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Efferent fibers and daily rhabdomal changes in the anteromedial eye of the liphistiid spider, *Heptathela kimurai*

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Abstract. The presence of efferent fibers in the retina of liphistiid spiders, kept in natural daily cycles of illuminance, was examined by electron microscopy. The efferent fibers were observed to extend their processes through the ocellar nerve to the retina. They contained characteristic large electron-dense granules and branched repeatedly within the retina with varicosities, to provide synaptoid contacts with the receptor cells. They ran mostly among receptor cells and glial cells but sometimes protruded into receptor cells to establish invaginated synaptoid contacts. The synaptoid structures were characterized by spherical clear vesicles located at the presynaptic region, with electron-dense material adhering to the plasma membranes of the receptor cell and the efferent fiber, and a cleft about 10 nm wide formed by the two opposed parallel membranes. The clear vesicles and the electron-dense granules were secreted by exocytosis. The efferent fiber was characteristically presynaptic in relation to the receptor cell. In addition, the rhabdoms differed in size from day to night.

Key words: Retina – Innervation – Rhabdomeres – Synaptoid contacts – *Heptathela kimurai* (Chelicerata)

Introduction

Efferent fibers in the eye have been reported in a few arthropods. The lateral eye of the horseshoe crab, *Limulus polyphemus,* has numerous centrifugal neurosecretory nerve fibers which terminate onto pigment cells and receptor cells with a synaptoid structure (Fahrenbach 1969, 1973). These eyes show a circadian oscillation in both the photoreceptor response and the optic nerve response which is mediated by efferent impulses in the optic nerve (Barlow et al. 1977; Barlow 1983; Chamberlain and Barlow 1987). In the median eye of the scorpion, *Androctonus australis,* the screening pigments within the visual cells exhibit migrations corresponding to the circadian day and night states (Fleissner 1972; 1974). The retina and the optic nerve of *Androctonus* have numerous neurosecretory fibers which terminate in a synaptoid manner onto the visual cells in the median eye (Fleissner and Schliwa 1977). The somata of the efferent neurosecretory fibers that control the circadian sensitivity rhythm in the median eye of *Androctonus* are located in the brain (Fleissner and Heinrichs 1982). Therefore, it is deduced that these efferent neurosecretory fibers contribute to the circadian sensitivity change and control of the transductive membrane turnover in the eyes of both *Limulus* and scorpions.

With respect to spiders, the posterior median eyes of the nocturnal *Dinopis* exhibit changes in rhabdom size in synchronization with natural daily cycles of illuminance (Blest 1978). The sensitivity of photoreceptors of the orb weaving spiders, *Argiope bruennichii* and *A. amoena,* is modulated by efferent inputs, including some derived from photosensitive neurons in the protocerebrum (Yamashita and Tateda 1981; 1983). These reports imply that spider eyes contain efferent fibers. However, the secondary eyes of a salcitid (Hardie and Duelli 1978) and the posteromedial eyes of the nocturnal *Dinopis* (Laughlin et al. 1980) are reported to lack efferent innervation. Additionally, the retina of the wolf spider, *Lycosa erythrognatha* (Lucas) or *Lycosa thorelli* (Keysterling), is only reported to have "clear fibers" that contain numerous granules and clusters of vesicles and maybe centrifugal elements (Melamed and Trujillo-Cenoz 1966). It remains unclear whether efferent fibers are a universal feature of the retinae of spiders or not, or, if so, whether they contribute to the circadian sensitivity changes in their eyes.

The liphistiid spider, *Heptathela kimurai* has vestigial segments in its abdomen, poorly developed mandibles, two pairs of book lungs and no tracheae, and seven or eight spinnerets. It belongs to the suborder Archaeothelae, considered to be a primitive type in the order

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Araneae. This spider has a subterranean habitat and exhibits nocturnal behavior. To examine for the presence of efferent fibers and daily changes in the retina, the eyes of the liphistiid spiders were kept in natural daily cycles of illuminance before being examined by light and electron microscopes. We found the efferent fibers to be characteristically present in the anteromedial eye. The rhabdom size varied from daytime to night. The present paper reports the structural features of the efferent fibers and the daily changes of the rhabdoms.

