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Extraocular photoreceptors in the brain of *Epilachna varivestis* (Coleoptera, Coccinellidae)*

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Summary. Within the optic lobe of adult Mexican bean beetles, *Epilachna varivestis*, three complex areas were detected that display elements with the morphological appearance of photoreceptors. Each of these complexes contains about eight cells that show fully differentiated rhabdomeres, screening pigments and other features typical of photoreceptors. It was shown that these areas originate from larval stemmata, which move into the depth of the head capsule during metamorphosis and are finally embraced by the outgrowing optic lobes. Although the function of these formations is not yet known, there are some indications that they may play a role in the entrainment of circadian clocks.

Key words: Photoreceptors, extraocular – Optic lobe – Metamorphosis – Circadian clocks – Insects (*Epilachna varivestis*)

Among numerous insects extraocular photoreceptors have been postulated to function in the photoperiodic control of development and in the entrainment of circadian rhythms. The effects of light on the daily and seasonal regulation of brain neuroendocrine activity are mediated solely through extraocular photoreceptors (e.g., Truman 1976; Bennett 1979; Fleissner 1982).

One may assume that photoreceptors of this type have not been verified morphologically. However, there are some reports that show an attachment of the larval stemmata to the optic lobe during metamorphosis; histologically they remain visible in the adult animal (for references, see Paulus and Schmidt 1978). In early publications (e.g., Bott 1928; Umbach 1934) these structures were regarded as rudiments associated with degenerated stemmal nerves. In contrast, based on ultrastructural findings Wachmann (1981) interpreted them as functional elements. During histological investigations on the brain of the Mexican bean beetle, *Epilachna varivestis*, three pigmented areas were found in each optic lobe, which proved to display typical photoreceptor structures. In the following, the structure of these formations as well as their origin during metamorphosis are described.

Materials and methods

Larvae, prepupae, pupae, and adults of different ages of *Epilachna varivestis* were used for the investigations (in addition, some individuals of the lady-beetle, *Adalia bipunctata*, served for comparison). The insects were decapitated, submersed in cold Karnovsky fixative (Karnovsky 1965) and their mouth-parts were removed to allow better infiltration. The specimens were fixed for 4 h at 4° C, then washed twice in cacodylate buffer, pH 7.4 (+5% sucrose), and postfixed in 2% OsO_4/cacodylate buffer for 2 h. After washing twice in buffer solution the specimens were dehydrated in an acetone series and embedded in Araldite. Semithin sections were stained with toluidine blue; contrast of the ultrathin sections was enhanced by uranyl acetate (10 min) and lead citrate (1 min). The sections were viewed with a Zeiss EM 9A electron microscope.

Results

a) Topography and fine structure of the extraocular receptor cells

The pigmented areas (Fig. 1, pr) are observed within the distal optic lobes between the lamina ganglionaris and the medulla on the ventral side at the level of the outer chiasma. The receptor regions each contain about eight cells which, on the one hand, are arranged in a different manner and occur at different levels, but form, on the other hand, a compact body (Fig. 2). Sporadic intercellular junctions may display a meander-like pattern. In electron-microscopic sections the receptor-like cells resemble retinula cells (Fig. 3). This impression is based on microvillar structures that correspond to rhabdomeres (rh). The Figures 2, 3, and 4 show these structures in different planes within various cells, as well as long rhabdomeres the microvilli of which are cut longitudinally or in an oblique direction.

The apical cytoplasm only contains few organelles. Somewhat more centrally numerous small dumbbell- or ring-shaped mitochondria occur (Fig. 3, m). Interspersed between the latter, granules of medium electron density display typical, round to elliptical shapes. In addition, many "fenestrated bodies" (Fernández-Morán 1958; Fahrenbach 1964) can be observed in this region (Figs. 3, 4, arrowheads). Small cisternae of the rough endoplasmic reticulum and free ribosomes are only rarely seen.

