Neural Organization of the Median Ocellus of the Dragonfly

II. Synaptic structure

JOHN E. DOWLING and RICHARD L. CHAPPELL

From The Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, and the Department of Biological Sciences, Hunter College of the City University of New York, New York 10021

ABSTRACT Two types of presumed synaptic contacts have been recognized by electron microscopy in the synaptic plexus of the median ocellus of the dragonfly. The first type is characterized by an electron-opaque, button-like organelle in the presynaptic cytoplasm, surrounded by a cluster of synaptic vesicles. Two postsynaptic elements are associated with these junctions, which we have termed button synapses. The second synaptic type is characterized by a dense cluster of synaptic vesicles adjacent to the presumed presynaptic membrane. One postsynaptic element is observed at these junctions. The overwhelming majority of synapses seen in the plexus are button synapses. They are found most commonly in the receptor cell axons where they synaptically contact ocellar nerve dendrites and adjacent receptor cell axons. Button synapses are also seen in the ocellar nerve dendrites where they appear to make synapses back onto receptor axon terminals as well as onto adjacent ocellar nerve dendrites. Reciprocal and serial synaptic arrangements between receptor cell axon terminals, and between receptor cell axon terminals and ocellar nerve dendrites are occasionally seen. It is suggested that the lateral and feedback synapses in the median ocellus of the dragonfly play a role in enhancing transients in the postsynaptic responses.

INTRODUCTION

The dorsal ocelli of insects are simple eyes that are advantageous for the study of receptor and synaptic mechanisms. The preceding paper described the pre- and postsynaptic potentials recorded intracellularly in the median ocellus of the dragonfly (Chappell and Dowling, 1972). It was concluded from these experiments that (*a*) light evokes a graded depolarizing potential in the receptors which is responsible for mediating synaptic transmission between receptors and ocellar nerve dendrites, and (*b*) the primary effect of the receptor cell synapse is to hyperpolarize the dendrites of the second-order fibers, thus

inhibiting the spontaneous activity of these units for as long as the light persists.

As preface to further work characterizing the properties of this well-isolated synapse, study of the synaptic structure and organization in the median ocellus of the dragonfly was undertaken. In particular it was hoped that some insight might be obtained on possible mechanisms underlying the alterations in the form of the responses that are observed across the synapse. For example, it was shown in the preceding paper that postsynaptic responses are much more phasic than is presynaptic activity. No electron micrographs of the synaptic structures in the dragonfly ocelli have been published, although Ruck and Edwards (1964) in a preliminary report on the fine structure of the dragonfly ocellus have provided some low power micrographs of the neuropil region (see also Goodman, 1970).

METHODS AND MATERIALS

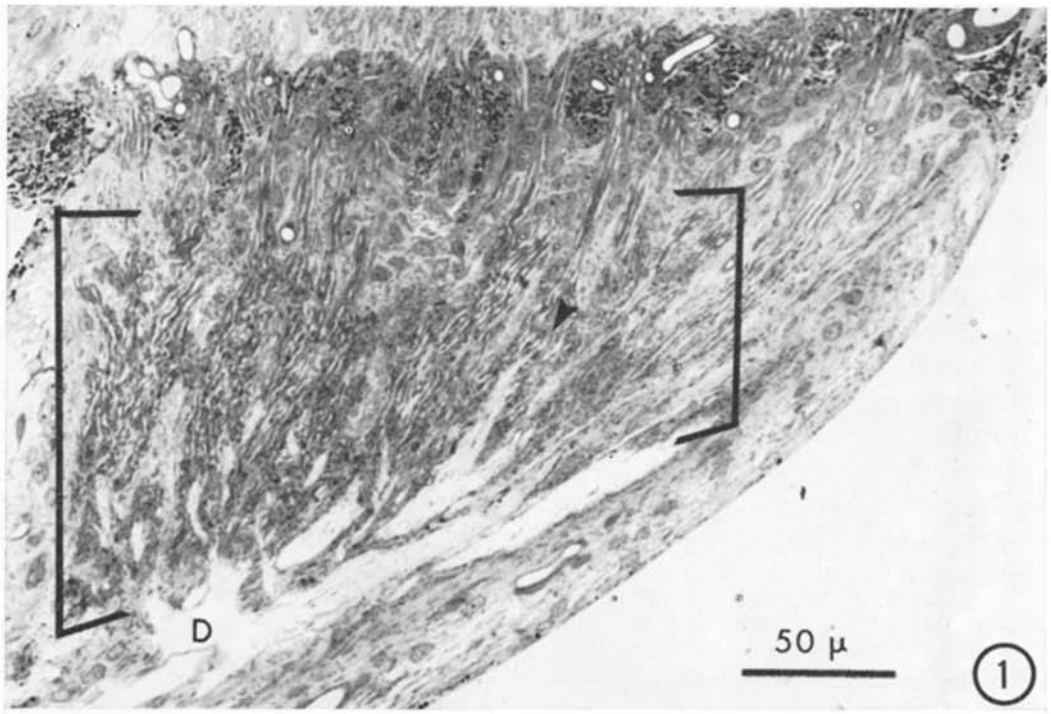
Two species of dragonfly were used in the present study, *Anax junius* and *Aeschna tuberculifera*; no differences in synaptic structure or organization were observed between these species. The median ocellus was exposed as it would have been for physiological recording (Chappell and Dowling, 1972) and flooded with cold fixative. After 10–20 min, the ocellus was carefully dissected out intact, and fixation was continued at room temperature for an additional hour. The fixative consisted of 2% OsO_4 buffered with 0.14 M Veronal acetate containing 1% $CaCl_2$ and 45 mg/ml sucrose. Following fixation, the tissues were dehydrated in graded ethanol-water mixtures and embedded in Araldite epoxy resin (Ciba Products Co., Summit, N.J.).

For light microscopy 2–3-μ thick sections were cut on a Porter-Blum MT-2 microtome (Ivan Sorvall, Inc., Norwalk, Conn.) and stained by the Richardson (1964) method. For electron microscopy, thin sections were cut on the same microtome, doubly stained with uranyl acetate and lead citrate, and studied in a JEM 100-b electron microscope (JEOL U.S.A., Inc., Medford, Mass.).

RESULTS

Light Microscopy

Figs. 1 and 2 are light micrographs of the synaptic region of the median ocellus of the dragonfly. The synaptic area (in brackets, Fig. 1) lies between the thin layer of shielding pigment cells and the initial branching points of the larger ocellar nerve dendrites (*D*). Fig. 1 is a section cut in parallel with several ocellar nerve dendrites; Fig. 2 is a section cut in parallel with receptor axons and across two large ocellar nerve dendrites. Branching of the dendrites into smaller and smaller processes as they ascend into the synaptic region can be seen in both micrographs. Cajal (1918) counted more than 1500 retinular cell axons entering the retinal plexus in the median ocellus of the dragonfly. On the other hand, only 25–30 fibers are observed in the ocellar nerve, of which two to three are particularly large (15–20 μ in diameter) (Cajal, 1918).