Materials and methods

Adult female liphistiid spiders *Heptathela kimurai* were collected in the fields of Southern Kyushu district, Japan. The spiders were kept in glass tubes stuffed with moist soil and were fed small insects. In order to examine whether the daily rhabdomal change occurred or not, each of the glass tubes was kept in the field for more than a week.

The spiders were immobilized by cooling, and the frontal parts of the cephalopils were dissected out with a sharp blade. Some of the spiders, conditioned to the natural rhythm of light and darkness, were decapitated at noon in the light and some were decapitated at midnight under a dim red light. Samples were immediately fixed for 2 h in 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.3 and then post-fixed for 2 h in 2% osmium tetroxide in the same buffer. After dehydration through ethanol series, specimens were embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a H-500 electron microscope.

Results

Distribution of efferent fibers

Some fine processes of the efferent fibers extended between the rhabdomic layer in the eye and the forebrain as seen in serial sections. Within this range, somata were not observed. The efferent fibers were distinguishable from the receptor cells and glial cells by characteristic large electron-dense granules with a diameter of about 100 nm. In cross-sections of the optic nerve, a small number of the efferent fibers or the processes of the efferent fibers lay among the axons of the receptor cells, making contact with the axon, without interposition of glial cells. They were surrounded by Schwann's cells (Fig. 1).

In the proximal region of the retina, processes of the efferent fibers increased in number and ran through the retina in various positions with respect to receptor cells and pigment cells (Figs. 2a, 2b). Toward the distal region of the retina, the efferent fibers branched repeatedly and formed varicosities whereby they made synaptoid contacts with the receptor cells (Fig. 3). They sporadically established synaptoid contacts with the receptor cells not only peripherally but also within cellular invaginations (Fig. 4).

Synaptoid structure and exocytosis of vesicles

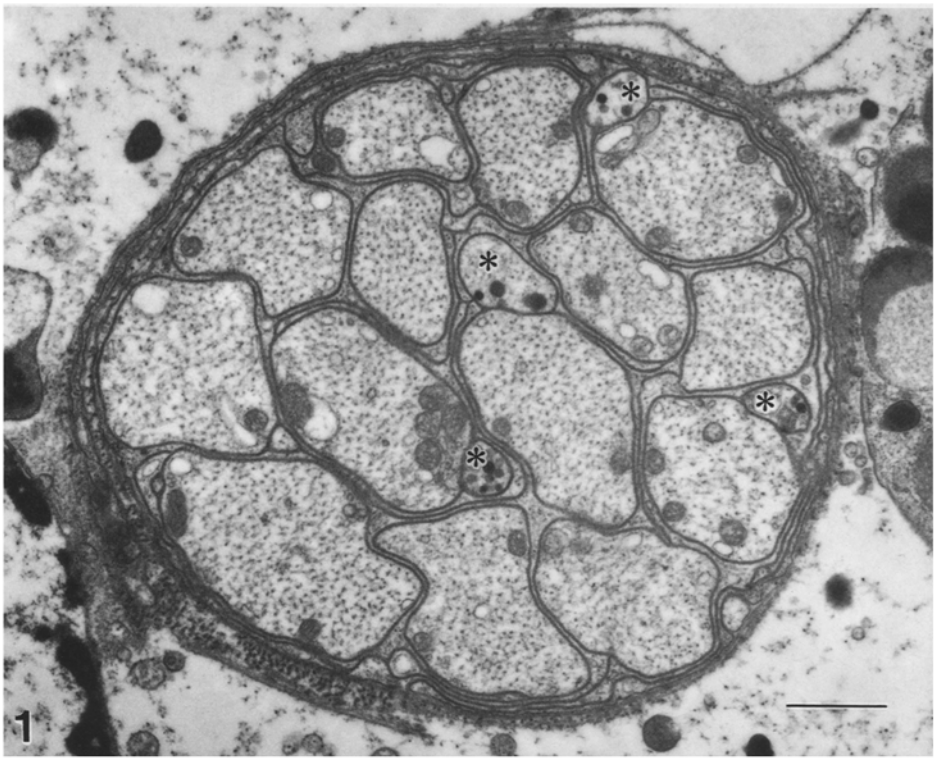
Though there was no presynaptic ribbon, synaptoid structures were easily recognized by characteristic structures. Spherical clear vesicles about 50 nm in diameter gathered at the presynaptic region, electron-dense material adhered to the plasma membranes of the receptor cells and of the efferent fiber, and a cleft about 10 nm wide existed between the two opposed parallel plasma membranes. The synaptic electron-dense material on the plasma membranes of the receptor cells was characteristically thicker than that of efferent fibers. The active zones of a synapse were wide and faced the cell body of a receptor cell, but did not face the membranes of microvilli or of pigment cells (Figs. 3, 4, 5a, 5b). The spherical clear vesicles were commonly seen to be secreted by exocytosis in the synaptoid structure (Fig. 5a), while the large granules were rarely observed to be discharged by exocytosis (Fig. 5b).

