DISTRIBUTION AND STRUCTURE OF OCELLI IN LEPIDOPTERA PREVIOUSLY REPORTED TO BE ANOCELLATE AND MORPHOLOGY OF A NERVE COMPLEX ASSOCIATED WITH THE OCELLI

by

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INTRODUCTION

Insects generally possess two types of photoreceptor structures: lateral compound eyes composed of numerous ommatidia each with its own lens and dorsal ocelli composed of a single lens with numerous receptor cells beneath it. In larval holometabolous insects stemmata, or lateral ocelli are present. These receptors are probably related to the compound eyes. Dorsal ocelli occur only in nymphal hemimetabolous and adult holometabolous insects. Many adult insects lack obvious external ocelli and thus have been described as anocellate.

The presence of internal ocelli in anocellate moths was reported only recently (Eaton, 1971), although Berlese (1909) claims to have seen ocelli within the brain of a sphingid his results are questionable. At the beginning, the purpose of this research was to study the distribution and structure of internal ocelli in Lepidoptera previously reported to be anocellate. In the structural studies of the internal ocellus particular attention was to be given to determining the existence of rhabdoms and, if present, describing their structure.

In the course of this study a nerve complex associated with the internal ocellus was discovered. Research was then directed to include the morphology of this complex. Also in studying the heads of anocellate moths small external ocelli were observed here for the first time. Anatomical, histological and scanning electron microscopical studies were then made of this external ocellus. It is hoped that these studies will lay the structural basis for further functional studies.

LITERATURE REVIEW

I. Distribution of Dorsal Ocelli in Insects

Dorsal ocelli are generally present in most orders of insects. They are found in nymphal hemimetabolous insects and adult hemimetabolous and holometabolous insects and are usually dorso-medial to the compound eyes. Three ocelli, two lateral and one median, are often present, but the number may vary from one to four (Goodman, 1970).

Kalmus (1945) demonstrated what he considered to be a good correlation between wings and ocelli among various insect orders (Table I) and an even better correlation in families with sexually dimorphic forms. He suggested that the rudimentary development of ocelli and wings in a species should be regarded as the retention of larval characters while becoming sexually mature, i.e., neoteny.

Conversely, Parry (1947) said the presence of ocelli in insects was extremely erratic in regard to both function and systematics (Table II). He found that in some closely related species living in similar habitats one may have well-developed ocelli, while the other may be anocellate.

Usually insects which are good fliers have ocelli, but there are exceptions. Tabanids, which are excellent fliers, are anocellate but have developed halteres which could possibly assume the tonus producing role of the ocelli (Buddenbrock, 1937). Homann (1924) and Buddenbrock (1937) indicate that the superposition eyes of the night-flying Sphingidae would be expected to admit more light than any ocelli and thus have

TABLE I. Presence of Dorsal Ocelli in Insect Orders

(Kalmus, 1945)

	Wings Present	Wings Present or Absent	Wings Absent
Ocelli present	Ephemeroptera Odonata		
Ocelli present or absent	Neuroptera Trichoptera	Orthoptera, Hemiptera Dermaptera, Mecoptera Plecoptera, Lepidoptera Isoptera, Coleoptera Psocoptera, Hymenoptera Thysanoptera, Diptera	
Ocelli absent		Embioptera, Strepsiptera	Protura Anoplura Aphaniptera

TABLE II. Distribution of Dorsal Ocelli in Insect Orders.

Compiled from Parry (1947)

Orthoptera:	always in Acridae, Gryllidae
	sometimes in Blattidae, Mantidae, Tettigoniidae
	absent in Grylloblattidae
Dermaptera:	absent
Plecoptera:	two or three present
Isoptera:	present
Embioptera:	absent
Psocoptera:	sometimes present
Anoplura:	absent
Ephemeroptera:	present
Odonata:	usually present
Thysanoptera:	present
Hemiptera:	great variation
Neuroptera:	conspicuous in some families, absent in others
Mecoptera:	some genera ocellate others anocellate
Trichoptera:	some families with ocelli, others without
Lepidoptera:	sometimes present
Coleoptera:	absent except in a few species
Strepsiptera:	absent
Hymenoptera:	usually present
Diptera:	sometimes present
Aphaniptera (Siphonaptera):	uncertain

replaced them. However, one report locates ocelli within the dorsal protocerebrum of the sphingid, Sphinx convoluvi (Berlese, 1909) and recently Eaton (1971) reported the presence of internal ocelli in several sphingids, saturniids and citheroniids. Among other Lepidoptera, Friederichs (1931) reported ocelli to be absent in the following Heterocera: Geometridae, Cossidae, Hepialidae, Castniidae, Bombycidae, Saturniidae, Zygaenidae, Pyralidae, Tineidae and Pterophoridae. No Ropalocera are reported to have ocelli (Borror and DeLong, 1971).