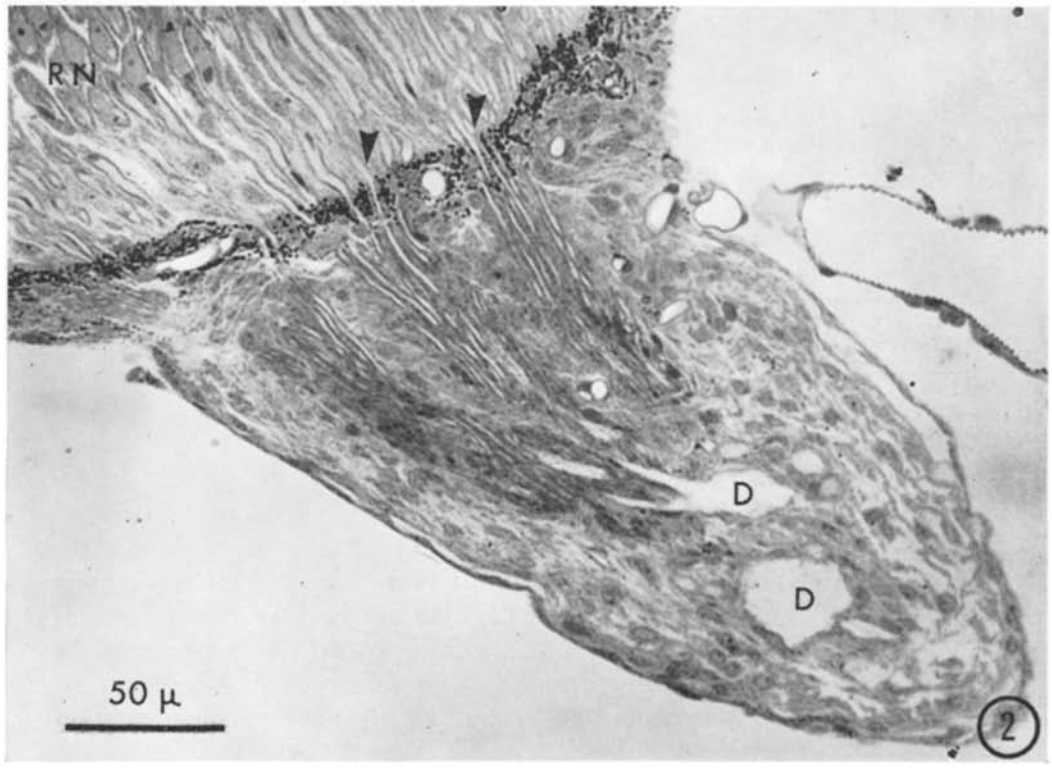


FIGURE 1. Light micrograph of the synaptic plexus in the median ocellus of the dragonfly (brackets). Ocellar nerve dendrites (*D*) branch into finer and finer processes (arrowhead) as they ascend into the synaptic region. × 400.

FIGURE 2. Light micrograph showing photoreceptor axons passing through the pigment layer (arrowheads) into the synaptic plexus. Receptor cell nuclei (*RN*) are seen in the upper left corner of the micrograph. Two large ocellar nerve dendrites (*D*), cut in cross-section, lie just below the synaptic zone. Two branches from one of the dendrites ascend into the plexus. × 425.

The photoreceptor axons extend from the receptor nuclei (*RN*) through the pigment layer well down into the synaptic region (arrowheads, Fig. 2). Cell perikarya seen around the synaptic region and occasionally within it are believed to be glial sheath cells (Cajal, 1918; Ruck and Edwards 1964). Brief examination of these cells by electron microscopy also suggests that these cells are nonneural elements. Thus, it appears that in the synaptic region of the dragonfly ocellus, only two types of neural processes interact, i.e., the photoreceptor axon terminals and the dendrites of the ocellar nerve fibers whose perikarya are located in the brain. No centrifugal fibers have been described in the ocellar nerve in the dragonfly (Cajal, 1918), and we have not observed any processes by either light or electron microscopy that appear to be centrifugal fibers.

Electron Microscopy

Fig. 3 is a survey electron micrograph of the synaptic region of the ocellus. Two ocellar nerve dendrites (*D*) are readily recognized at the bottom of the micrograph by virtue of their large size and relatively clear cytoplasm. Photoreceptor axon terminals (*RT*) may also be readily recognized by the abundant synaptic vesicles they contain, and which give many of the terminals an even, granular appearance at this low magnification. In the larger receptor axon terminals the synaptic vesicles are clustered near the plasma membrane, leaving the central core of the axon relatively clear. Nevertheless, with careful examination, the photoreceptor terminals are easily distinguishable from the ocellar nerve dendrites.

Two types of presumed synaptic contacts have been recognized in the synaptic region of the dragonfly median ocellus. The first type bears a striking resemblance to the dyad synapses seen in the inner plexiform layer of the vertebrate retina (Fig. 4 *a*) (Dowling and Boycott, 1966). Internal to small protrusions along the presynaptic cell membrane, a prominent, round, intracytoplasmic electron-opaque body, some 400–500 A in diameter, is observed surrounded by a cluster of synaptic vesicles. The dense body is separated from the plasma membrane by a clear space of about 50 A, and some substructure of the organelle, consisting of a denser rim closer to the plasma membrane, may often be distinguished (insert, Fig. 4 *a*). Two postsynaptic processes are associated with these synapses, and specializations consisting of added electron opacity and a subsynaptic web are usually seen on both postsynaptic membranes. A widened synaptic cleft is usually observed, and in the cleft, filamentous material may occasionally be resolved.

Associated with the dyad synapses of the inner plexiform layer in the vertebrate retina is an electron-opaque ribbon or bar (Kidd, 1962; Dowling, 1970). In the dragonfly ocellus the electron-opaque intracytoplasmic organelle associated with the synapse has more of a button appearance, but in other respects

