THE LOBSTER OPTIC LAMINA

II. TYPES OF SYNAPSE

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SUMMARY

The following interpretations are based on the assumption that the vesicles are presynaptic. Synapses between retinula cells are symmetrical contacts, with cisternae attached to both thickened membranes and the cleft is 8–10 μm wide. Synapses from retinula terminal bags to the numerous invaginating spines of the ganglion cell axon have presynaptic ribbons and filaments but few vesicles; the cleft is 7.5–13 μm wide. Synapses from retinula cell bags to secretory horizontal fibres have postsynaptic spines, typical vesicles one side and thickened presynaptic membrane (cleft 10–17 μm wide). Synapses from retinula fibres to empty (long) transverse fibres are similar. Synapses from secretory or empty transverse fibres to ganglion cell axons are axon-to-axon contacts; there are vesicles one side but both membranes are thickened; the cleft is 11–13 μm wide. Between empty transverse fibres the synapses are similar but symmetrical; from empty to secretory transverse they have vesicles one side. Synapses from secretory fibres to each other (symmetrical) or to empty transverse fibres (vesicles on one side and with only the postsynaptic membrane thickened) reveal a sharp distinction between synaptic vesicles and secretory vesicles. Serial synapses occur (a) from empty transverse fibre to secretory fibre to another empty transverse fibre, and (b) from retinula cell to secretory fibre to ganglion cell fibre. On account of its curious structure the optic cartridge probably has complex synaptic properties. Retinula terminals are probably inhibitory. Their light mitochondria, contrasting with the dense ones of the ganglion cells, are interpreted as aged.

INTRODUCTION

A previous paper (Hámori & Horridge, 1966) describes the general organization of the optic lamina of the lobster into five layers. Sandwiched between glial sheets and cell bodies lies the thickest of these, the central columnar region, where the retinula fibres terminate on the axons of the second-order ganglion cells at structures called optic cartridges. Other axons from cell bodies below the lamina penetrate the columnar region as transverse bundles and pass by many of the radially arranged optic cartridges. Four clearly distinguishable types of axons come together at the cartridges where the retinula endings terminate. In this paper the ultrastructure of the synaptic contacts between these different axons will be described.

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MATERIAL AND METHODS

Preparation of the optic lobe, and all electron-microscope techniques were as described in the previous paper.

RESULTS

Fig. 1 illustrates schematically the general structure of the lobster lamina. The main morphological units are the optic cartridges, connected by the horizontal fibres. The whole synaptic area is closed off by glial sheaths (g1 and g2). The cartridge itself consists of one or two central ganglion cell axons surrounded by several retinula fibre endings (re). The retinula fibres originating in different ommatidia can be seen crossing each other before entering the cartridges. The transverse fibres are of two types: 

(a) local, usually of secretory cells, with a distribution restricted to the lamina; and
(b) of normal nervous structure from some other, unknown region of the optic lobe.

No other fibre types have appeared in a large number of preparations by Gomori, Holmes and Golgi methods and by thin-section techniques. As may be inferred from
the silver preparations and from the only previous study, synaptic contacts between the
different fibres can be expected as follows: (a) retinula ending with ganglion cell axon;
(b) transverse fibres with ganglion cell axon and with retinula endings; (c) between
retinula endings from different ommatidia; and (d) between different transverse
fibres. Synaptic contacts between ganglion cell axons may theoretically be expected
only when the cartridge contains two such axons; synapses between side branches of
ganglion cell axons of different cartridges cannot be excluded, but we have no light- or
electron-microscope evidence of them.

Fig. 2. Schematic drawing of the cartridge in cross-section. The two central ganglion
cell axons (ga) send spines into the surrounding seven large retinula endings (re),
which contain numerous vesicles (sv) and large mitochondria. The synaptic ribbons (sr)
lie in the foldings between the spines. An enlarged ribbon surrounded by synaptic
vesicles is shown at lower left. A transverse section of an invaginated spine (st) is
shown enlarged at lower right. The presynaptic membrane (pm) is mainly thicker
than the postsynaptic (ps); the intercellular gap (ig) is 13 m. Also ending in the
cartridge are transverse fibres (tf), some of which contain neurosecretory vesicles.
Transverse fibres are mainly postsynaptic to the retinula endings and presynaptic to
ganglion cell axons, with typical synaptic contacts. Non-polarized synaptic contacts
lie between neighbouring retinula endings. The whole cartridge is ensheathed by
glial processes (gp).
Synapses of retinula fibres on ganglion cell axon spines