II. Structure of Dorsal Ocelli in Insects

A. General

Arthropods in general and insects in particular possess two basic types of visual architecture: large compound eyes composed of numerous ommatidia each with a lens-like structure, and simple eyes, ocelli, composed of a unicorneal lens with a small cup-shaped retina beneath (Trujillo-Cenóz, 1965). Sometimes the lens may be distinct and separate from the retinular cells or it may just be an area of transparent cuticle with the retinular cells beneath (Wigglesworth, 1965).

Not much variation exists in the general structure of ocelli. Usually a lens is present and is secreted by the underlying layer of hypodermis, the corneagen cells. The retina consists of sense cells, and pigment cells are usually present. The sense cells usually have short processes which syanpse with second order neurons from the protocerebrum (Parry, 1947). Often a tapetum composed of urate granules as in Schistocerca, supportive cells with numerous tracheoles as in Diptera,

or some other reflecting substance is present (Goodman, 1970).

The general structure of ocelli outlined above follows for all the ocelli listed in Table III with some minor modifications. Some of these will be mentioned when they occur in the following discussion.

B. The Rhabdom and Retinulae

Usually a few to several hundred monopolar sensory neurons, called retinular cells, are present in an ocellus. Each retinular cell is divided into three parts: a distal portion bearing the rhabdomere, an intermediate portion with axonal characteristics and a proximal synaptic portion (Trujillo-Cenóz, 1965). These retinular cells are usually arranged in groups of two, three, four or five to form retinulae but the number may vary even within the same ocellus (Mazokin-Porshyakov, 1969). The proximal ends of these cells form axons which synapse with fibers from second order neurons whose somata are located in the pars intercerebralis (Goodman, 1970).

Certain parts of the outer membrane of each retinular cell are thrown into numerous folds called microvilli which are continuous at their base with the retinular cell cytoplasm. Microvilli are characteristic of photoreceptors and their ordered plano-arrangement of photopigment is thought to be very efficient in the absorption of radiant energy. Straight lateral microvilli, which are not derived from cilia, are characteristic of Arthropods as well as certain Annelids, Cephalopods, Platyhelminths and Onychophorids (Eakin, 1965).

The microvilli from one cell form a rhabdomere and the combination

TABLE III. Studies on the Structure of Ocelli in Pterygote Insects

Star denotes the use of transmission electron microscopy in the study.

Pterygote

Paleoptera

Odonata

Anisoptera--Calopteryx splendens--adult--Redikorzew, 1900

Agrion--Hesse, 1908

*Libellula pulchella--Ruck and Edwards, 1964

Sympetrum rubicundulum--Ruck and Edwards, 1964

Orthopteroid orders

Dictyoptera

Blattoidea--*Periplaneta americana--Ruck, 1957

Orthoptera

Locustidae--*Schistocerca gregaria--Goodman, 1970

Plecoptera--Perla bicaudata--larva--Redikorzew, 1900

Hemipteroid orders

Hemiptera--Cimex sp.--larva--Redikorzew, 1900

Neuropteroid orders

Hymenoptera--*Apis mellifera--Redikorzew, 1900

*Yanase and Katoaka, 1963

Neuroptera--Neuronia ruficrus-- Link, 1909

Panorpa communis--Link, 1909

Osmylus chrysops--Link, 1909

Raphidia ophidiopsis--Link, 1909

Table III. (continued).