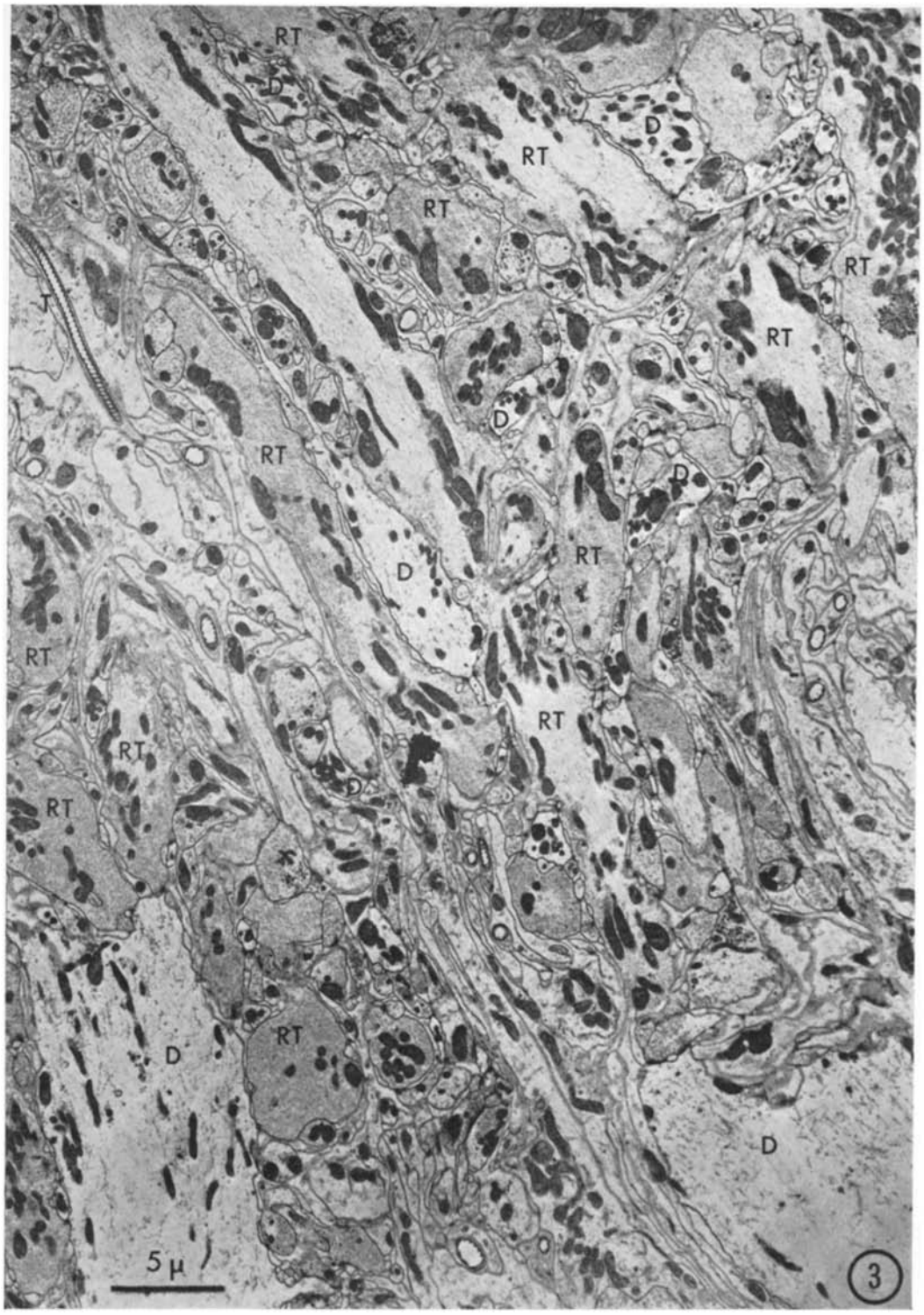


FIGURE 3. Low magnification electron micrograph of the synaptic plexus in the median ocellus of dragonfly. Dendrites (*D*) of the ocellar nerve fibers are recognized by their relatively clear cytoplasm and/or large size (bottom of micrograph). Receptor terminals (*RT*) contain numerous synaptic vesicles which give much of the terminal an even, granular appearance. *T*, tracheole. × 3500.

the two types of synapses appear remarkably alike. We shall refer to the dyad synapses in the ocellus of dragonfly as *button* synapses.

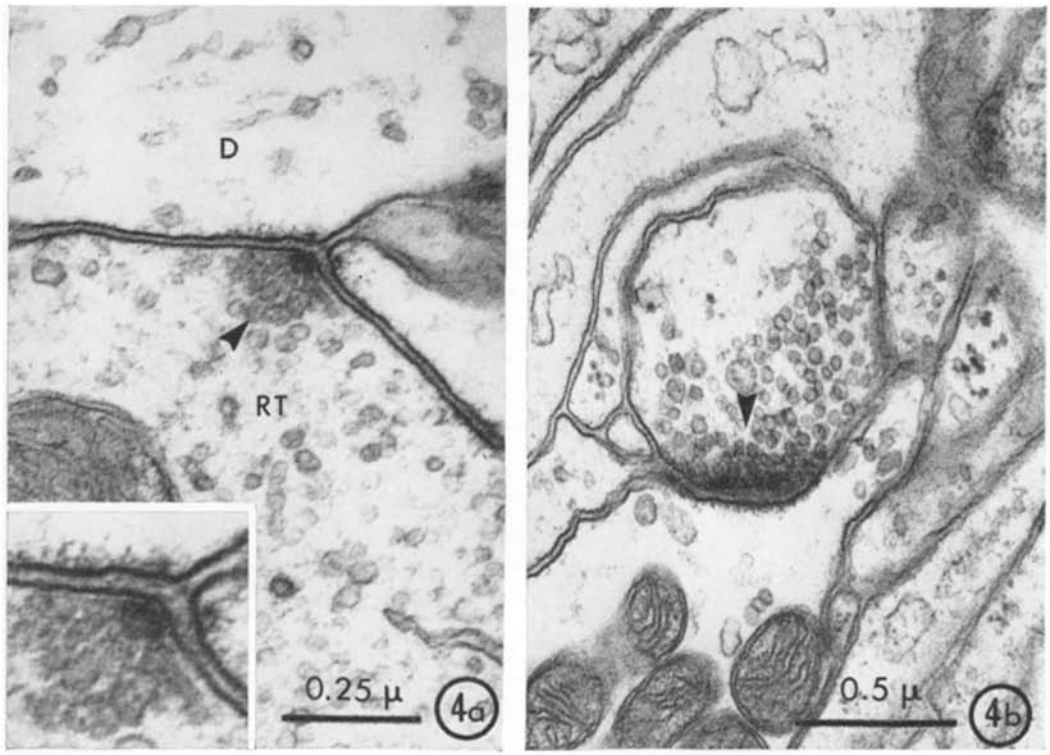
The second type of synapse observed in the dragonfly ocellus is similar in appearance to synapses described throughout vertebrate and invertebrate nervous systems (Gray and Guillery, 1966), and we shall call it a *conventional* synapse (Fig. 4 *b*). It is characterized by a dense aggregation of synaptic vesicles clustered close to the presumed presynaptic membrane. Some increased membrane density on both pre- and postsynaptic sides of the membrane is observed, as well as a subsynaptic web along the postsynaptic membrane. Only one postsynaptic element is present at these synapses.

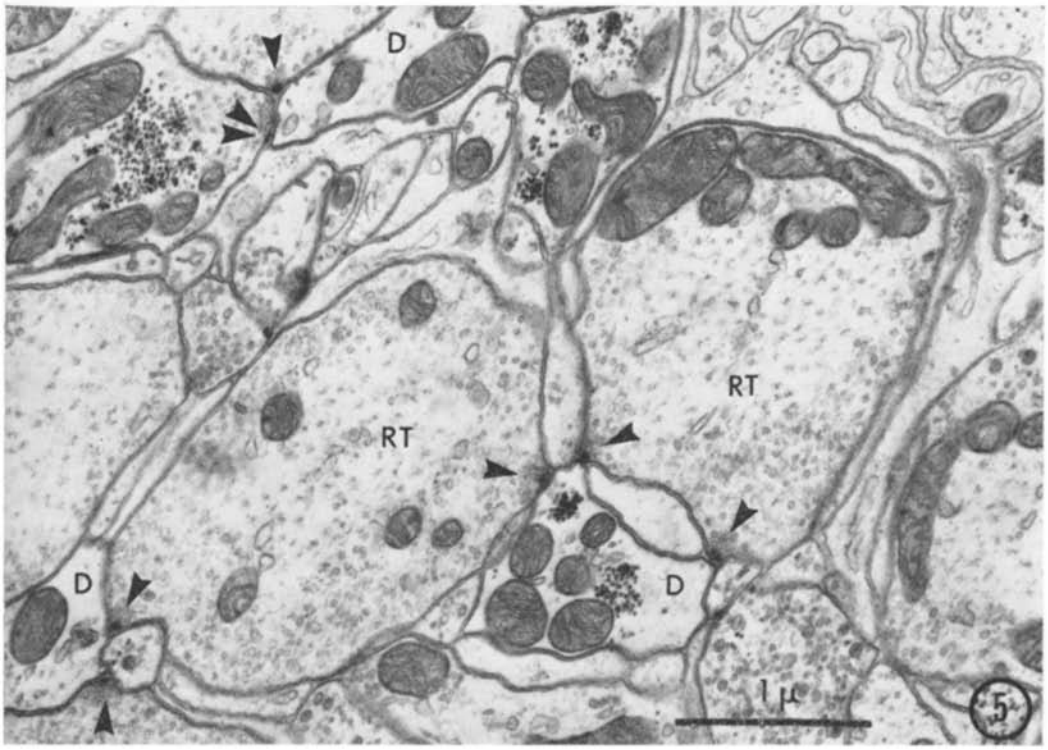
The overwhelming majority of synapses seen in the neuropil region of the dragonfly median ocellus are the button synapses. So far only about a dozen unequivocally identified conventional synapses have been seen; these were all in small processes containing abundant synaptic vesicles and presumably were receptor terminals. Fig. 5 shows a cross-section of several receptor terminals making numerous button synapses (arrowheads). Usually at the button synapses, one of the postsynaptic processes is an ocellar nerve dendrite. These are characterized, as noted above, by the relatively clear appearance of their cytoplasm. The second postsynaptic process at the dyad synapse often contains synaptic vesicles, and occasionally this process can itself be seen to make a button synapse (double arrowhead, upper left corner of Fig. 5).