The two elements of this synapse occupy the major part of the cartridge, which is drawn schematically in Fig. 2 and illustrated in section in Figs. 4 and 5. The retinula endings are expanded into terminal bags which together completely surround the ganglion cell axon or axons in the centre. In each cartridge there are about seven retinula fibres making numerous and extremely widespread synaptic contacts with the spines of one or two ganglion cell axons. Counts show that in the lobster there are the same number of cartridges as ommatidia and it is already known that each ommatidium has seven retinula cells (Rutherford & Horridge, 1965). The retinula cell endings are filled with synaptic vesicles (Figs. 4, 5, 8, 9, 10). These are 30–70 mμ in diameter, and similar to the commonest type of empty vesicles found at synapses of both vertebrates and invertebrates. They occur here only in the termination and not in the preterminal fibre. The mitochondria are rather large and sometimes seem shrunken, with rather few cristae. Out of all participating elements of the cartridge such mitochondria are found only in the retinula fibres and we used this feature to distinguish retinula endings from other axons containing vesicles in cases difficult to interpret. Retinula fibre endings never have branches or spines.

The ganglion cell axons are filled with neurotubules, 20 mμ in diameter (Figs. 4–9). The mitochondria are smaller than those of the retinula endings, with dense cristae, and the internal cavity or stroma is very dense after osmic fixation (Figs. 6, 11, 14). The mitochondria commonly have dark spots (Figs. 6, 9, 11, 14) about 12–15 mμ in diameter. Large vesicles of 80–150 mμ diameter can occasionally be found in these axons.

The principal feature of the ganglion cell axon in the synaptic region is its branched or spiny appearance (Figs. 6, 8, 9, 10). The spines lie in tunnels which invaginate deeply and branch as they enter the retinula endings (Fig. 8). With larger magnification the spines may be classified as primary, secondary and tertiary, as shown best in Fig. 7. While the primary and secondary spines contain neurotubules similar to those in the axon trunk, the very thin tertiary spines are usually empty (Figs. 13, 16), and often have a bulbous ending (Fig. 7). The tertiary spine is connected with its secondary by a thin neck (Figs. 6, 10) which only rarely lies in the plane of section. In most cases the tertiary spines are seen as small ovoid or round profiles embedded in the retinula endings, and are characterized by the double cell walls of their outline. There are occasionally transitional forms in which the tertiary spines seem to be connected by only two membranes to the secondary ones. Though penetrating the retinula endings deeply the spines never reach the peripheral region of the cartridge (Figs. 2, 4, 5). Similar spines have been described on the Purkinje cell dendrites which invaginate the parallel fibres in the cerebellar cortex of the cat (Gray, 1961; Hámori & Szentagothai, 1964), but with the essential difference that there the two cerebellar elements are separated by glial membranes with the spine as the only contact, whereas in the lobster lamina no glial elements penetrate the cartridge (Fig. 9). There are no spines or invaginations in the corresponding synapse of the lamina of the blowfly (Trujillo-Cenóz, 1965).
The question arises which part of this complex structure of dendritic spines embedded in a bag-like ending can be considered as the specialized synaptic attachment. Much more than half of the total contact area is established by the extensive system of tertiary spines, so they must play at least some role in synaptic transmission. The cleft between the unit membrane varies from 13 m\(\mu\) down to 7.5 m\(\mu\) at different points over its large area. An unusual feature is that the supposed presynaptic retinula fibre membrane is thicker than that of the postsynaptic spine which lies against it. No specific accumulations of synaptic vesicles occur against the presynaptic membrane opposite the invaginations of the ganglion cell spines (Figs. 4–6 and 8–10). However, as seen at high magnifications, the presynaptic membrane is backed by a system of microfilaments which lies close against it in the retinula fibre cytoplasm, resembling that first found by Gray (1959) and subsequently described by de Robertis et al. (1961) at the postsynaptic membrane in higher vertebrates. This filamentous web in the lamina is found at the places where the synaptic membrane is thickened. At the same place, and as further indication of the synaptic nature, there is a filamentous structure between the synaptic membranes and at right angles to them. Perhaps our presynaptic filaments are related to those of Gray (1963) who described the 'dense projections' attached to the presynaptic membrane of some synapses of mammalian spinal cord. In that case (unlike ours) the postsynaptic membrane was, as usual, thicker than the presynaptic one.