Diptera--*Drosophila melanogaster*--Hertweck, 1931

Heliophilus sp.--Hesse, 1908

Cristalis sp.--Redikorzew, 1900

**Boettcherisca peregrina*--Toh, Tominaga and Kuwabara, 1971

Lepidoptera

Zygaenidae--*Zygaena* sp.--Link, 1909

Arctiidae--*Callimorpha dominula*--Link, 1909

Arctia caja--Link, 1909

Phragmatobia fuliginosa--Link, 1909

Noctuidae--*Craniophora ligustri*--Link, 1909

Mamestra persicariae--Link, 1909

Catacola fraxini--Link, 1909

Catacola sponsa--Link, 1909

Xylotropha--*Sesia spheciformis*--Link, 1909

of the rhabdomeres from the cells of a given retinula make up the rhabdom. The number of retinular cells contributing rhabdomeres to make up the rhabdom may vary. Each rhabdom of the dorsal ocellus of the worker honeybee is composed of rhabdomeres from two adjacent cells coming together (Yanase and Katoaka, 1963). The retinulae of the American cockroach ocellus may be made up of three to five retinular cells (Ruck, 1957). More often the rhabdom appears as v-shaped strips formed when the rhabdomeres of several adjacent retinular cells come together. The rhabdoms of both the locust, Schistocerca gregaria, and the dragonfly, Libellula, are composed of microvillar v-shaped strips with four and three retinular cells per retinula, respectively (Goodman, 1970; Ruck and Edwards, 1964). The rhabdom of the flesh fly, Boettcherisca peregrina, however, is ringlike and encircles the distal end of each retinular cell (Toh, et al., 1971).

C. Retinular Cell Cytoplasm

The cytoplasm of the retinular cells is rich in organelles such as smooth and rough endoplasmic reticulum, multivesicular bodies, onion bodies, Golgi complexes and numerous mitochondria. Generally the cell nuclei are large and ovoid, and are situated either proximally in the retinular cells as in Libellula (Ruck and Edwards, 1964), the worker honeybee (Yanase and Fujimoto, 1961) and the flesh fly, Boettcherisca peregrina (Toh, et al., 1971), or distally as in Schistocerca gregaria (Goodman, 1970).

The endoplasmic reticulum is most predominant in the distal end of

the retinular cells where the rhabdomere is located in both ocelli and compound eyes (Goodman, 1970). Ruck and Edwards (1964), in the dragonfly ocellus, traced membranes of endoplasmic reticulum to an area just below the rhabdomeres and then found channels of endoplasmic reticulum which they thought to be confluent with the extracellular space in the region of the axon. Similar situations exist in the compound eyes of Dissosteira, Anax, Apis, Musca and the skipper butterfly, where structures described as ultratracheoles connect rhabdomeres with extracellular spaces (Fernández-Morán, 1958). Ruck and Edwards (1964) suggested that the limited extracellular space in photoreceptors is not adequate to support enough ions to sustain the prolonged positive entry phase characteristic of the electrical activity of photoreceptors. They suggested the extracellular space described above may serve as the site of the generator potential. Goodman (1970) does not agree with the hypothesis put forth by Ruck and Edwards (1964). He cites two objections: firstly, he found no connections between the endoplasmic reticulum and extracellular space in the retinular cells of Schistocerca; secondly, he says that the proliferation of narrow tubules would not significantly increase the useful extracellular space available since the volume of extracellular space per unit area of membrane is what is important in ionic exchange.

An alternative function of the vacuoles of the endoplasmic reticulum in the retinular cell cytoplasm is suggested by Horridge and Barnard (1965). In their study of the compound eye of Locusta migratoria, the cisternae of the endoplasmic reticulum were located in a region 2 μ to

4 μ across in the dark-adapted eye, but the vacuoles were spread throughout the cytoplasm in the light-adapted eye with mitochondria crowded around the rhabdom. This same effect is also observed in the ocellus of Schistocerca (Goodman, 1970). Horridge and Barnard suggested that the vacuoles in the cytoplasm immediately surrounding the rhabdom in the dark-adapted eye are of a low refractive index and thus allow for a larger amount of light acceptance by the individual cells.