These observations suggest that photoreceptor axon terminals synapse on adjacent photoreceptor axon terminals, as well as second-order fibers. That this is indeed the case is shown in Figs. 6 and 7. Synapses between receptor terminals are most easily identified more distally in the synaptic region where the photoreceptor axons run closely parallel to one another (Fig. 6). Small branches from adjacent photoreceptor axons extend outward and contact neighboring photoreceptor axons. In Fig. 7 *a*, a button synapse is made by the axon terminal onto the contacting branch; in Fig. 7 *b*, the synapse is made by the branch process extending over to the adjacent receptor terminal.

Fig. 8 shows some of the complex arrangements of contacts between receptor terminals. The terminal (RT_1) at the bottom of the micrograph synapses onto two vesicle-filled processes (arrowhead); one of these, a larger receptor terminal (RT_2), synapses in turn onto two further processes (arrowhead). One of these postsynaptic elements is clearly a dendrite of a second-order fiber (D); the other (RT_3) contains vesicles and appears to be making another synapse (double arrowhead) on yet another terminal (RT_4). Three or more *serial* synapses between four receptor terminals have been seen, as here, in several single sections, and this suggests that in consecutive sections more extensive serial synapses between receptor terminal axons would undoubtedly be observed.

The terminal marked RT_4 in Fig. 8 also makes a button synapse back onto


D
RT
0.25 μ
4a
0.5 μ
4b


D
RT
RT
D
D
1 μ
5

terminal RT_3. Thus a *reciprocal* synaptic junction is suggested between terminals RT_3 and RT_4. Fig. 9 shows a clearer example of a reciprocal synaptic arrangement between two large receptor terminals. Reciprocal synapses between receptor terminals have been observed in single sections several times, as here, and we have no doubt that they would commonly be found if serial sections were studied.

The large ocellar nerve dendrites are easy to identify unequivocally in the plexus simply because of their size. While following some of these large dendrites and their branches into the plexus region, a surprising observation was made. Small groups of synaptic vesicles are occasionally observed in the dendrites, clustered close to the plasma membrane. More careful searching shows typical button synapses in the dendrites in association with the vesicles. The two postsynaptic elements contacted at these button synapses are usually another second-order dendrite and a synaptic vesicle-containing process, which itself may make a button synapse.

Fig. 10 shows a portion of a dendrite branch that was followed into the plexus from a large primary dendrite. Overall it has the clear appearance of a typical dendrite branch. Synapses onto the dendrite from typical receptor terminals (RT) are marked by arrowheads. At the bottom of the micrograph a portion of the dendrite, which is magnified in the insert of Fig. 10, is seen to contain a cluster of vesicles and make a typical button synapse. More synaptic vesicles are seen here than are usually observed near the button synapses of the second-order fibers.

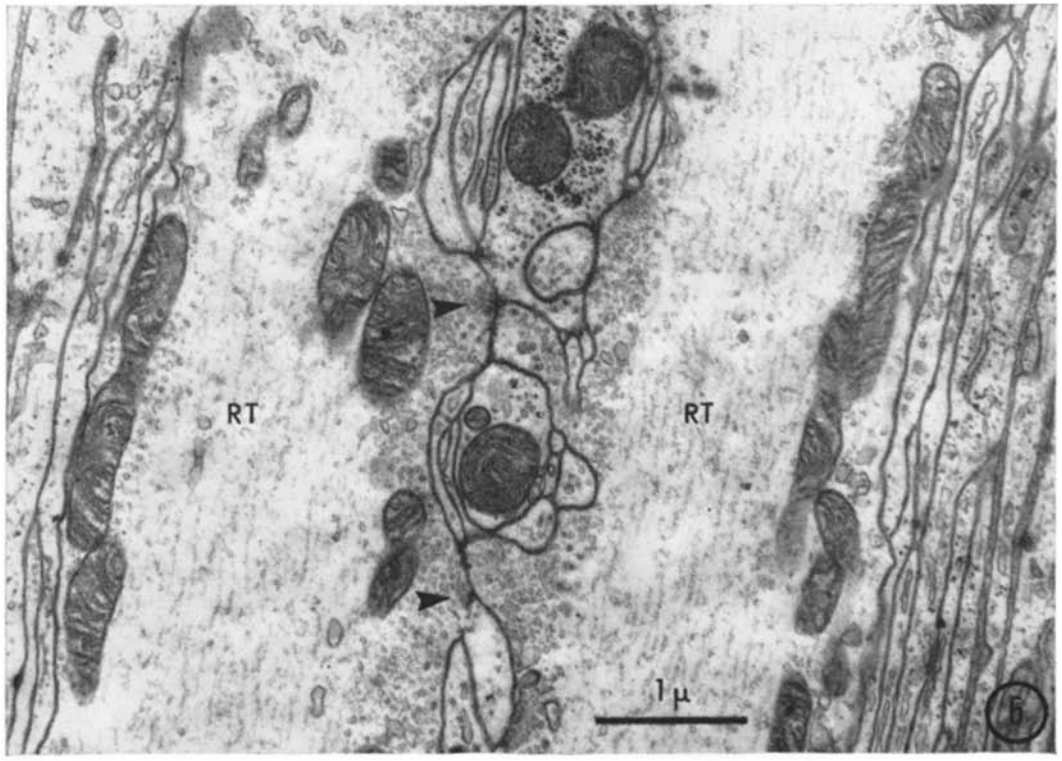
Fig. 11 shows a more typical distribution of synaptic vesicles in relation to a button synapse found in a very large dendrite (D_1) just as it passed into the plexus region. This button synapse synapses on a second dendrite (D_2) as well as a vesicle-filled process. The vesicle-filled process (probably a receptor ter-

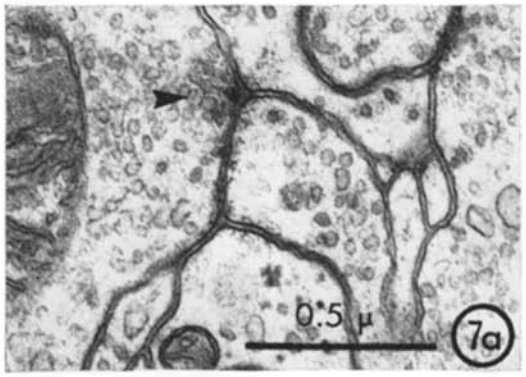
---

FIGURE 4 *a*. Typical synapse in the plexus of the dragonfly ocellus. The synapse is characterized by an electron-opaque button-like structure in the presynaptic cytoplasm (*insert*) which is surrounded by a cluster of synaptic vesicles. Two postsynaptic processes are associated with these synapses. Filamentous material may be resolved in the widened synaptic cleft, and a subsynaptic web is seen on the membrane of both postsynaptic processes. × 74,000; *insert*, × 150,000.

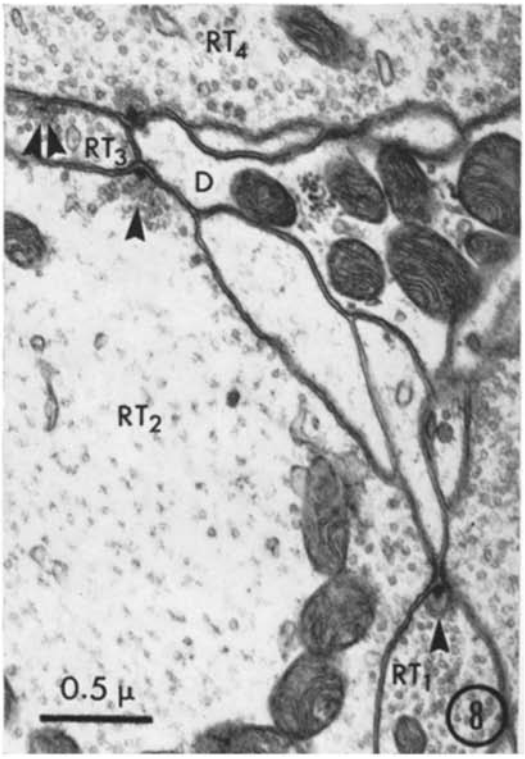
FIGURE 4 *b*. A rare conventional-type synapse in the synaptic plexus (arrowhead). These synapses are characterized by a dense aggregation of synaptic vesicles close to the presumed presynaptic membrane, a widened synaptic cleft, and a subsynaptic web on the membrane of the single postsynaptic process. × 42,000.