Synaptic ribbons, which elsewhere in invertebrates have appeared only in the insect lamina, are osmiophilic lamellae 100–150 m\(\mu\) by 500 m\(\mu\), and 30–40 m\(\mu\) thick, lying at right angles to the presynaptic membrane (Figs. 11–17). Characteristically they are situated in incisions between the primary and secondary spines (Fig. 6) and are covered on each side by a row of synaptic vesicles. They are strikingly similar to the presynaptic ribbons in vertebrate photosensory cell endings, or in other primary sensory cells (see Discussion). The ribbon lamella is rectangular, and rather narrow, so that it is rarely cut along the long axis, as in Figs. 8 and 14. The synaptic ribbon lies in a thin process in the cytoplasm of the retinula fibre usually between the fork where two tertiary spines branch out. This means that in most cases the ribbon lies parallel to the presynaptic membranes on both sides of it, while the 30–50 m\(\mu\) gap between the ribbon and the presynaptic membranes on both sides is filled with a row of synaptic vesicles against those membranes. The end of the ribbon lies close to the membrane of the tip of the presynaptic process, but there is no sign of fusion between them. Both the pre- and postsynaptic membranes in this region are electron-dense but attached to the latter there is an additional layer, 20–30 m\(\mu\) thick and presumably of protein (Figs. 11, 13–15), which gives an impression that the postsynaptic membrane is thicker than the presynaptic one. The presynaptic ribbons appear only along the contact between retinula endings and ganglion cell axons and have never been observed along the contact areas between retinula endings or between these and transverse axons. There are none of the capitate projections of glial cells which are the most characteristic feature of the terminal bags of the fly's photoreceptor axon terminals.
Synapses of transverse fibres with ganglion cell axons

The main axon trunks of the transverse fibres run in a layer at the distal end of the cartridge (Fig. 4). Here they appear in section as large profiles which may be (a) 'empty', having no inclusions other than a few synaptic vesicles of normal size, or (b) contain secretory granules or occasionally dense-core vesicles (Figs. 2, 4, 22, 24). The few mitochondria in the preterminal part are always smaller and denser than the very large mitochondria which distinguish the retinula endings. Though synaptic contacts with the retinula endings occasionally lie at the peripheral region of the cartridges (Figs. 4, 22), the majority of synaptic contacts are made by thin lateral branches of the transverse axons which penetrate to the centre of the cartridge and lie between a ganglion cell axon and its surrounding retinula endings. One transverse fibre sends terminal branches to many cartridges, each of which may contain several such endings. The transverse fibre arborizations can be distinguished from the retinula endings by their empty appearance, their smaller size (Figs. 19, 20), and by their small dense mitochondria (Figs. 4, 5, 20). Although both are very thin with the same spiny appearance (Figs. 19, 20) there is no problem in separating transverse fibre terminals from ganglion cell axons because the latter are filled with neurotubules while the former have none. A further clear distinction is that the transverse fibre spines, but not those of the ganglion cells, contain 'normal' synaptic vesicles (Figs. 18-20). Larger fibres with secretory vesicles also penetrate as far as the centre of the cartridge (Fig. 13) although the majority of such fibres usually lie at the periphery.

The 'empty' transverse fibres make synapses (a) with ganglion cell axons (Fig. 18), in which case there is no invagination and the transverse fibre is presynaptic as judged by synaptic vesicle accumulation, and (b) with the retinula fibre endings, where the transverse fibre is postsynaptic as judged by the accumulation of vesicles in the retinula fibre side, but occasionally this contact may appear as unpolarized (Figs. 20, 24), with synaptic vesicles on both sides. In any case there is a considerable thickening of the synaptic membranes.