The basal regions of the receptor cells are filled with granules of high electron density (Figs. 2–4, p). They measure about 800–1100 nm in diameter and are almost spherical in shape. Between these screening-pigment granules,

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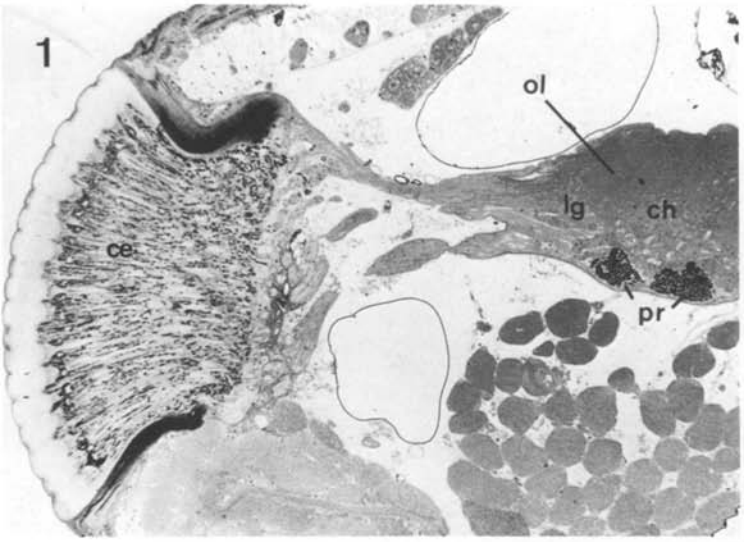
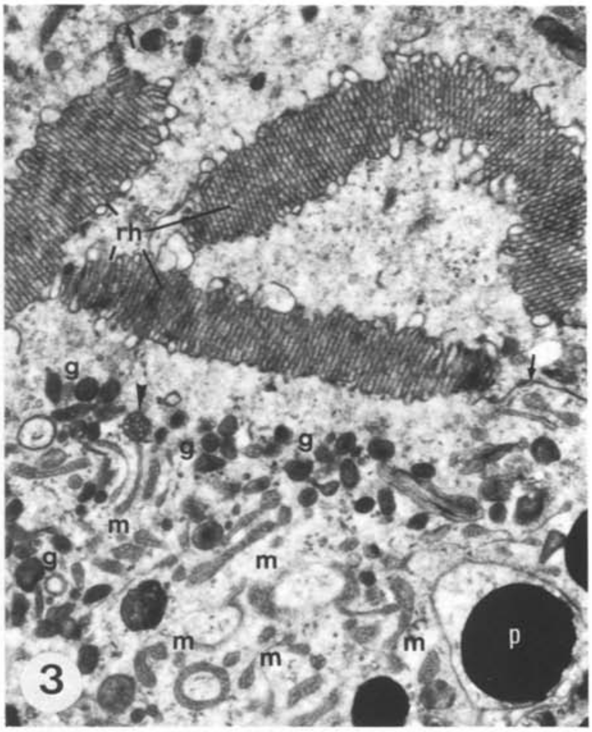
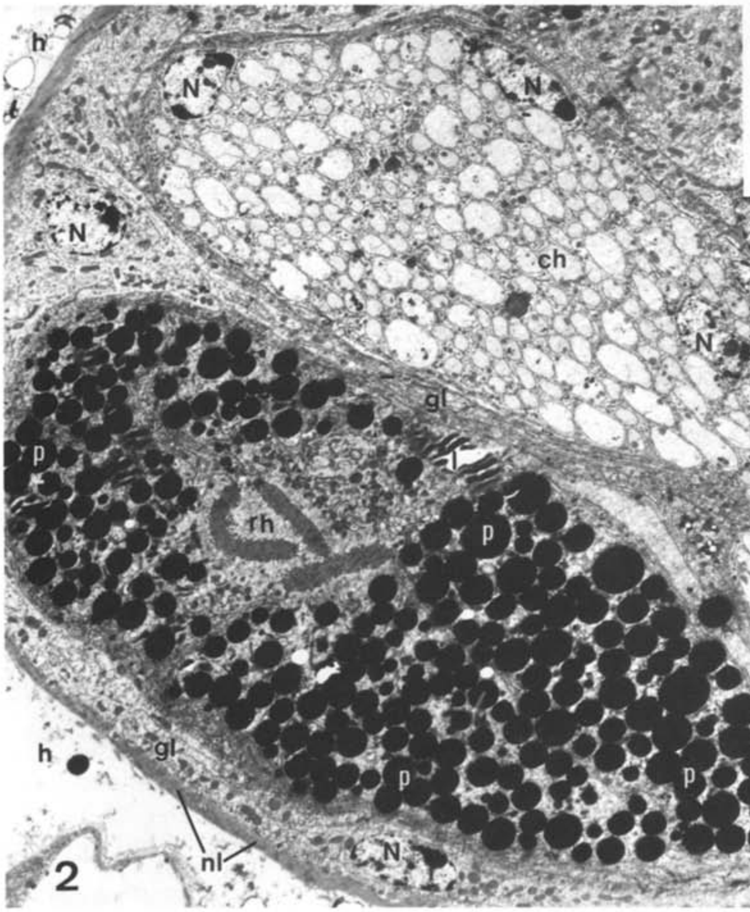