Multivesicular bodies, spherical organelles containing small vesicles, have been found in numerous Arthropod photoreceptors including ocelli. The numerous vesicles are usually membrane bound though they may occur in non-membrane bound groups or dispersed through the cytoplasm (Goodman, 1970). When first discovered in the developing compound eye of Drosophila, multivesicular bodies were thought to function in membrane formation (Waddington and Parry, 1961). In the fat body of Calpodes ethlius, multivesicular bodies were found to contain some acid phosphatase and were involved in protein uptake. Since they did not accumulate, they were assumed to be involved in protein turnover (Locke and Collins, 1968). Trujillo-Cenóz (1962) suggests multivesicular bodies are involved in neurosecretion. Curtis (1969) described a perirhabdomeric reticulum in the retinular cells of the spider, Mitopus morio, consisting of tubular and vesicular elements. Some vesicles were even found in the microvilli. These vesicles were shown to have acetylcholinesterase activity and Curtis suggested that the acetylcholine-acetylcholinesterase system might be involved in the transduction of radiant stimulation into electrical activity.

Onion or myeloid bodies are composed of concentric spherical membranes and are found in retinular cells of both compound eyes and ocelli (Goodman, 1970) as well as other nervous tissue (Tung and Pipa, 1970). Bernstein (1961) suggests they function to register changes in light intensity for pigment migration, but no change is observed in the onion bodies of light- and dark-adapted locusts (Horridge and Barnard, 1965.

Golgi complexes in Arthropod photoreceptors are generally found near the rhabdom and may be either involved in lysosome formation or associated with multivesicular bodies (Trujillo-Cenóz, 1962; Rutherford and Horridge, 1965). Mitochondria present in retinular cells provide the energy necessary for the transduction of photic stimuli and other cell processes.

D. Synaptic Connections Between the Retinular Cells and Second Order Neurons

Usually many retinular cell axons converge on just a few larger second order fibers whose cell bodies lie in the pars intercerebralis (Goodman, 1970; Toh, et al., 1971). Indeed, the tripart ocellus of Boettcherisca peregrina, representing the fusion of a median and two lateral ocelli, receives only twelve second order fibers with four going to each ocellus, however, these main fibers have numerous branches (Toh, et al., 1971).

The retinular cell axon is generally the pre-synaptic unit of the synapse, but Goodman (1970) reports synapses where the second order neuron appears to be the presynaptic unit. The synapses of ocellar

nerves with second order axons from somata in the pars intercerebralis are supposedly inhibitory (Ruck, 1961a). Uchizono (1965) in studying the central nervous system of the cat describes two morphological types of synaptic vesicles: E-type, spherical, synaptic vesicles characteristic of an excitatory synapse and smaller I-type, ellipsoidal, synaptic vesicles characteristic of an inhibitory synapse. Recently in the crayfish, Procambarus clarki, an Arthropod, Atwood and Lang (1972) confirmed Uchizono's results. Goodman (1970) says that some E-type synaptic vesicles characteristic of an excitatory synapse are definitely present at synapses of retinular cell axons with second order neurons in the ocellus of Schistocerca, but says that I-type vesicles may also be present.

E. Screening Pigment

Opaque pigment granules are generally present in ocelli but may be absent entirely in some such as Periplaneta americana (Ruck, 1958a). The pigment may be present: in the retinular cells themselves proximal to the rhabdomere region, as in Helophilus and Synconastes marginatus (Hesse, 1901), Zygaena sp. (Link, 1909), Drosophila melanogaster (Hertweck, 1931), and Apis mellifera (Redikorzew, 1900; Yanase and Fujimoto, 1961); in pigment cells separate from the retinular cells which may be in the form of a thin ring of pigment cells surrounding the distal end of the ocellus as in Schistocerca (Goodman, 1970); a proximal retinular cell sheath through which retinular cell axons run as in Sympetrum (Ruck and Edwards, 1964); or a pigmented epithelium surround-

ing the entire ocellus as in Calopteryx splendens (Redikorzew, 1900) or Cleon, Ceratopsyllus canis and Agrion where migration supposedly occurs with changes in light intensity (Lammert, 1925). Though pigment migration has been reported in the compound eyes of insects several times (Goldsmith, 1964) there is only one report of it in ocelli (Lammert, 1925), thus the main function of opaque pigment in ocelli is to limit the entrance of light to the ocellus except through the corneal lens (Goodman, 1970).