FIGURE 5. Cross-section of receptor terminals (RT) showing button synapses (arrowheads). Two postsynaptic processes are seen typically at each contact. One of the postsynaptic processes can often be identified as an ocellar nerve dendrite (D). The other postsynaptic process usually contains synaptic vesicles and occasionally can be observed to make a button synapse (double arrowhead, upper left corner). × 26,000.


RT
RT
1 μ
5


0.5 μ
7a


0.5 μ
7b


RT4
RT3
D
RT2
RT1
0.5 μ
8

minal) appears to be making a reciprocal synapse back onto the dendrite (double arrowhead). Synapses made by the ocellar nerve dendrites are seen much less frequently than those made by the photoreceptor axons. Nevertheless, a search along any good-sized dendrite for any significant distance invariably shows up one or more synapses.

Fig. 12 is a summary diagram of the pattern of synaptic contacts believed to exist in the plexus of the median ocellus of the dragonfly. Dyad synapses of the receptor terminals (*RT*) are made onto the ocellar nerve dendrites (*D*) as well as adjacent receptor terminals. Occasionally, both postsynaptic processes may be receptor terminals or both ocellar nerve dendrites. Reciprocal synapses between receptor terminals are also seen. Button synapses are observed in the dendrites of the ocellar nerve fibers; these synapses appear to be made back onto receptor terminals as well as adjacent second-order dendrites. Reciprocal synaptic relationships between second-order dendrites and receptor terminals are also suggested. Conventional synapses are only rarely observed in the plexus. Those seen so far were made by receptor terminals onto dendrites of the second-order fibers and also onto other receptor terminals (not shown).

DISCUSSION

The striking similarity between the dyad synapses in the ocellus of the dragonfly and the dyad synapses in the inner plexiform layer of the vertebrate retina is intriguing. Similar dyad synapses have also been described at the first synaptic relay of the spider eye (Trujillo-Cenoz, 1965 *a*) and in the neural plexus of the *Limulus* lateral eye (Whitehead and Purple, 1970). The principal morphologic variation between these synapses appears to be the form of the intracytoplasmic dense body associated with the synapse. In the vertebrate retina it is a dense ribbon-like structure (Kidd, 1962; Dowling and Boycott, 1966), and in the dragonfly, a dense, button-like organelle. In the spider visual system, density near the presynaptic plasma membrane is observed, but

---

FIGURE 6. A longitudinal section of two receptor axons in the distal half of the plexus. Fine branches extend outward from receptor axons to contact adjacent axons. Synaptic junctions between receptor terminals at these contact points are marked by arrowheads. *RT*, receptor terminals. × 20,000.

FIGURES 7 *a* and 7 *b*. Higher magnification micrographs of the synaptic junctions (arrowheads) between receptor terminals. In (*a*), a button synapse is made by the axon terminal onto the contacting branch; in (*b*), the synapse is made by the branch onto the axon terminal. (*a*), × 50,000; (*b*), × 40,000.

FIGURE 8. Complex synaptic arrangements in the plexus of the ocellus. Terminal RT_1 synapses onto RT_2, which in turn synapses onto RT_3 forming a serial synaptic arrangement (arrowheads). RT_3 may also be making a synaptic contact on terminal RT_4 (double arrowhead). RT_4 synapses back onto RT_3 suggesting a reciprocal synaptic arrangement between terminals RT_3 and RT_4. × 30,000.

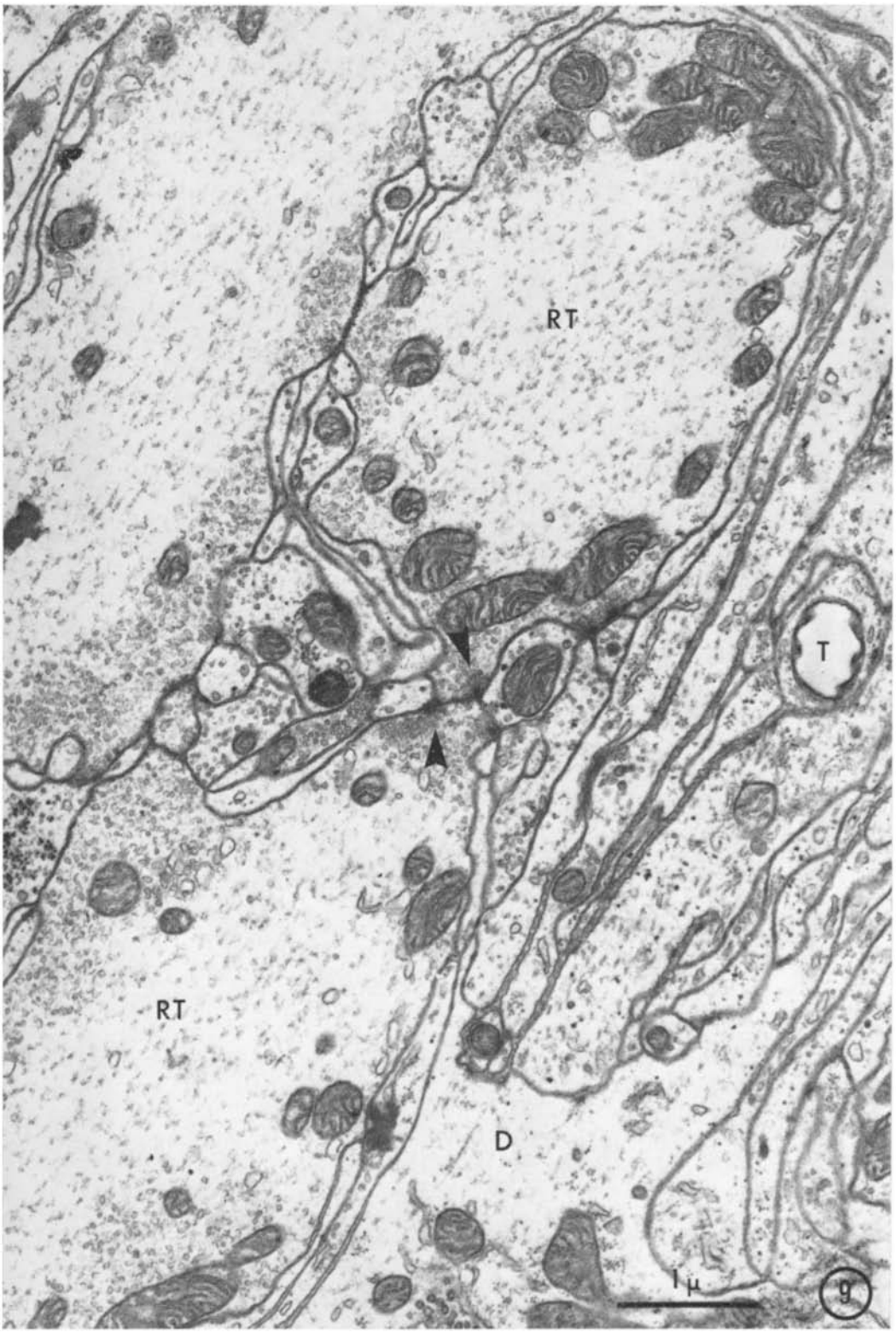
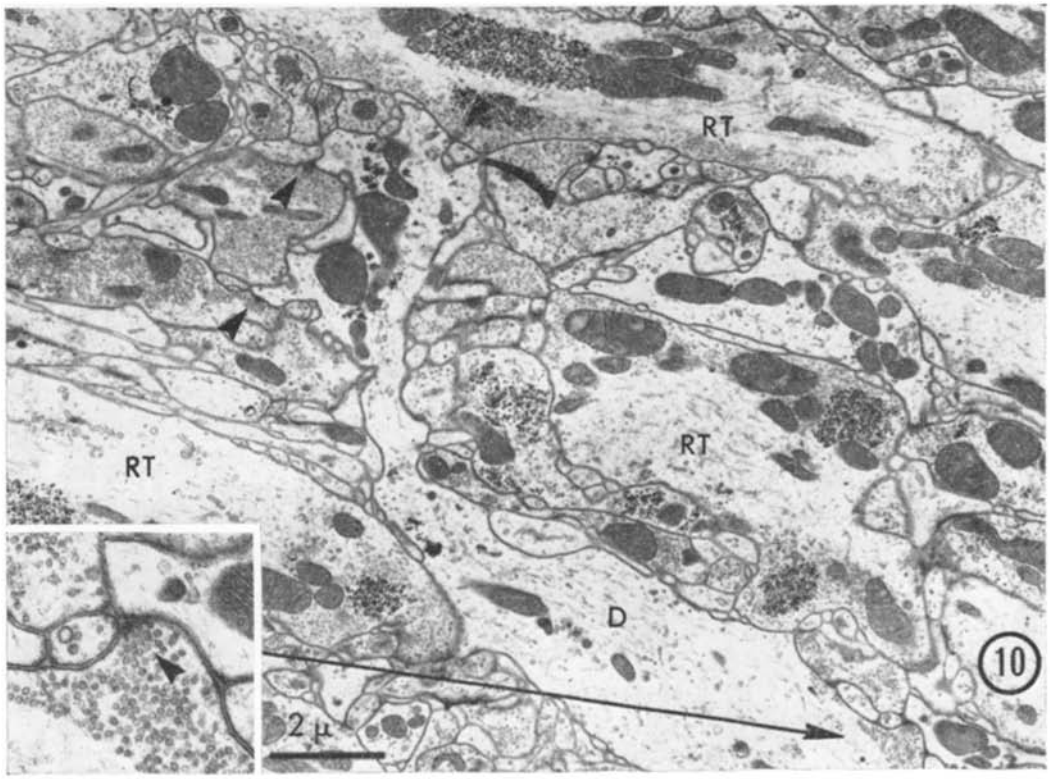