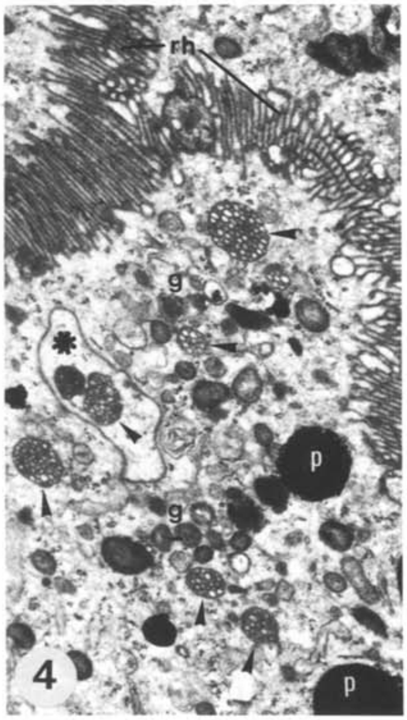
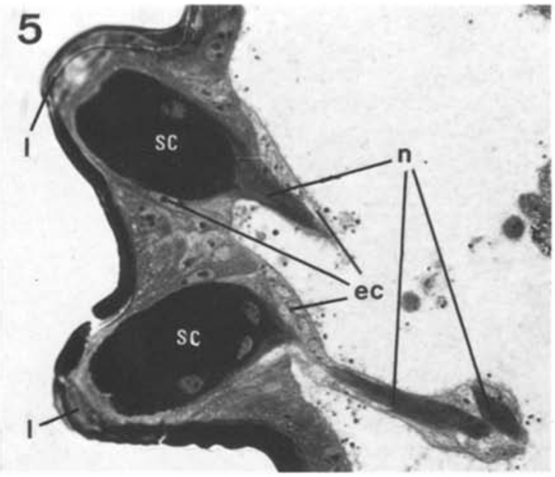


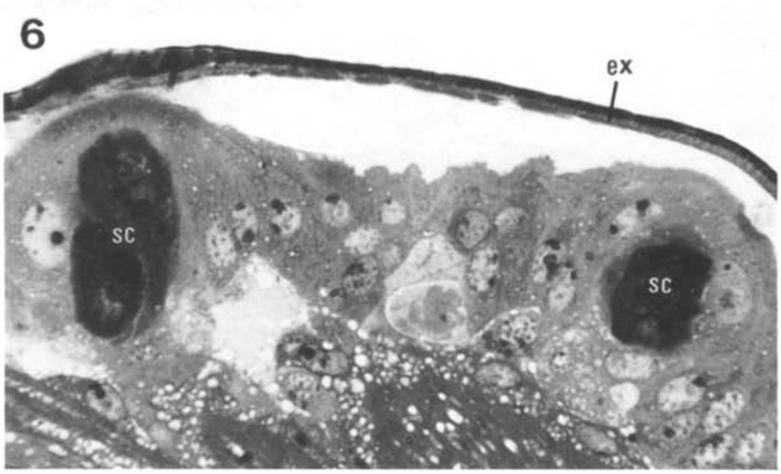
Fig. 1. Transverse section through the head of an adult *E. varivestis* including the optic lobe (*ol*) and one of the compound eyes (*ce*). In this section two of the three pigmented areas (*pr*) can be seen ventral to the outer chiasma (*ch*), proximal to the lamina ganglionaris (*lg*). × 130