F. Glial Cells

Glial cells are present to some extent in all insect nervous tissue and ocelli are no exception. Glial cells are usually elongate and flattened with numerous microtubules present in their cytoplasm. Several functions have been postulated for glial cells including: support of neurons (Goodman, 1970); fulfillment of the metabolic requirements of nerve cells; disposal of transmitter substances and to serve as a source of ions (Bullock and Horridge, 1965). In flesh fly ocelli the retinular cells are surrounded by glial cells except for the rhabdom and desmosomes occurring between adjacent retinular cells. The glial cells contain numerous microtubules and glial cell processes sometimes penetrate the retinular cells (Toh, et al., 1971). In Schistocerca glial cells surrounding the retinular cell axons extend distally to become intimately associated with these cells. Cell junctions often occur between retinular cells in the region of the rhabdom and glial cells. Vesicles similar to those composing multivesicular bodies are

found in the glial cell cytoplasm here. This is thought to possibly lend support to Bullock and Horridge's idea of the multivesicular bodies being involved in neurosecretion (Goodman, 1970).

III. Function of Dorsal Ocelli in Insects

A. General

Early work on the function of ocelli was misleading. Kolbe (1893), Hesse (1908) and Link (1909a, 1909b) thought ocelli functioned to perceive distant objects. Others, including Müller (1826), Lubbock (1889) and Lowne (1870) conversely thought the perception of only near objects was the function of ocelli. Buddenbrock (1937) thought ocelli could perceive either close or distant objects. Demoll and Scheuring (1912) considered ocelli to function to assess distance. All these functions first attributed to ocelli assumed form vision (Cornwell, 1955) which has been disproved in several cases (Homann, 1924; Wolsky, 1930; Parry, 1947; Cornwell, 1955). That ocelli might function to increase the insect's visual field has been suggested but mostly discarded when the visual field of the ocelli of Locusta was found to be smaller than that of the adjacent compound eyes (Goodman, 1970).

Since the image formed by the ocellar lens did not fall in the region of the rhabdom, Homann (1924) thought ocelli functioned to register light intensity changes. Lowne (1878), Lubbock (1889), Homann (1924), Wolsky (1933) and Parry (1947) considered ocelli not only to perceive changes in light intensity but also to register absolute light

intensity. Wolsky (1933) also considered ocelli to have a general stimulatory function in addition to their sensory function.

Ocelli are also thought to play a role in phototaxis (Müller, 1931; Friederichs, 1931; Kalmus, 1945; Von Buddenbrock, 1937; Cornwell, 1955) and photokinesis (Bozler, 1926; Parry, 1947; Buddenbrock, 1937). In at least one instance ocelli are thought to be responsible for the entrainment of daily rhythms (Harker, 1956).

B. Behavior

1. Form perception

Prerequisite to form perception are: the ability of a lens system to form an image on the retinal layer; little convergence between receptor axons and higher order neurons; and the differentiation and utilization of the visual information received by the central nervous system (Goodman, 1970; Goldsmith, 1964). In all cases examined thus far, the insect dorsal ocellus satisfies none of these prerequisites.

The principal focal plane formed by the lens system of every ocellus examined has been found to fall beyond the level of the rhabdomeres (Hesse, 1901; Link, 1908, 1909; Homann, 1924; Wolsky, 1930; Parry, 1947; Cornwell, 1955). Cornwell (1955) studied the ocellar lens systems of Calliphora (Diptera) and Locusta (Orthoptera) using corrected refractive indices for the lenses and depth of the rhabdom layers, and still found the principal focal plane to fall beyond the retinal layer.

A high degree of convergence of the retinular cell axons on to a few second order neurons also exists. Generally 500 to 1,000 retinular

cells converge on a maximum of 25 to 30 nerve fibers (Goodman, 1970). Convergence occurs to an especially high degree in the dorsal ocellus of the flesh fly, Boettcherisca peregrina, where each ocellus receives only four second order axons (Toh, et al., 1971). In all cases examined thus far, form vision is generally not assumed for insect dorsal ocelli (Goodman, 1970; Wigglesworth, 1965; Goldsmith, 1964).