FIGURE 9. A reciprocal synaptic arrangement between two receptor terminals (*RT*). Arrowheads point to the two button synapses contributing to the junction. *D*, ocellar nerve dendrite; *T*, tracheole. × 22,000.



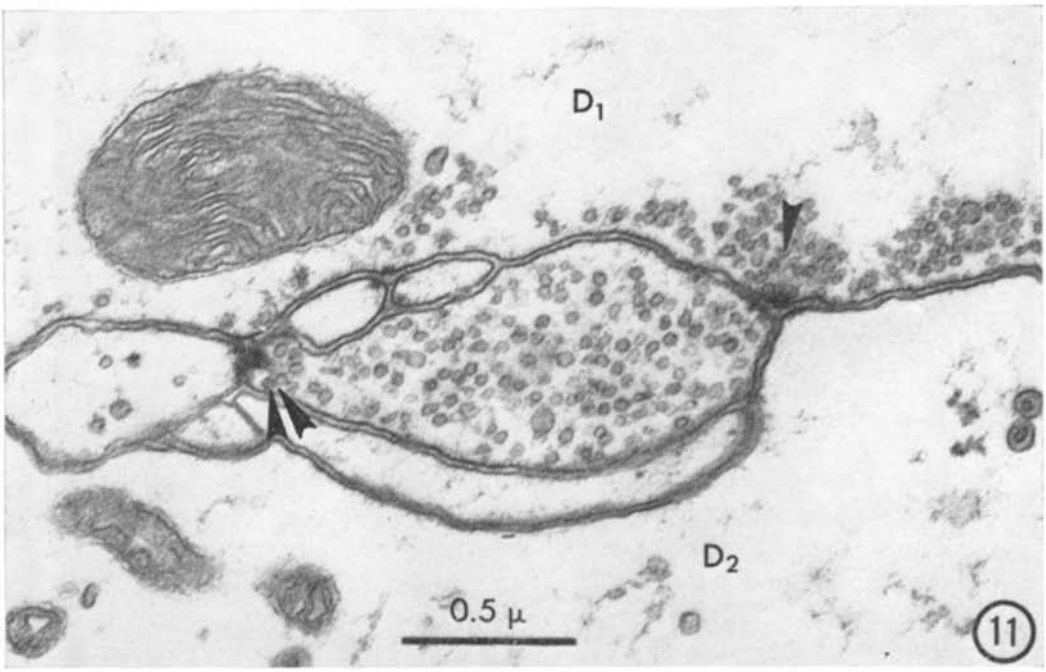


FIGURE 10. An ocellar nerve dendrite (D) in the synaptic plexus. Synaptic contacts onto the dendrite are marked by arrowheads. The *insert* shows at higher magnification a button synapse made by the dendrite onto two other processes. RT, receptor terminal. × 8000; *insert*, × 27,000.

FIGURE 11. A button synapse (single arrowhead) in a large ocellar nerve dendritic branch (D_1). One of the postsynaptic processes is another dendritic branch (D_2); the other contains numerous synaptic vesicles and appears to be making a reciprocal synapse back onto dendrite D_1 (double arrowhead). × 46,000.

also a much less dense rodlike structure extends away from the synapse (Trujillo-Cenoz, 1965 *a*). In *Limulus*, an elongated triangular density, its base adjacent to the plasma membrane, has been described (Whitehead and Purple, 1970). In other respects, however, these various synapses strongly resemble each other. The intracytoplasmic dense body fits in a corner or small evagination of the presynaptic process and is directed between two postsynaptic elements. Some specializations are observed on the presynaptic and both postsynaptic membranes, and a widened synaptic cleft is usually present.

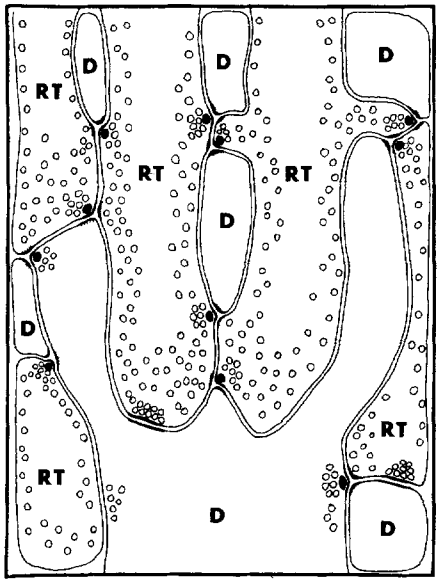


FIGURE 12. A summary diagram of the arrangement of synaptic contacts observed in the plexus of the dragonfly median ocellus. Button synapses are made by the receptor terminals (*RT*) onto other receptor axon terminals and onto the dendrites of ocellar nerve fibers (*D*). Button synapses are also observed in ocellar nerve dendrites feeding back onto receptor terminals and onto other ocellar nerve dendrites. Occasional conventional contacts are observed in the plexus; these are contacts between receptor terminals and ocellar nerve dendrites or between receptor axon terminals (not shown).