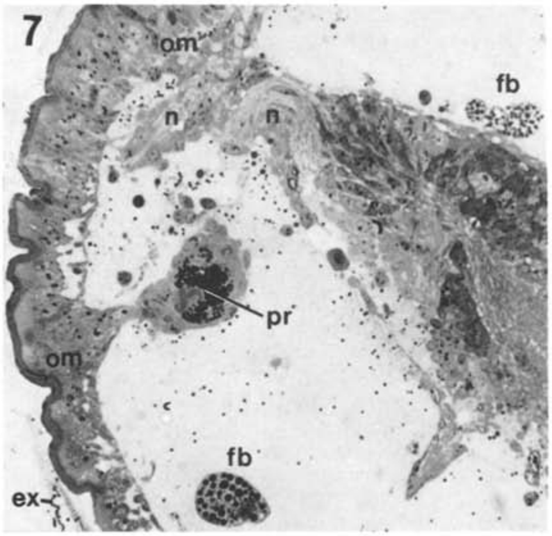
Fig. 2. Longitudinal section through the distal region of the optic lobe containing the outer chiasma (*ch*) and one of the pigmented areas. The latter is surrounded by glial cells (*gl*), the outer layer of which forms the neurilemma with the neural lamella (*nl*) bordering against the hemocoele (*h*). The photoreceptor-like cells display rhabdomeres (*rh*) and more peripherally numerous pigment granules (*p*). Note at the right side of the receptor complex an axon bundle arising from receptor cells (*n*); *l* lipid droplet, *N* glial nuclei. × 3500

Fig. 3. Electron micrograph of a cross section through the apical regions of photoreceptor cells connected by cellular junctions (*arrows*). Beneath the rhabdomere structure, only few organelles are found in the cytoplasm, but at some distance many slender mitochondria (*m*) and granules displaying a medium electron-dense content (*g*) occur. Note more basally large pigment granules (*p*). *Arrowhead* points at a fenestrated body. × 14800

Fig. 4. Electron micrograph of a similar section to that shown in Fig. 3, however, containing numerous fenestrated bodies (*arrowheads*). *Asterisk* marks the slender apex of a receptor cell surrounded completely by another cell; *g* electron-dense granules, *p* screening pigment granules, *rh* rhabdomeres. × 14800







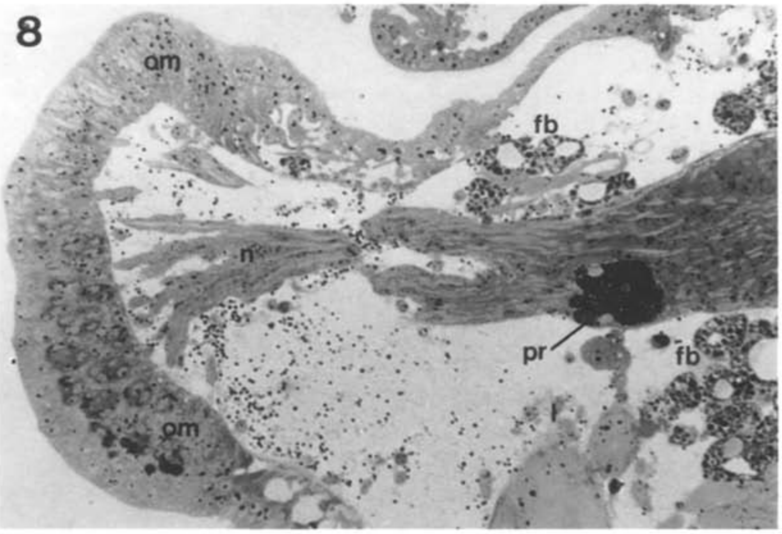


Fig. 5. Light micrograph of two longitudinally sectioned stemmata from a L_4-larva; *ec* enveloping cells, *l* cuticular lens, *n* optic nerves of the stemmata, *sc* region of the sensory cells × 320

Fig. 6. Section through two larval stemmata at the early prepupal stage; *ex* exuvia of L_4-larva, *sc* sensory cells. × 320

Fig. 7. Transverse section through the ocular head region of a late prepupal stage; *ex* exuvia of L_4-larva, *fb* undifferentiated fat-body lobes in front of and behind the optic lobe, *n* compressed optic nerves, *om* region of differentiating ommatidia of the compound eye, *pr* photoreceptor complex of the former stemma. × 200

Fig. 8. Transverse section through the ocular head region of the pupa; *fb* fat-body lobes, *n* stretched optic nerves, *om* differentiating ommatidia of the compound eye, *pr* one of the displaced stemmata. × 200

slender dumbbell-shaped mitochondria, small granules of medium density, and single lipid droplets can be observed (Fig. 2, l).