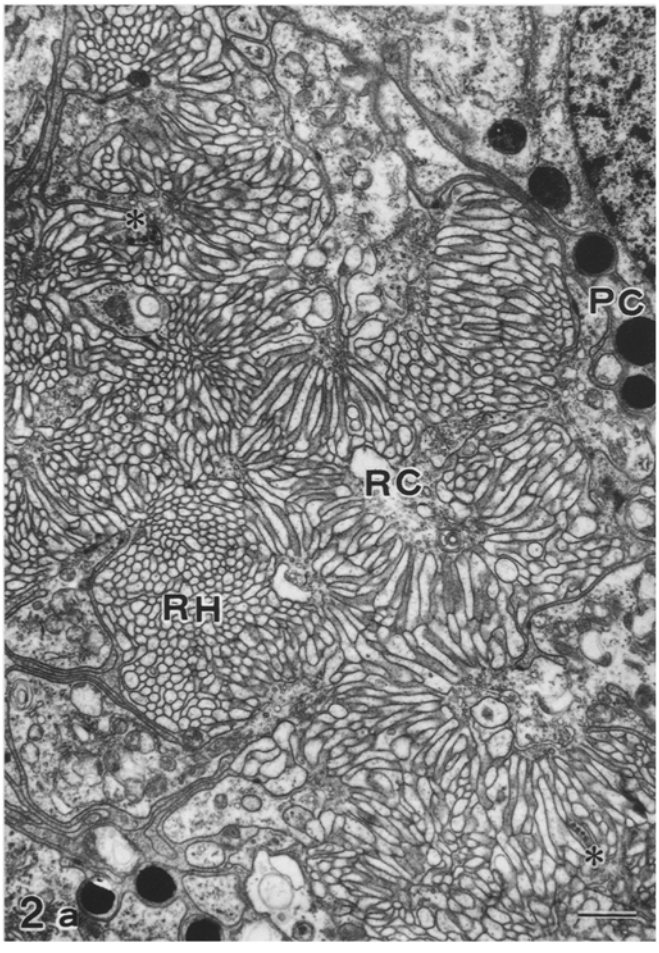
Daily rhabdomal changes

Differences were observed in measurements taken of rhabdom sizes of receptor cells between daytime and nighttime. At night microvilli of the rhabdoms were well developed and filled the receptor cells (Fig. 2a), while in the daytime they were short and occupied a small proportion of the area in the receptor cell in cross-sections (Fig. 2b). Furthermore, multivesicular bodies that were packed with small vesicles were scattered in the receptor cells in the daytime (Fig. 2b). The plasma membrane of the rhabdomeral microvilli was frequently invaginated, forming buddings coated with electron-dense material at each base (Fig. 6a, arrow). Near the buddings small vesicles with similar sizes and multivesicular bodies were observed (Fig. 6a). Occasionally, multivesicular bodies accumulated near the efferent fibers (Fig. 6b).

