967): (*a*) that it was eccentric cell to eccentric cell, and that the synaptic transmitter agent within eccentric cell branches was inhibitory; (*b*) that a possible internuncial pathway featuring either RTA collaterals or another neuron (Graziadie, as quoted by Schwartz, 1971) existed—the synaptic transmitter of eccentric cells being excitatory and the transmitter agent of retinular cells or another internuncial being inhibitory. We reject the second hypothesis in favor of the first.

The second point is that eccentric cell collaterals make synaptic contacts with collaterals from the same ECA. For self-inhibition in the eye of *Limulus* (Stevens, 1964; Purple and Dodge, 1966), we now hold that two possible pathways may exist which are not mutually exclusive. We have reported here the pathway of collateral to collateral from the same cell. Previously (Whitehead and Purple, 1970), the demonstration of ribbon synapses directly opposite each other was interpreted as morphological evidence favoring the hypothesis of a synapse whose transmitter agent could affect both the pre- and postsynaptic endings (two-way chemical transmission as postulated by Purple and Dodge, 1966). The existence of collateral-to-collateral connections from the same cell does not alter the status of the "two-way chemical synapse" pathway. Indeed one can contend that this relatively primitive neuronal system in which neurons appear incapable of discriminating between branches from themselves and another cell, might have just the biochemical substratum required for a nonselective, two-way synapse.

The third point is that the branching pattern of the lateral plexus involves ECA collaterals which literally produce a shower of subbranches near the ECA. The same large collaterals must make up the lateral plexus which functionally connects the ommatidia. M. Behrens of the Masonic Medical Research Laboratory has kindly allowed us to view her slides of sections from eccentric and retinular cells that were injected with Procion yellow. The fluorescent pattern of stain in her sections and in those of Schwartz (1971)

show a clustering of points in neuropile regions, and also indicate only short RTA collaterals. Thus, results of Procion yellow staining are in general agreement with our findings, although the resolution of the fluorescent stain is too limited to be taken as direct confirmation of the ultrastructural patterns reported here.

Given the three points above, we conclude that the physiological mechanisms of self-inhibition (Stevens, 1964; Purple and Dodge, 1966), of synaptic delay and threshold for lateral inhibition (Hartline et al., 1956; Hartline and Ratliff, 1957), and also of the depolarizing and hyperpolarizing components of lateral inhibition (Tomita et al., 1960; Purple, 1964; Knight et al., 1970) must be resolved within the structural context of the ribbon synapses in the fine collateral branches of the neuropile. Finally, Purple and Dodge (1965) have proposed a cable model for interpreting intracellular recordings from the eccentric cell. As part of this cable model, the requirement exists for a length of passive axon and/or axon collaterals between the cell body and both the site of impulse encoding and the site of postsynaptic inhibition. We suggest that a major site of the passive cable resides in the fine collaterals of the neuropile. Propagated action potentials must be generated somewhere in the collaterals giving rise to the fine fiber system of the neuropile, since virtually all of these larger collaterals (except the distal ones which end on each other) are the only available links to more distant ommatidia. The dimensions of the small collaterals within the neuropile interior, together with their abundance of synaptic vesicles and ribbons, suggest their function to be primarily nonelectrogenic (see Grundfest, 1965, 1971).

3. *A Working Hypothesis for the Structure of the Lateral Plexus* We suggest that the depth profile of ECA collaterals and neuropile topographically mirrors the functional organization of lateral inhibition that has been elucidated by Hartline and his colleagues (for a review, see Hartline, 1969). In essence, we suggest that the most distal collaterals make contact with themselves, and those collaterals lying successively proximal make contacts with ommatidia successively farther away (Fig. 9). We term this suggested arrangement a working hypothesis because the present study confirms the first part of it but only hints at the successive distribution of the collateral system with depth in the lateral plexus.

The hypothesis has the following merits. (*a*) It can account for the general profiles of lateral inhibitory magnitude vs. distance, and is flexible enough to account exactly for individual variations. Averaged maps of inhibitory coefficients for lateral inhibition (Hartline and Ratliff, 1957; Kirschfeld and Reichardt, 1964; Barlow, 1967, 1969) show a gradual reduction with distance in the strength of inhibition exerted by one cell upon its neighbors. Considerable variation exists for the strength of lateral inhibition exerted by any one cell on neighbors when compared to a large population that has been averaged

(Barlow, 1967). Self-inhibitory coefficients, however, are about as great as the combined sum of lateral inhibitory coefficients operating on a cell (Stevens, 1964; Purple and Dodge, 1966; Knight et al., 1970). An "average" profile of all the cores studied does suggest a strong magnitude of self-inhibition, and the variation of the depth profiles of individual cores appears to approach the order of magnitude of variation seen by Barlow (1967) in detailed maps of lateral inhibitory coefficients. (*b*) With the additional assumption that collaterals close to the eccentric cell body develop before more proximal

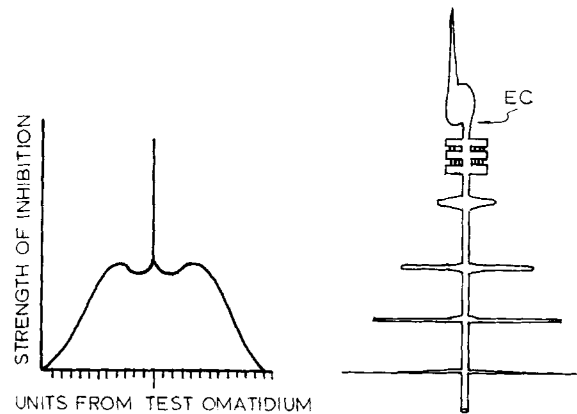


FIGURE 9. Schematic diagram to illustrate working hypothesis of the lateral plexus branching pattern. On the left is a schematic of lateral spread of inhibition (abstracted and modified from Hartline and Ratliff, 1957; Kirschfeld and Reichardt, 1964; and Barlow, 1967). Diagram is not to scale since the magnitude of the self-inhibitory coefficient (line at the center) is some 10^3 times greater than any lateral inhibitory coefficient. On the right is abstract of an eccentric cell (*EC*) and its branching pattern with depth. The interconnected short lines schematize the self-inhibitory pathway. Widths of the other horizontal lines indicate relative numbers of collaterals contributing to plexus and neuropile of neighboring ommatidia. The lengths of the horizontal lines indicate relative lengths of collateral pathways.

collaterals, well-developed ommatidia will have formed synaptic connections with near neighbors before more distant neighbors are developed sufficiently to establish contacts. Such a pattern would insure that the distal collaterals would be in contact with near neighbors, and later-developing collaterals would reach farther away from their parent ommatidium. Growth of the *Limulus* eye is from the center outwards, i.e., the eye adds ommatidia at the periphery (Waterman, 1954). (*c*) Our hypothesis implies that basic, orderly mechanisms of cellular biology and genetics are in operation in determining the structure of the lateral plexus, even though at first sight the lateral plexus appears to be a hopeless tangle. (*d*) Finally, our working hypothesis is amenable to testing. With neuronal staining techniques applied to the eyes of

developing embryos, it should be possible to trace the maturation stages of the lateral plexus.

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