2. "Stimulationsorgane"

Wolsky (1933) introduced the term "stimulationsorgane" to refer to organs which had both sensory and excitatory functions. He said that these stimulatory organs sent to the central nervous system continuous stimuli which were essential to the normal functioning of the neuromuscular system. These stimuli were essential for two reasons which were not always separable: the production and maintenance of muscle tone (the tonus producing effect) and the maintenance of the normal contractibility of the muscles (the kinetic effect). He said that most sense organs including the eyes of vertebrates have at least some stimulatory function. Indeed, Mast (1924) had found earlier that different regions of the compound eye of the robber fly, Proctacanthus philadelphicus, have different degrees of importance in the production and maintenance of phototonus.

The claim that ocelli are stimulatory organs is usually based on observations of depressed activity with the occlusion of the ocelli. The rate of movement or the latency of response to photic stimuli in normal and ocelli occluded insects are often used as a measure of the

depression of activity (Goodman, 1970).

The photokinetic effects of ocelli of different species are variable (Table IV). The ocelli of Drosophila were found to be stimulatory to walking speed with a general photokinetic effect being their primary function (Bozler, 1926; Médioni, 1959). Conversely, the compound eyes of Drosophila were found to inhibit running speed (Médioni, 1959). In the German roach, Blatella germanica, however, the occlusion of the ocelli increases walking speed, so the ocelli are inhibitory (Goustard, 1958). In addition, Goustard found that occlusion of the compound eyes of the German roach reduced its walking speed.

Recently Goodman (1968) observed the interaction between the ocelli and compound eyes in Schistocerca gregaria. He found that occluding the ocelli produced a reduction in walking speed at both low and high light intensities, but the reduction was larger at higher intensities. Also, at a given light intensity the reduction in walking speed observed was generally proportional not only to the number of ocelli covered but also to the degree which the ocelli were occluded (Goodman, 1970).

The latency of response to light stimuli in ocellar blinded migratory locusts and noctuid moths was measured by Cassier (1965) and Dufay (1964). In Locusta migratoria migratoriodes, Cassier found the latency period shortened up to an illumination of 50 lux, but at higher illuminations it increased again. No activity was observed at low intensities with the ocelli occluded, but as the illumination increased the latency period shortened and no reversal occurred at higher intensities. Occlusion of the median ocellus alone resulted in an increased latency period.

TABLE IV. A Summary of the More Recent Results of Studies on the Ocellar Contribution to Photokinetic Behavior (Speed of Locomotion) (From Goodman, 1970)

Author	Insect	Intact Insects		Ocelli Occluded		Compound Eyes Occluded		Blind
		Dim light	Bright light	Dim light	Bright light	Dim light	Bright light	
Bayramoglu-Ergene (1964, 1965)	*Schistocerca*, *Anacridium aegyptium*	Flight speed increases very slightly at higher intensities		Reduced	Reduced	No effect	No effect	Cease Flying
	Mantis religiosa	--	--	--	Climbing speed reduced	--	--	--
Cassier (1965)	*Locusta*	Walking speed independent of light intensity over three orders of magnitude		Reduced	Reduced	No effect	No effect	--
Goodman (1968)	*Schistocerca*	Walking speed independent of light intensity		No effect	Reduced	No effect	No effect	Reduced
	Periplaneta	Walking speed increases at higher intensities		No effect	Reduced	No effect	Reduced	Much Reduced
	Calliphora	Walking speed independent of light intensity		No effect	Reduced	No effect	Reduced	--
	Apis	Walking speed independent of light intensity		No effect	Reduced	No effect	No effect	--
Goustard (1958)	*Blatella*	In photopositive state walking speed decreases at higher intensities, in photonegative state it increases. With experience of tests it becomes independent of light intensity.		Increased	Increased	Reduced	Reduced	Reduced
Jander and Barry (1968)	*Gryllus*	Runs slightly faster at higher intensities		Reduced	Reduced	Reduced	Reduced, but less than in dim light	--
Médioni (1959)	*Drosophila*	--	--	Reduced	Reduced	Increased	Increased	--
Richard (1950)	*Calotermes*	Walking speed varies with age. Speed decreases at higher intensities with older insects.		--	Reduced	--	Reduced	--
Schricker (1965)	*Apis*	--	--	No effect on foraging flights		--	--	--

Dufay (1964) looked at the latency period before a walking or flying response to illumination in various noctuid moths. He found that darkening of either the compound eyes or ocelli caused approximately the same increase in latency time and thus that the ocelli could replace the compound eyes with regard to sensitivity to light. Further, he found a reverse correlation between latency period and light intensity in normal moths. If only the ocelli were functioning, however, the latency period shortened up to a certain level of illumination above which it increased. From the results of Cassier and Dufay, Goodman (1970) concludes that if latency period is a measure of responsiveness of an insect then ocelli appear to function to enhance the responsiveness of the insect at low light intensities and inhibit it at high intensities.