Discussion

Efferent fibers were found in the eye of a liphistiid spider. They contained both small clear vesicles and large dense granules and extended their process through the ocellar nerve to the retina and terminated in the rhabdomic layer. The somata of the efferent fibers were considered to lie behind the protocerebrum because they were not present in serial sections from the retina to the protocerebrum. The efferent fibers made synaptic contact with the receptor cells and were always presynaptic in relation to a receptor cell judging from the thickness of the electron-dense material which adhered to the plasma membranes, and the spherical clear vesicles. It is suggested that they exert some efferent effect on the receptor cell. There has been no morphological evidence showing that efferent fibers exist in the eyes of the other species of spiders. However, there is physiological evidence that efferent fibers are present in the eyes of the orb-web spider and are capable to modulate the sensitivity of photoreceptors (Yamashita and Tateda 1981; 1983). Additionally, it is well known that efferent fibers innervate the eyes of *Limulus* and the scorpion, chelicerate arthropods related to spiders (Fahrenbach 1981, 1985; Fleiss-





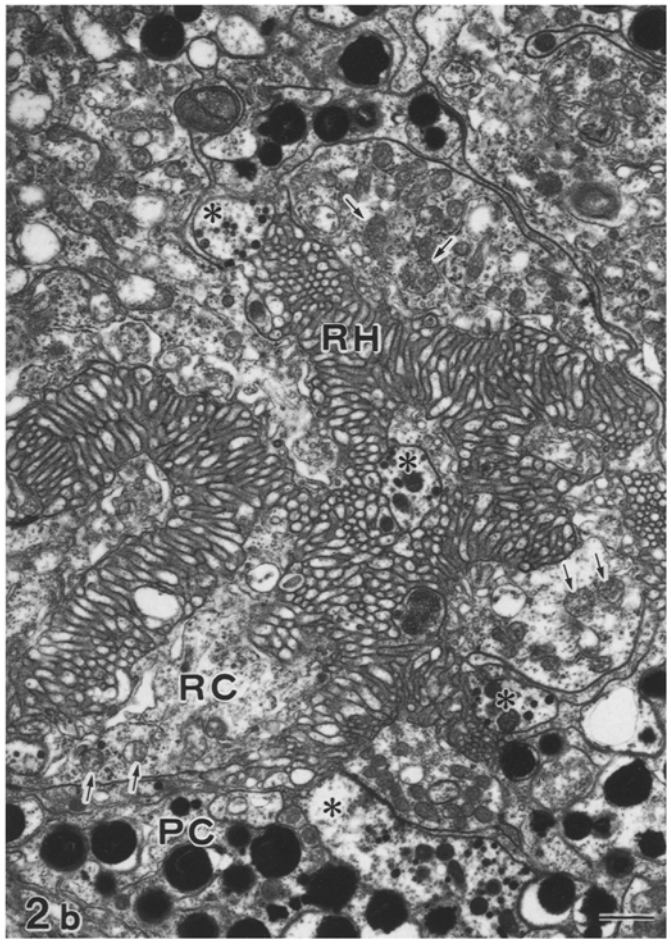
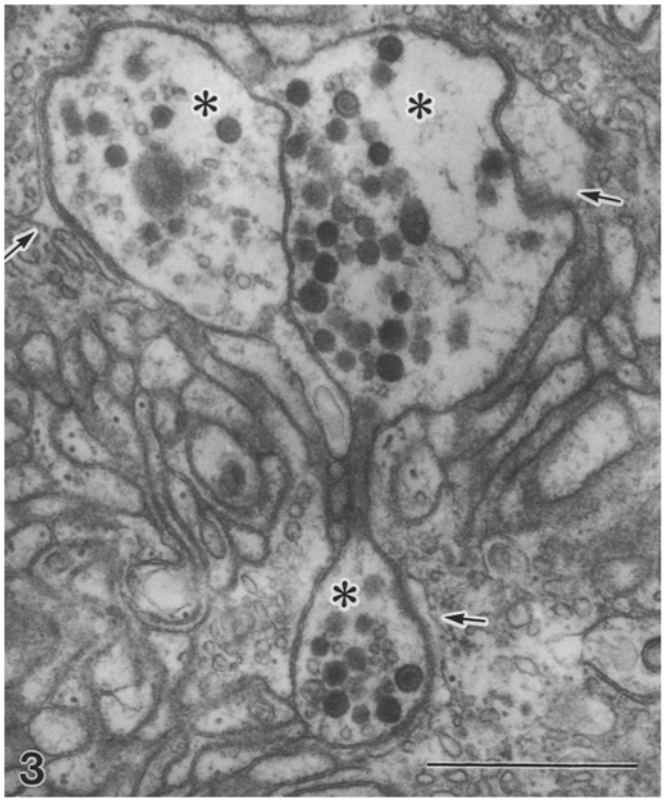