Synapses between retinula endings and transverse endings are established in the central region of the cartridge exclusively by very thin transverse fibre processes which also send long spines into the retinula ending. As a result, we have two different types of spine invaginating the retinula bag endings; the majority belonging to the ganglion cell without synaptic vesicle accumulation (except at synaptic rods), and others to transverse fibre spines having the specialization characteristic of synapses generally, with vesicles on the retinula cell side. The transverse fibre processes are interpolated here and there between the ganglion cell trunk and its retinula endings (Figs. 19, 20).

Secretory fibres, which are distributed mainly around the periphery of the cartridges and sandwiched between other transverse fibres of typical neurons, form unpolarized synaptic contacts with retinula fibre endings and the vesicles on both sides are of normal type (Fig. 24). In the fly lamina Trujillo-Cenoz (1965) finds two presynaptic centrifugal fibres to each retinula ending, with synapses on to all six retinula endings as well as to the two ganglion cell axons.
Lobster lamina synapses

Synaptic contacts between transverse fibres

There are transverse fibres of at least two different origins: (a) secretory fibres from local cells, and (b) others of normal appearance, probably with long axons to other neuropile regions. These run side by side in the transverse fibre layer, giving off thin terminations to the cartridges as they pass, and it is not surprising to find some synaptic contacts between them. Synapses between transverse fibres can be recognized most clearly when formed by secretory fibres, and such synapses are found mainly in the peripheral part of the cartridges, or occasionally in the glial area between two cartridges. The contact between a secretory and a normal transverse fibre is usually polarized, with a thickened presynaptic membrane and accumulation of synaptic vesicles in the secretory fibre (Figs. 23, 25), though some with normal vesicles are presynaptic to the secretory fibres (Figs. 23, 24). In a few cases sandwich synapses have been observed, as in Fig. 23, where a normal fibre is presynaptic to the secretory fibre, which in turn is presynaptic to the small axon containing normal vesicles at the right. This sequential or sandwich arrangement is now known to be widespread in the animal kingdom.

A point of general importance is that when a fibre of secretory nature establishes synaptic contact, the synaptic vesicles, of 30–60 mμ diameter, which are accumulated opposite to the thickened presynaptic membrane, are typical of normal synapses in every way and quite distinct from the neurosecretory vesicles. A similar segregation of normal and secretory vesicles is found in the glomeruli of the accessory olfactory lobe of the lobster. Moreover the non-synaptic portion of the secretory fibre contains none of the normal vesicles.

The secretory vesicles in these cells are identified as such by their similarity to the neurosecretory fibres observed in the pars nervosa of vertebrates (Palay, 1957), the corpus cardiacum of insects (Meyer & Pflugfelder, 1958), the brain of the leech (Hagadorn & Nishioka, 1961), the brain of annelids (Scharrr & Brown, 1961) and the post-commissural organ of crustaceans (Knowles, 1958), but here in the lamina there is no end-organ and no functional reason for calling them neurosecretory. The secreted droplets in them have typical limiting unit membranes, as have the normal synaptic vesicles (Fig. 24).

Synapses between retinula endings

Unpolarized synaptic contacts are found occasionally between two adjacent retinula fibre bags in the cartridge. At these places the synaptic membrane is thickened and reveals an unusual membrane structure of four osmiophilic and three osmiophobic layers, with the outer pair thicker. This implies that two extra osmiophilic membranes adhere to the cell membranes on each side. Strangely enough the whole thickness of this multilayered structure is only about 40 mμ (arrows in Fig. 27). The two outer membranes are modifications of subsurface cisternae, one of which is very large in Fig. 27. Similar formations have been described by Rosenbluth (1962) and by Smith & Sjöstrand (1961) in the hair-cell processes of the vertebrate organ of Corti. Besides the specialization of the membrane the synapse is marked by the accumulation of
synaptic vesicles of normal type on both sides. No corresponding synaptic arrangement was suggested by Trujillo-Cenoz for the fly lamina, where the retinula-fibre terminals have much more glial investment than is found in the lobster.

Occasionally one can find desmosome attachments between primary or secondary spines of two ganglion cell axons where these occur in one cartridge. Between the symmetrical membrane thickening an intermediate osmiophilic layer lies along the middle of the intercellular gap; no vesicles are aggregated in the vicinity of these desmosomes (Fig. 21).