In other invertebrate visual systems also, synapses with features suggestive of ribbon-dyad contacts have been reported. For example, a presynaptic ribbon-like structure has been observed at synapses in the optic lamina of the lobster (Hamori and Horridge, 1966) and, in the fly lamina (Trujillo-Cenoz, 1965 *b*; Boschek, 1971) and fleshfly ocellus (Toh et al., 1971), a presynaptic structure having the form of a T is seen at sites of presumed synaptic contact. In the lamina of the honeybee, "electron-opaque material" has been reported as a feature of the presynaptic side of the synapse (Varela, 1970), but further resolution of this presynaptic structure has not been provided.

The significance of ribbon synapses in the vertebrate retina and elsewhere

in the vertebrate nervous system has long been a puzzle. They are found associated with several types of receptors including photoreceptors (Sjostrand, 1958), cochlear hair cells (Smith and Sjostrand, 1961), and electroreceptors (Barets and Szabo, 1962), as well as the bipolar cells in the inner plexiform layer of the retina (Dowling and Boycott, 1966). Only the ribbon synapses of the bipolar terminals consistently have two postsynaptic terminals, and it is these synapses that most closely resemble the dyad synapses of the invertebrate visual systems. Many of the processes postsynaptic at these dyad synapses have been shown to make reciprocal synapses. This has been demonstrated anatomically in the inner plexiform layer of the vertebrate retina (Dowling and Boycott, 1966; Dowling, 1970), in the dragonfly ocellus, spider visual system (Trujillo-Cenoz, 1965 *a*), and *Limulus* plexus (Whitehead and Purple, 1970). In the *Limulus* eye, physiological evidence for reciprocal synaptic interaction between units has been provided (Ratliff et al., 1963; Lange et al., 1966).

Observations made by Trujillo-Cenoz (1965 *a*) on the first synaptic region of the spider eye resemble closely many of the observations reported here on the dragonfly ocellus. This is perhaps not surprising since the spider visual system consists only of ocelli-type eyes. For example, in the spider eye, reciprocal dyad synapses between the photoreceptor axons and one of the postsynaptic elements were shown. However, all the postsynaptic elements at the dyad synapses of the spider photoreceptor terminals were described as "second-order fibers," and it was not suggested that the postsynaptic element feeding back could be a process from an adjacent photoreceptor terminal. In the ocellus of the dragonfly, it appears that reciprocal, feedback synapses onto the photoreceptor axons are made both by processes from adjacent photoreceptor terminals and the dendrites of second-order terminals.

The role of the conventional synapses in the dragonfly ocellus is unclear. Like conventional synapses seen elsewhere, they contact only a single postsynaptic element. Conventional synapses have been seen on the dendrites of the second-order fibers as well as on photoreceptor axon terminals. Too few observations on the conventional synapses in the dragonfly ocellus have been made so far to suggest what special role, if any, they may have.

Largely due to the elegant work carried out in the *Limulus* visual system, it is generally assumed that the observation of lateral and reciprocal innervation between receptors or higher-order units along sensory pathways indicates an inhibitory system which serves to enhance contrast between elements and hence improve image discrimination (see, for example, Sjostrand, 1961; Dowling and Boycott, 1966). It seems unlikely that this is the primary role of the lateral and reciprocal interconnections between both the photoreceptor axon terminals and the second-order dendrites that we have described here. From simple anatomical considerations, it would appear that ocelli are involved more in detecting changes in levels of illumination than in discriminat-

ing images. For example, Cajal (1918) counted some 1500 retinular cells in the median ocellus of the dragonfly which he stated synapse with the dendrites of just 25–30 postsynaptic neurons. Image discrimination at best must be very rudimentary in these eyes.

On the other hand, the ocellus is organized as an efficient light detector. The high degree of convergence of the photoreceptors on the large second-order units appears designed to enhance effective photosensitivity of the eye. That this is the case is demonstrated in the preceding paper which shows that the second-order units have an apparent sensitivity 1–2 log units greater than the individual receptors (Chappell and Dowling, 1972). In a photoreceptor system such as this, designed primarily for high photosensitivity, extensive lateral and feedback interactions between units for enhancement of image contrast would not appear useful.

However, it has recently been emphasized that the lateral and self-inhibitory mechanisms in *Limulus* serve another function, and that is to sharpen "on" activity and to generate an "off" overshoot in the eccentric cell response (Ratliff et al., 1963; Hartline, 1969). As a result of the interplay of excitatory and inhibitory interactions in the *Limulus* eye, eccentric cell activity is made much more phasic, and activity in the optic nerve may be limited to "on-off" and pure "off" responses by appropriate adjustment of background and stimulus conditions (Ratliff and Mueller, 1957).

In the dragonfly ocellus transient "off" responses are readily produced postsynaptically, and in some situations "on-off" responses can be generated in the ocellar nerve by proper adjustment of background and stimulus conditions. Examination of the intracellular activity in the ocellus shows that postsynaptic activity is much more phasic in nature than presynaptic activity even in the dark-adapted eye, and with dim background light on the ocellus, all postsynaptic activity is reduced to "on" and "off" transients. We postulate, therefore, that the extensive system of lateral and feedback synapses suggested by our anatomical observations are related to the enhancement of the "on" transient and to the generation of the prominent "off" depolarizing overshoot in the postsynaptic response of the dragonfly median ocellus.

It is difficult to suggest specifically the mechanisms by which the lateral and feedback synapses can enhance transients in the postsynaptic activity in the dragonfly ocellus. As in *Limulus* we presume that the enhancement of transients is dependent on time-delayed negative feedback. However, unlike *Limulus* such interactions in the dragonfly ocellus would not appear dependent on spike activity in either pre- or postsynaptic units since postsynaptic activity is not altered by tetrodotoxin. Also the *recorded* presynaptic activity does not show obvious enhancement of transients. It is possible that the input fed back onto the receptor terminal results primarily in local changes that are not recorded distally in the photoreceptor cell.

In the vertebrate retina, the transition from tonic-to-phasic type of response occurs at the bipolar-amacrine junction (Werblin and Dowling, 1969; Dowling, 1970) which has been shown in all vertebrate retinas so far examined to be a reciprocal synaptic arrangement. That is, the amacrine process synaptically feeds back onto the bipolar terminal just adjacent to the synapse of the bipolar terminal onto the amacrine process (Dowling and Boycott, 1966; Dowling, 1970). However, evidence for a transient input from amacrine cells is not recorded in bipolar cells even when amacrine cells are specifically stimulated (Werblin, 1972).

Examination of the receptor response in dragonfly does show some suggestion of feedback activity, however, especially at the termination of the light stimulus. A fast, oscillatory "off" response that may transiently drive the membrane potential below resting membrane potential is observed in the receptor intracellular response (Chappell and Dowling, 1972). This "off" response is particularly obvious when low intensity sustained flashes are used, and it may sometimes be seen when the rest of the receptor potential is of too low an amplitude to be detected. Occasionally, similar oscillatory activity is observed at the beginning of the receptor potential, just subsequent to the spike. Fig. 13 shows such oscillatory activity in the receptor response at both "on" and "off" of light and in the presence of tetrodotoxin at a concentration of 2×10^{-7} g/ml Ringer solution. The oscillatory activity in the receptor response is not affected by tetrodotoxin; it therefore is not impulse dependent, and presumably not the result of activity back from the brain. It would appear rather to be locally mediated, presumably by slow potentials, and we suggest it may be as a result of the synapses back onto the receptor terminals.

It is interesting to note, in light of the above postulate, that Alawi and Pak (1971) have recently observed a similar small oscillatory potential at "off" of the Drosophila retinular cell response recorded intracellularly. This "off" response is absent in a mutant of Drosophila, *X-7*, which otherwise has a receptor response identical to the wild type. Alawi and Pak (1971) report that there are no obvious histological differences between the retina and lamina of mutant *X-7* and the wild type, but no details of synapses in the Drosophila lamina have been provided. It would appear worthwhile to compare synaptic organization in mutant *X-7* and wild-type forms of Drosophila to see if the absence of the "off" response in the mutant is related to a lack of reciprocal or lateral synapses in the lamina.