Fig. 1. A cross-section of the optic nerve. The efferent fiber is characterized by large electron-dense granules. Four processes of the efferent fiber (*asterisk*) and 14 receptor axons are seen to be sheeted together in Schwann's cells. ×13000. *Bar:* 1 μm

Fig. 2 a, b. Circadian rhabdomal changes. **a** A cross-section through rhabdoms at night. Rhabdomeral microvilli run in different directions and appear to fill the space. ×7500. *Bar:* 0.1 μm. **b** A cross-section through rhabdoms during the daytime. The rhabdoms decrease their surface area markedly when compared to those at night. Multivesicular (*arrow*) bodies are scattered in the receptor cells. ×7500. *Bar:* 0.1 μm. *RC* Receptor cell; *RH* rhabdomeral microvilli; *PC* pigment cell; *asterisk* efferent fiber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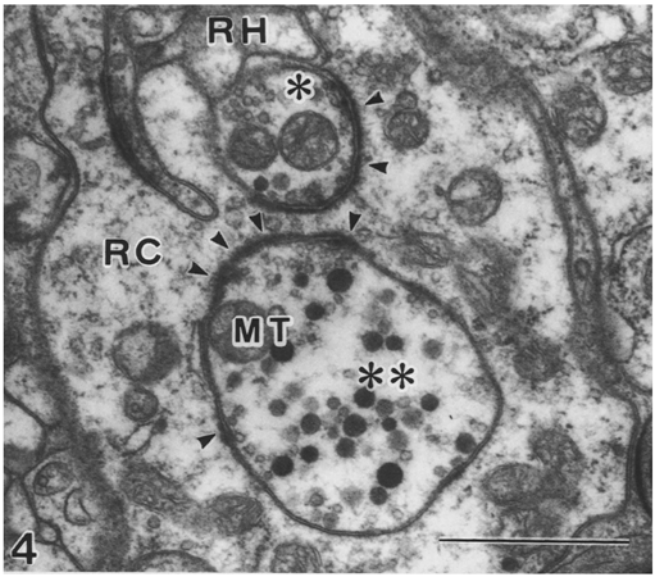
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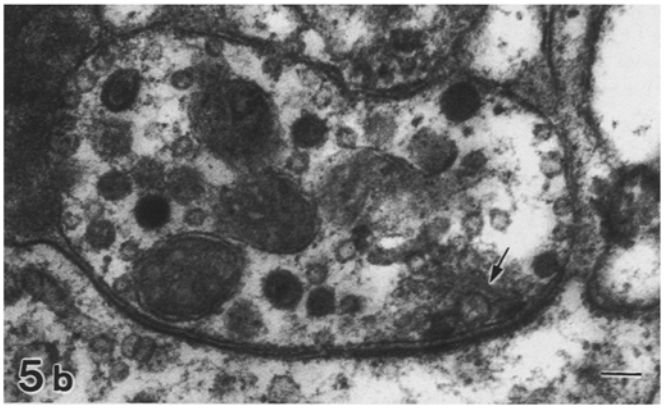
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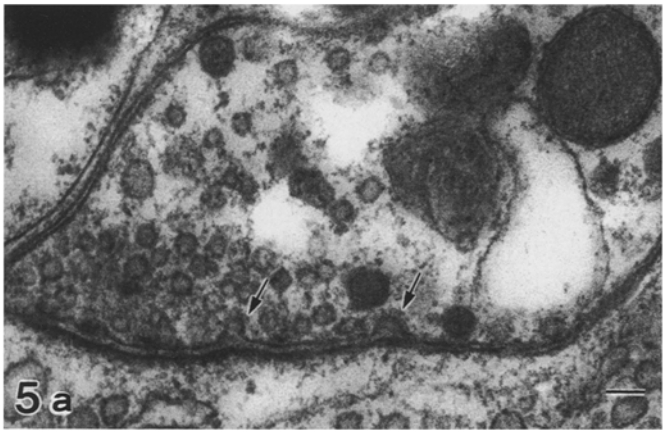
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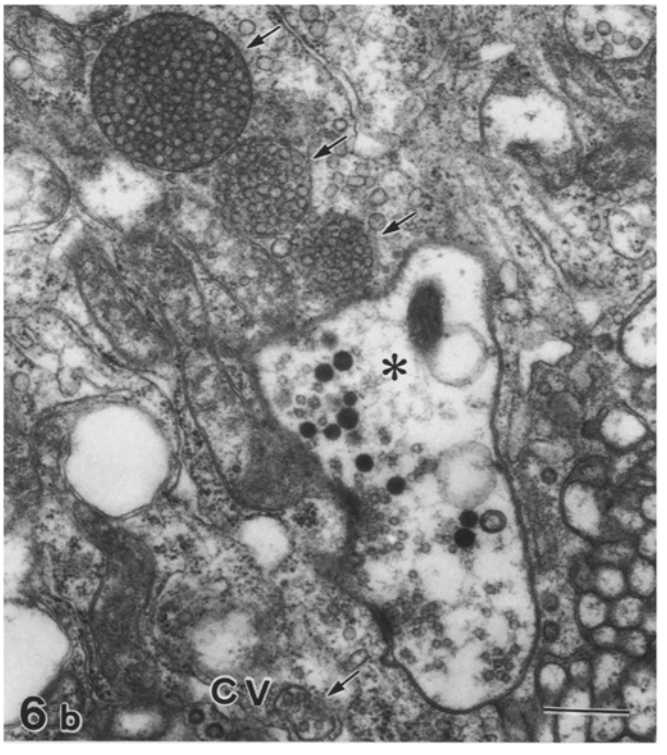
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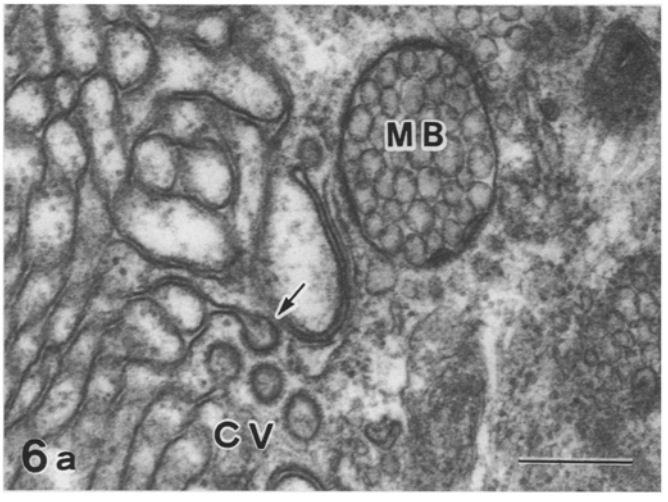
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ner and Schliwa 1977; Fleissner and Heinrichs 1982). Hence, it remains a possibility that, in other spiders efferent fibers are present.