**DISCUSSION**

Newly described synaptic structures are summarized in Table 1, where polarity is based on the general assumption, which has been established by morphological examination of many types of synapse, that vesicle concentrations are presynaptic. We are aware that this criterion of synaptic polarity is not universally valid but here it has not led us to any ridiculous conclusions. The optic cartridges occur in a 1:1 ratio to the ommatidia, and like them lie in a regular array. No eccentric cells or their axons have been found. There is considerable interweaving between the retinula fibres just before they enter the optic cartridges, and the retinula endings have no side-branches. This necessarily has the effect of passing excitation to a number of ganglion cells from each ommatidium—not at all conducive to greater acuity unless a number of ganglion cells are taken into account together at a lower level. The synapses between adjacent similar retinula cell bags are most likely to be inhibitory, as may be argued from the advantage and wide occurrence of lateral inhibition behind the retina and the absence of other inter-retinula synapses. The extraordinary sensitivity of the crustacean eye to small movements of contrasting edges down to 0.05° is combined with a failure to resolve stripes less than about 4°, suggesting that receptors have overlapping fields of acceptance but showing a great sensitivity to movement of any contrasts which can be resolved. We do not yet know how the anatomy and the performance can be fitted together.

The main synapse of the lamina is from the retinula fibres to the ganglion cell axons; the synaptic units are the optical cartridges which are made up of about seven retinula endings and either one or two ganglion cell axons. The contact area is a combination of the 'en passant' and the invagination types, as in the rod to bipolar cell synapse of vertebrates (Sjöstrand, 1958, 1961), and evidently the geometry of the structure plays an essential part in its function.

The penetration of primary, secondary and tertiary spines into the bags of the retinula endings enlarges the whole contact area and at the same time the synapse is electrically isolated in a fashion which must be important physiologically. In the vertebrates, the presynaptic bags of the rods and cones have similar contacts, but the invaginations are restricted to a region in the base of the bag. As in the vertebrates the retinula cell presynaptic bag is crowded with synaptic vesicles of normal size but in the lobster these do not show the special aggregation of typical synapses. This and the narrowness of the cleft (7.5–13 m/μ) suggests that transmission is electrical. The pre-
### Table 1. Summary of the newly described synaptic structures

<table>
<thead>
<tr>
<th>Class of Synapse</th>
<th>Structure of Synapse</th>
<th>Specializations</th>
<th>Vesicles</th>
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<tr>
<td>Retinula cell to retinula cell</td>
<td>Symmetrical lateral contact of terminal bags</td>
<td>Synaptic vesicles both sides with flat cisternae attached to both thickened membranes. Presynaptic filaments and thick membrane. Presynaptic ribbons but very few vesicles</td>
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<tr>
<td>Retinula cell to ganglion cell axon</td>
<td>Axon to axon with invagination</td>
<td>Primary, secondary and tertiary postsynaptic spines ramify into bags. Postsynaptic spines.</td>
<td>As above</td>
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<tr>
<td>Retinula cell to neurosecretory transverse fibres</td>
<td>Axon to axon with invagination</td>
<td>Postsynaptic spines.</td>
<td>As above</td>
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<tr>
<td>Empty transverse fibres to ganglion cell axon</td>
<td>Axon to axon</td>
<td>Axon lateral contact. En passant sometimes symmetrical. Post-synaptic membrane thicker.</td>
<td>As above</td>
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<td>Empty transverse fibres to empty transverse fibres</td>
<td>Axon to axon</td>
<td>Axon lateral contact frequently followed by 9 vesicles.</td>
<td>As above</td>
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<tr>
<td>Empty transverse fibres to neurosecretory transverse fibre</td>
<td>Axon to axon</td>
<td>Axon contact from symmetrical transverse fibres at empty side</td>
<td>As above</td>
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<tr>
<td>Empty transverse fibre to neurosecretory fibre</td>
<td>Axon to axon</td>
<td>Axon lateral contact. As above</td>
<td>As above</td>
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<tr>
<td>Neurosecretory fibres to empty transverse fibres</td>
<td>Axon to axon</td>
<td>Axon lateral contact. As above</td>
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<td>Neurosecretory fibres to ganglion cell axon</td>
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<td>As above</td>
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<tr>
<td>Neurosecretory fibres to neurosecretory fibres</td>
<td>Axon to axon</td>
<td>Axon lateral contact. As above</td>
<td>As above</td>
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**Sandwich Types:** 7 followed by 9, 3 followed by 10.