The receptor cell complex is surrounded by several layers (5–8) of flattened glial cells devoid of pigment granules (Fig. 2, gl). The ventral aspect of the optic lobe is separated from the hemocoele (h) by the neurolemma with the neural lamella (nl). A layer of specialized pigment cells surrounding the receptor cells is missing. The screening pigment is restricted to the visual cells that obviously produce it. Possibly, the granules of medium electron density represent immature stages of screening pigment granules.

The axons of the receptor cells are directed ventromedially from the chiasma to the medulla (Fig. 2, n). Their further pathway and projections are not yet known. In principle, however, this appears to be a typical complex of retinula cells containing apical rhabdomeres and screening pigments. The entire structure is enclosed by glial cells.

b) Origin of the extraocular photoreceptor cells

Since the above-mentioned receptor areas always appear in triplicate within each of the optic lobes, it seemed plausible that these might be derived from larval stemmata. For clarification, L_4-larvae, and various prepupal and pupal stages were examined histologically. In larvae, the stemmata are prominently elevated above the level of the head capsule (Fig. 5). The overlying cuticle is thickened forming a transparent lens (l). An optic nerve (n) leaves each stemma in a proximal direction. Between each stemma and the surrounding epidermis, the enveloping cells (ec) of the stemma itself and of the optic nerve are observed. The number of sensory cells (sc) is estimated to be eight. In light-microscopic sections the rhabdomeres are superimposed by pigment granules.

In the young prepupa the region of the lens is flattened shortly after apolysis (Fig. 6). The epidermal cells situated between the stemmata, now having proliferated, have overgrown the sensory cells, thus pushing the latter in a proximal direction. This process continues with the commencement of metamorphosis. Since in the region of the stemmata the complex eyes differentiate with their elongating ommatidia (Figs. 7, 8, om), the larval eyes shift more and more into the body cavity and are finally associated with the optic lobe. Moreover, during early metamorphosis, axons of the retinula cells of the compound eye grow in a centripetal direction against the optic lobe. In the late prepupa these axons are compressed due to a lack of space (Fig. 7, n). They stretch in the pupa thus enlarging the distance between the optic ganglia and the complex eyes; or, in other words, the latter are pushed outwards (Fig. 8). Thus, the larval stemmata move even further into the interior of the head capsule. The position of the fat-body lobes lying frontal and caudal to the optic lobe in the late prepupa and pupa (Figs. 7, 8, fb) clearly demonstrates these movements within the complex-eye region; they, too, gradually move into the interior of the head capsule.

Discussion

The extraocular receptor areas in the distal optic lobes of the imaginal *Epilachna varivestis* have without doubt their origin in the larval stemmata. This also holds true for the lady-beetle, *Adalia bipunctata*, which was examined for purposes of comparison. In a similar position, Fleissner (personal communication) found multicellular and pigmented areas on the caudal edge of each optic lobe of the carabid beetle, *Anthia sexguttata*. There are reports in the literature emphasizing a similar fate of the stemmata in other holometabolous insects (see Introduction).

It is impossible to conclude from our morphological observations that the described structures have significance with reference to the circadian clock(s): systematic physiological investigations concerning this question are needed. Nevertheless, these structures are situated within that region of the brain where in *Anthia sexguttata* the circadian clock is thought to be located (Fleissner 1982; Fleissner and Fleissner 1982). We are also not able to make any conclusive statements concerning the possible neurophysiological function of these complexes. However, based on empirical data, the retained structures of the stemmata (rhabdomeres, screening pigment, "fenestrated bodies", and the numerous specialized mitochondria) lead to the speculation that these elements are still functional. Microvilli, for example, are often either prevented from differentiation in a status without function (e.g., Heyer 1982) or they are withdrawn immediately (e.g., Haase 1975). The same holds true for the primary photoreceptive pineal complex of vertebrates; the sensory pinealocytes reduce their outer segment during evolution and are transformed into glandular elements (e.g., Oksche 1971; Ueck 1974; Oksche and Hartwig 1979; Hartwig and Oksche 1981; Ueck 1982). The importance of the screening pigment is very difficult to understand; light entry seems to be anything but intensive since a transparent cuticle overlying the receptor region is absent. It also remains unclear whether entrance of light onto the rhabdomeres is directed via a specific arrangement of the pigment.

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