Daily rhabdomal changes are described in the anteromedial eye of a liphistiid spider. In many invertebrates circadian rhabdomeral changes occur, and regular renewal of transductive membrane is achieved by a variety of mechanisms in different species (Schwemer 1986; Blest 1988; Stowe et al. 1990). In the posterior median eye of the nocturnal spider *Dinopis* (Blest 1978), the membranes of rhabdoms are removed by endocytosis and incorporated into multivesicular bodies during the transition period from night to day. New membrane is produced and added to the rhabdoms during the transition period from day to night. In the present liphistiid spider, there are many endocytotic vesicles, multivesicular bodies, and a well-developed endoplasmic reticulum in the receptor cells. Therefore, the rhabdoms may be renewed in the same manner as that of *Dinopis*.

In a scorpion (Fleissner and Fleissner 1978; Fleissner and Heinrichs 1982; Heinrichs and Fleissner 1987) and *Limulus* (Barlow et al. 1980; Chamberlain and Barlow 1987), efferent neurons regulate circadian changes in the structure and function of the eyes and contain the same clear vesicles and characteristic large electron-dense granules as those of the liphistiid spider. Especially, these in the scorpion are very similar to those in the liphistiid spider. Moreover, efferent fibers in *Limulus* eyes synthesize, store, and release octopamine, a neurotransmitter that mimics many of the effects of endogenous efferent innervation (Battelle 1991), and octopamine is suggested to be released in response to efferent nerve activation in situ by either electrical stimulation or a circadian clock (Edwards et al. 1990). Since circadian sensitivity in the eye of an orb-web spider has been reported (Yamashita and Tateda 1981; 1983), the possibility that the efferent fibers observed in this study regulate the daily rhabdomal changes of the anteromedial eye in the liphistiid spider must be considered.

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Fig. 3. Branching of the efferent fiber and synaptoid contact. The efferent fiber (*asterisk*) branches repeatedly within the retina. Here, it forms bulges and makes synaptoid contact with retinula cells (*arrow*). ×28000. *Bar:* 1 μm

Fig. 4. Synaptoid junctions of the efferent fiber in the distal region of the retina. Two efferent fibers are observed. One efferent fiber (*asterisk*) lies at the periphery of the receptor cell (*RC*) in close apposition to the rhabdomeral microvilli (*RH*), and the other (*double asterisks*) lies in the center of the receptor cell. The synaptoid structure faces the cytoplasm (*arrowheads*). Small clear vesicles are gathered at the synaptoid junction in the efferent fiber. *MT* Mitochondrion. ×25000. *Bar:* 1 μm

Fig. 5. a Exocytosis of small clear vesicle (*arrow*) at a synaptoid junction. ×51000. *Bar:* 0.1 μm. **b** Exocytosis of large electron-dense granule (*arrow*). It is very rare to observe the exocytosis of large granules. ×52000. *Bar:* 0.1 μm

Fig. 6a, b. Multivesicular bodies in the receptor cells. **a** The plasma membrane of microvilli is invaginated at their base to form pinocytotic vesicles with undersurface coat (*arrow*). Coated vesicles (*CV*) are seen lying in the neighborhood of the pinocytotic vesicles. *MB* Multivesicular body. ×31000. *Bar:* 0.5 μm. **b** Multivesicular bodies (*arrow*) near the efferent fiber (*asterisk*). *MT* Mitochondrion; *CV* coated vesicle. ×22000. *Bar:* 0.5 μm

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