**Unit Membrane:** U.m.
and postsynaptic membranes, on the other hand, show no reliable sign of fusion, which is sometimes accepted as an indicator of electrical transmission as in crayfish giant-fibre synapses (Hama, 1961; Robertson, 1962). Although a special rigidity and clarity distinguish membrane which we term synaptic from other membrane, the absolute widths of synaptic clefts must be treated with reserve because they may have been changed by fixation. Retinula cell synapses, however, are distinctly narrower than others and apparent fusion of membranes is considered to be an artifact arising from twisting of the membrane. According to Eccles (1964), effective chemical transmission requires the resistance between the synaptic cleft and the interstitial space to be low and it is difficult to see how this condition can be met where a presynaptic bag is penetrated by spines. The enormous surface area of spine contact provides a situation which is more favourable for electrical transmission, provided that the whole synaptic system is isolated sufficiently to force ionic current to be channelled into the primary spine axoplasm. The similar discussion about mechanisms in smooth muscle is relevant (Lane & Rhodin, 1964), and the glial processes encapsulating the whole cartridges must be taken into account. In the chick ciliary ganglion Martin & Pilar (1963) found a dual, electric and chemical, transmission mechanism. There, the synapse develops into a labyrinthine structure like the spines within a bag described here, but having presynaptic vesicles, membrane thickenings, close membrane apposition in places, and loosely woven myelination (Szentagothai, 1964; Hámori & Dyachkova, 1964; Takahashi & Hama, 1965). A similar dual or even purely electrical transmission for the 1st synapse on the lobster visual pathway is indicated from our morphological findings. Any final statement, however, can be made only after a careful electrophysiological investigation of the cartridges, and in view of their complexity this will be a formidable task.

Whether the retinula fibre synapses are excitatory or inhibitory is a matter of speculation as yet, but comparative physiological data are of interest here. The following examples of arthropod visual cell endings appear to be inhibitory: (a) retinula axon arborizations upon each other in Limulus (presumed from electron-microscope studies); (b) eccentric cell axon arborizations on each other (Tomita, 1958) and at the optic lobe (Wilska & Hartline, 1941); (c) whereas insect retinula cells are small field 'on' units, it is possible to find small field 'off' units rather far peripherally in the insect optic lobe (Horridge, Scholes, Shaw & Tunstall, 1965); and (d) insect ocellus primary visual fibres (Ruck, 1961). Besides these vague indications one can argue that the synapses of one retinula bag upon a neighbouring bag are unlikely to be excitatory, as that would reduce visual acuity, whereas lateral inhibition at this level would enhance contrast. On the other hand we can think of no reason to suggest that the retinula endings are excitatory.

The appearance of synaptic ribbons here and in the insect lamina means that now any generally applicable theory as to their function can be tested on invertebrate as well as vertebrate material. Ribbons are typical of vertebrate primary optic terminations, even in the pineal eye (Kelly & Smith, 1964), and elsewhere in sensory cells are found in the ampulla of Torpedo (Barets & Szabo, 1962) and in the organ of Corti (Sjöstrand, 1958, 1961; Smith & Sjöstrand, 1961). They also occur in the inner plexiform
layer of vertebrates (Sjöstrand, 1960; Kidd, 1962) and are usually between two invaginations from a postsynaptic process and always near to the synaptic membrane. Corresponding studies on invertebrate primary visual endings of the octopus (Dilly, Gray & Young, 1963) and fly (Trujillo-Cenóz & Melamed, 1963) do not mention synaptic ribbons. After destruction of the retinula cell bodies the ribbons disappear very quickly from the retinula bags (to be described in a forthcoming paper, on degeneration), but as yet nothing can be said about their function.

![Fig. 3. Hypothetical scheme of likely interactions between retinula terminals (r), ganglion cell axons (g), empty transverse fibres (e), and neurosecretory transverse fibres (n).](image)

Besides the usual electron-microscope morphological criteria of the direction of excitation at the synapses (see Fig. 3), we have one other piece of evidence that transverse fibres are presynaptic to the ganglion cells. After degeneration of all retinula cell endings following retinal damage the ganglion cells do not show signs of transneuronal degeneration, as might be expected if all their synaptic input had been destroyed.