Only two authors have reported the loss of muscular tone with ocellar occlusion. Götze (1927) reported a decrease in tonus in bees when the ocelli were covered. More recently Schremmer (1950) noted that the metathoracic legs of the honeybee are dragged when the ocelli are occluded.

Concerning the functioning of ocelli as stimulatory organs, Goodman (1970) concludes that none of the results of numerous behavioral studies present conclusive evidence that these photoreceptors do function as stimulatory organs. Bullock and Horridge (1965) agree saying that the idea of a stimulatory organ is perhaps good, but no organ in the animal kingdom has been shown to function in this manner.

3. Phototaxis

A taxis is defined as a directed reaction to a stimulus (Fraenkel and Gunn, 1961). Jander (1963) chose to limit the term taxis to refer to oriented turning movements. Phototaxis is thus defined as an oriented turning movement to a light stimulus.

Photic orientation was first looked at in plants where De Candolle (1832) observed this to be the reason plants indoors turn toward an open window. His hypothesis corrected Ray's (1693) idea that temperature was the controlling factor. Verworn (1894) applied De Candolle's theory of photic orientation to lower animals, but said the amount of penetrating energy was not as important as the direction in which the rays penetrated. He later abandoned the latter theory for the former (Mast, 1924). Mast said that photic orientation in bilaterally symmetrical animals was the result of the amount of energy received by receptors on either side. By blinding the compound eye on either side of the robber fly, Proctacanthus philadelphicus, he found the specimens would lean toward the side of the functional eye and tend to turn in that direction.

With regard to the phototactic function of ocelli, Götze (1927) found a loss of phototactic accuracy when the ocelli of bees were blinded and he thought ocelli functioned as phototactic organs. Müller (1931) observed that by blinding the median and one lateral ocellus, bees walking on a path between two lights of equal intensity would deviate toward the side of the blinded ocellus. Cornwell (1955) noted in Calliphora and Locusta that even though ocelli were unable to mediate

phototactic orientation, it was not as efficient when the ocelli were occluded.

More recently Jander and Barry (1968) conducted numerous experiments designed to demonstrate the phototactic function of ocelli in Locusta and Gryllus. In one experiment crickets were placed between two parallel light beams of equal intensity which intersected at 90° and were allowed to orientate. The average orientation angle between the light sources was 45° for intact insects, insects with only the median ocellus blinded or insects with both lateral ocelli occluded. If, however, either one of the lateral ocelli were blinded the specimen would orientate towards the light on that side, which suggested that in the intact insect there was antagonistic action between the lateral ocellus and compound eye on the same side. The ocellus, however, appeared to act synergistically with the compound eye when the median ocellus was blinded since the orientation of the insect was then toward the light on the unblinded side. The switching action of the median ocellus was confirmed in other experiments by Jander and Barry (1968) on the turning tendency of the cricket in a homogeneous white environment. They proposed a model for the functional connections necessary to achieve this negative feedback in which they take into account both behavioral and electrophysiological studies.

Goodman (1970) concludes that perhaps the photokinetic effect of ocelli "may just be an epiphenomenon of their phototactic role which until now has not been fully appreciated." He also says that apparently the interactions between ocelli and compound eyes in various

insect species may be different and studies on insects with only two lateral ocelli are needed for comparative purposes.

4. Detection of absolute level of and changes in light intensity

The structure of ocelli suggests they may be especially sensitive to changes in light intensity (Goodman, 1970). Ruck (1961b, 1961c) demonstrated that the dragonfly ocellus is not only able to signal changes in light intensity but also information on the absolute level of light. Several behavioral studies have been conducted to assess the role of the ocelli in the detection of changes in light intensity and the absolute level of illumination, but since ocelli alone are seldom responsible for the initiation of a response the ocellar contribution is difficult to evaluate (Goodman, 1970).