In conclusion, a surprisingly complex organization of lateral and reciprocal synapses is suggested in the plexus of the dragonfly ocellus. It seems possible that these synapses play a role of enhancing transients in the postsynaptic response and in generating the prominent "off" discharge in the ocellar nerve. In the vertebrate retina, similar complex synaptic arrangements are found in the inner plexiform layer, and it is in this synaptic plexus in the vertebrate that

light-evoked activity changes from predominately sustained (tonic) responses to predominately transient (phasic) responses (Werblin and Dowling, 1969). A further interesting analogy is that across both synaptic plexuses there is a change in polarity of the principal light-evoked responses. In the vertebrate, responses presynaptic to the inner plexiform layer are primarily hyperpolarizing, while postsynaptic responses are primarily depolarizing. In the dragonfly ocellus (and in the first lamina of other invertebrate visual systems) presynaptic responses are depolarizing, while postsynaptic responses are primarily hyperpolarizing. The transient responses in the inner plexiform layer of the vertebrate are believed to play an important role in accentuating temporal

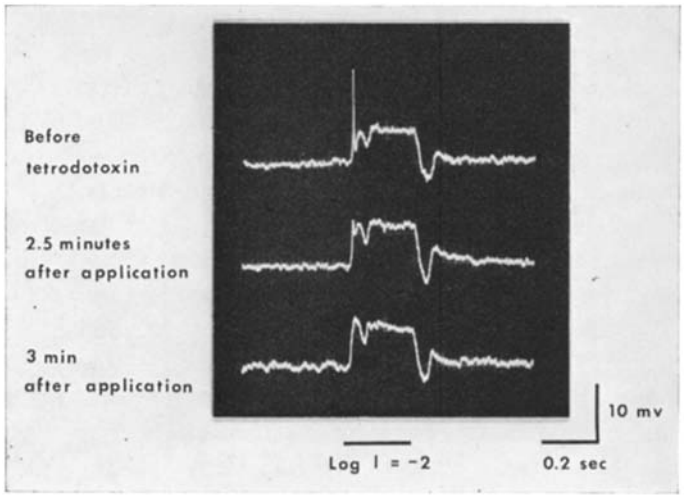


FIGURE 13. An intracellularly recorded receptor response showing oscillations both at "on" and "off" of illumination. Tetrodotoxin at a concentration of 2×10^{-7} g/ml Ringer solution rapidly eliminates the initial spike at "on" of illumination but does not affect the oscillations or the rest of the receptor potential.

changes in illumination and in detecting moving stimuli (Werblin and Dowling, 1969; Dowling, 1970). It seems reasonable to suggest that the transient responses recorded in the postsynaptic units in the dragonfly ocellus are serving similar functions.

This research was supported by National Institutes of Health research Grant EY-00470 and NIH training Grant 5-T01-GM-00716.
Patricia A. Sheppard provided superb technical assistance and prepared the figures for these studies.

Received for publication 4 January 1972.

REFERENCES

ALAWI, A. A., and W. L. PAK. 1971. On-transient of insect electroretinogram: its cellular origin. *Science (Wash. D.C.)*. **172**:1055.

BARETS, A., and T. SZABO. 1962. Appareil synaptique des cellules sensorielles de l'ampoule de Lorenzini chez la torpille *Torpedo* maomorata. *J. Microsc. (Paris).* **1**:47.

BOSCHEK, C. B. 1971. On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica. Z. Zellforsch. Mikrosk. Anat.* **118**:369.

CAJAL, S. R. 1918. Observaciones sobre la estructura de los ocelos y vias nerviosas ocelares de algunos insectos. *Trab. Lab. Invest. Biol. Univ. Madrid.* **16**:109.

CHAPPELL, R. L., and J. E. DOWLING. 1972. Neural organization of the median ocellus of the dragonfly. I. Intracellular electrical activity. *J. Gen. Physiol.* **60**:121.

DOWLING, J. E. 1970. Organization of vertebrate retinas. *Invest. Ophthalmol.* **9**:655.

DOWLING, J. E., and B. B. BOYCOTT. 1966. Organization of the primate retina: electron microscopy. *Proc. R. Soc. Lond. B Biol. Sci.* **166**:80.

GOODMAN, L. J. 1970. The structure and function of the insect dorsal ocellus. *Adv. Insect Physiol.* **7**:97.

GRAY, E. G., and R. W. GUILLERY. 1966. Synaptic morphology in the normal and degenerating nervous system. *Int. Rev. Cytol.* **19**:111.

HAMORI, J., and G. A. HORRIDGE. 1966. The lobster optic lamina. *J. Cell. Sci.* **1**:257.

HARTLINE, H. K. 1969. Inhibitory interaction in the retina. The retina: morphology, function and clinical characteristics. *U.C.L.A. Forum Med. Sci.* **8**:297.

KIDD, M. 1962. Electron microscopy of the inner plexiform layer of the retina in the cat and the pigeon. *J. Anat.* **96**:179.

LANGE, D., H. K. HARTLINE, and F. RATLIFF. 1966. The dynamics of lateral inhibition in the compound eye of *Limulus. In* Functional Organization of the Compound Eye. C. G. Bernhard, editor. Pergamon Press Ltd., Oxford. 425.

RATLIFF, F., H. K. HARTLINE, and W. H. MILLER. 1963. Spatial and temporal aspects of retinal inhibitory interaction. *J. Opt. Soc. Am.* **53**:110.

RATLIFF, F., and C. G. MUELLER. 1957. Synthesis of "on-off" and "off" responses in a visual neural system. *Science (Wash. D.C.).* **126**:840.

RICHARDSON, K. C. 1964. The fine structure of the albino rabbit iris with special reference to the identification of adrenergic and cholinergic nerves and nerve endings in its intrinsic muscles. *Am. J. Anat.* **114**:173.

RUCK, P., and G. A. EDWARDS. 1964. The structure of the insect dorsal ocellus. I. General organization of the ocellus in dragonflies. *J. Morphol.* **115**:1.

SJOSTRAND, F. S. 1958. Ultrastructure of retinal rod synapses of the guinea pig eye as revealed by three-dimensional reconstructions from serial sections. *J. Ultrastruct. Res.* **2**:122.

SJOSTRAND, F. S. 1961. Electron microscopy of the retina. *In* The Structure of the Eye. G. K. Smelser, editor. Academic Press, Inc., New York. 1.

SMITH, C. A., and F. S. SJOSTRAND. 1961. A synaptic structure in the hair cells of the guinea pig cochlea. *J. Ultrastruct. Res.* **5**:184.

TOH, Y., Y. TOMINAGA, and M. KUWABARA. 1971. The fine structure of the dorsal ocellus of the fleshfly. *J. Electron Microsc.* **20**:56.

TRUJILLO-CENOZ, O. 1965 *a*. Some aspects of the structural organization of the arthropod eye. *Cold Spring Harbor Symp. Quant. Biol.* **30**:371.

TRUJILLO-CENOZ, O. 1965 *b*. Some aspects of the structural organization of the intermediate retinal of *Dipterans. J. Ultrastruct. Res.* **13**:1.

VARELA, F. G. 1970. Fine structure of the visual system of the honey bee (*Apis mellifera*). II. The lamina. *J. Ultrastruct. Res.* **31**:178.

WERBLIN, F. S. 1972. Lateral interactions at the inner plexiform layer of vertebrate retina: antagonistic responses to change. *Science (Wash. D. C.)* **175**:1008.

WERBLIN, F. S., and J. E. DOWLING. 1969. Organization of the retina of the mudpuppy, *Necturus maculosus.* II. Intracellular recording. *J. Neurophysiol.* **32**:339.

WHITEHEAD, R., and R. L. PURPLE. 1970. Synaptic organization in the neuropil of the lateral eye of *Limulus. Vision Res.* **10**:129.