The striking difference in the ultrastructure of the mitochondria between retinula and other axons, especially those of the ganglion cells, suggests that the mitochondria of retinula endings are aged. A similar interpretation has been made for ctenophore combplate cells, where the mitochondria far from the nucleus have fewer cristae and are shrunken, while those nearer to the nucleus are normal or small and dense (Horridge, 1964). The dense particles within the mitochondria are interpreted as ribosomes (Berger, 1964), and in the lobster lamina the dense mitochondria of the ganglion cell contain a relatively great number of such opaque particles (Fig. 11), while the huge, but withered and empty mitochondria of retinula fibre endings contain few or none. The same differences in density of pre- and postsynaptic mitochondria may be observed (Szentagothai, 1963) in the mammalian lateral geniculate body where the axons of the optic nerve terminate. It seems probable that young mitochondria which contain opaque granules are formed in or near the perikaryon and they have aged by the time that they...
accumulate in the terminations. As a whole, this observation seems to support the hypothesis of axoplasmic flow and accompanying disintegration of mitochondria.

The ultrastructure of the synapses between local transverse fibres presents another feature of general interest. These synapses are specialized, with thickened membranes, and the synaptic vesicles are of the usual size (30–60 m\(\mu\)) and empty even when the fibre itself is crowded with dense secretory vesicles. A similar pattern of vesicles can occasionally be discerned in molluscan ganglia figured by Gerschenfeld (1963). In the glomeruli of the accessory olfactory lobe in the lobster, nerve endings filled with dense-core vesicles have only the normal, empty vesicles opposite the presynaptic membranes. Secretory granules and synaptic vesicles are clearly separate. The reviews of Eccles (1964) and Bullock & Horridge (1965) show that the presence of vesicles, while characteristic of synapses, does not indicate the electrical or chemical nature of transmission.

In conclusion, the columnar region of the optic lamina in the lobster is a synaptic layer of four types of fibre, where the primary visual impulses are integrated in a highly complex manner.

One question which we would like to answer, but cannot for lack of physiological data, is whether the lamina achieves the abstraction of movement perception, as can be done by the ganglion cells of the rabbit (Barlow & Levick, 1965) from the changing pattern of intensities on the retina.

REFERENCES

Lobster lamina synapses


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ABBREVIATIONS

\( as \) ganglion cell spine
\( av \) aposynaptic vesicles
\( ds \) desmosome
\( ga \) ganglion cell axon
\( gp \) glial process
\( m \) mitochondrion
\( mg \) mitochondrial granule
\( ng \) neurosecretory granule
\( nt \) neurotubule
\( ntf \) neurosecretory transverse fibre
\( pm \) presynaptic membrane
\( ps \) postsynaptic membrane
\( re \) retinula ending
\( s \) synapse
\( sp \) primary axon spine
\( sr \) synaptic ribbon
\( ss \) secondary axon spine
\( st \) tertiary axon spine
\( sv \) synaptic vesicle
\( tf \) transverse fibre
\( tfe \) transverse fibre ending
\( ts \) transverse fibre spine

Fig. 4. Electron micrograph of a cartridge in oblique section showing a central ganglion cell axon (\( ga \)) filled with neurotubules, surrounded by large retinula endings (\( re \)) filled with synaptic vesicles and characteristic mitochondria (\( m \)). The retinula endings are penetrated by many small axon spines (\( sp \)) near the centre of the cartridge. At the periphery are a few transverse fibres (\( tf \)) one of which (\( tfe \)), with both normal and neurosecretory vesicles, penetrates the retinula endings with spines (arrows).

Fig. 5. Longitudinal section through the cartridge, with ganglion cell (\( ga \)) identified by neurotubules, large retinula bags (\( re \)) and transverse fibre ending (\( tfe \)) containing synaptic vesicles and dense mitochondria. Many spines are invading the retinula endings, the outer surfaces of which are covered by glial processes (\( gp \)).
Fig. 6. Ganglion cell axon spines, demonstrating the relation between the trunk of the main axon (ga) with the primary (sp), secondary (ss) and tertiary (st) spines. The axon trunk, primary and secondary spines contain neurotubules (nt), but the tertiary spines only large vesicles (av). Synaptic ribbons (sr) in the retinula endings (re) can be seen lying against the bases of the tertiary spines. Mitochondria of retinula endings contrast with those of the ganglion cell.

Fig. 7. High-magnification picture of tertiary spine, with the presynaptic membrane (pm) thicker than the postsynaptic (ps). A few synaptic vesicles (sv) lie near the presynaptic membrane.

Fig. 8. Low-magnification picture of the repeated branching of primary spines from the ganglion cell axon (as shown in Golgi preparations). Synaptic ribbons here lie adjacent to the primary spines.

Fig. 9. Tertiary spines (st) connected to the axon trunk at the point shown by the arrow.

Fig. 10. Secondary and tertiary spines. Neurotubules occur only in the secondary ones; the tertiary are connected with the secondary only by a thin neck.
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Fig. 11. Synaptic ribbons in the retinula endings (re). Close to the presynaptic membrane four synaptic ribbons (sr) lie in a row in relation to the primary (sp) and secondary (st) spine membranes. Near the synaptic ribbons both synaptic membranes are thickened. Other features shown are granules (mg) in the mitochondria, aposynaptic vesicles (av), and tertiary spines (st).

Fig. 12. Detail of retinula fibre endings on ganglion cell axons. Note the symmetrical membrane thickening (ds) between two axon spines and the opaque particles in ganglion cell mitochondria.

Fig. 13. Detail within the cartridge showing retinula ending on ganglion spines, synaptic ribbons (sr) and a neurosecretory transverse fibre (ntf).
Figs. 14–17. Synaptic ribbons (sr) in cross-section (Figs. 14–16) and in longitudinal section (Fig. 17). They are closely packed around with synaptic vesicles. The retinula endings (re) are distinguished from the secondary spines (ss) by the usual criteria.
Fig. 18. Transverse fibre ending \((tfe)\) on the ganglion cell trunk \((ga)\). Arrows show a region of thickened membrane with synaptic vesicles in the transverse fibre.

Fig. 19. Retinula ending \((re)\) upon a transverse fibre which sends a long spine into the retinula ending. Unlike the contacts with ganglion cell spines, there is here a specific accumulation of synaptic vesicles on the retinula fibre side. Arrows show contact areas which stand out as if specifically synaptic.

Fig. 20. Retinula ending upon a transverse fibre, showing synaptic vesicles on both sides. At areas where membranes are thickened, however, (arrows) vesicles mostly accumulate adjacent to the synaptic membrane on the retinula fibre side.

Fig. 21. A symmetrical desmosome \((ds)\) between secondary ganglion cell spines \((ss)\), with thickened membranes and an osmiophilic line along the middle of the intercellular gap.
Fig. 22. Neurosecretory transverse fibres (ntf) in contact with retinula ending (re).

Fig. 23. Contacts (s) between transverse fibres. The neurosecretory fibre containing dense elementary granules (ng) is sandwiched between two small transverse fibre endings (tfe) which contain only normal vesicles.

Fig. 24. Sandwich synapse between three transverse fibres, tfe₁, ntf (with neurosecretory granules, ng), and tfe₂. The large arrows show membrane thickenings. The fibre tfe₁ is also sandwiched between a retinula ending and the neurosecretory fibre ntf. Special relations which are identified as contacts between retinula endings (re₁ and re₂), as well as between retinula endings and transverse fibres, are marked by small arrows.

Fig. 25. Synapse (s) between two neurosecretory fibres. Only normal vesicles accumulate against the region of membrane thickening. Between the neurosecretory fibre (ntf₂) and the retinula ending (re) lies an unidentified axon with microtubules.
Fig. 26. Contacts between two neighbouring retinula endings (re); also a ganglion cell spine (as) with neurotubules.

Fig. 27. Higher magnification of Fig. 26, showing details of the specialized contact between two cells. Synaptic vesicles accumulate on both sides (arrows). At the contact area there are four unit membranes, two of which belong to a cisterna of the endoplasmic reticulum lying against the cell membrane.
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