Bozler (1926) found that Drosophila whose ocelli were painted reacted less rapidly to a sudden exposure to light than intact specimens. Dufay (1964) reported that in noctuids the ocelli could actually replace the compound eyes with regard to light sensitivity. More recently Schricker (1965) found that if given a choice between two light sources most honeybees would choose the brightest one. With the occlusion of the ocelli, however, the differences in brightness had to be larger and the size of the difference was proportional to the number of ocelli occluded. In further experiments he found that bees with occluded ocelli began foraging later in the morning and terminated foraging earlier in the evening than normal bees. Schricker therefore suggested that ocelli in bees provide them with not only a register of

changes in illumination but also information concerning absolute illumination. Goodman (1968) found that the locust, Schistocerca gregaria, was sensitive to small changes in light intensity only if the ocelli were functioning along with the compound eyes. Goodman (1970) also said that undoubtedly as more cases are studied an ocellar contribution to the detection of the absolute level of illumination and changes in light intensity will be found.

5. Spectral sensitivity and the detection of the plane of polarized light

The spectral sensitivity of the visual system of the dragonfly, Libellula luctuosa (Ruck, 1965), the honeybee, Apis mellifera (Ruck and Goldsmith, 1958), the cockroach, Periplaneta americana (Ruck and Goldsmith, 1958) and the praying mantis, Tenodera sinensis (Sontag, 1971), have been recorded. To record a spectral response the electroretinogram (ERG) technique is used where the whole ocellus is stimulated and thus the resulting ERG represents the response of a number of cells (Goodman, 1970). Goodman also points out that the possibility of functional interactions among classes of photoreceptor cells is reduced due to the threshold stimulation.

Ruck (1965) reported that the spectral sensitivity curve for the dragonfly had a peak at 518 nm in the visible range and a second peak in the ultraviolet around 380 nm with a valley at about 400 nm. Similar spectral sensitivity curves were found for both the honeybee with peaks at 490 nm and 335-340 nm (Ruck and Goldsmith, 1958) and the pray-

ing mantis with peaks at 510-520 nm and 370 nm (Sontag, 1971). In the ocellus of the American roach, Periplaneta americana, only one class of photoreceptor with a peak at 500 nm was found by Ruck and Goldsmith (1958).

The presence of two sensitivity peaks is supposed to indicate two classes of receptors as well as two visual pigments (Ruck and Goldsmith, 1958). Indeed, Sontag (1971) found that the retinular cells composing the ocellus of the praying mantis were separated into two distinct groups each making its own contribution to the ocellar nerve. That two distinct regions of cells were present corresponded with the two-peaked spectral sensitivity curve of this ocellus.

The capacity to detect the plane of polarized light is characteristic of the compound eyes of many Arthropods (von Frisch, 1950; Waterman, 1950; Kuwabara and Naka, 1959). Waterman and Horch (1966) postulated that the polarized light analyzer lay within a single ommatidium and evidence from electron microscopy suggested that the rhabdom with its oriented microvilli must be the place. Wellington (1953) reported that the ocelli of the flesh fly, Sarcophaga aldrichi, could detect and cause a response to a change in the plane of polarized light. This is the only report of ocelli being sensitive to the plane polarized light. Recently, however, examination of the structure of the rhabdom of the dorsal ocellus of the flesh fly, Boettcherisca peregrina, revealed that microvilli project from the periphery of each retinular cell in all directions so that analysis of the plane of polarized light was not possible.

6. Circadian rhythms

The synchronization of animals with factors in their environment is of great survival value (De Wilde, 1962). The synchronization of both inter- and intraspecific relationships is the primary function of circadian rhythms. The synchronization of this interspecific relationship is of especial importance when it concerns reproduction. Circadian rhythms must also be of importance in the production of lunar and seasonal cycles (Danilevsky, 1970).

Several authors report ocelli to be involved in the entrainment of circadian rhythms. Cloudsley-Thompson (1953) reported that occlusion of the ocelli caused a loss of circadian rhythm in the American cockroach, Periplaneta americana. He did, however, say that the compound eyes were also involved. A few years later, Harker (1956) said ocelli alone were responsible for the establishment of a circadian rhythm in the American cockroach. She further implicated the sub-oesophageal ganglion as the source of either an inhibitory or stimulatory secretion which was released by ocellar stimulation. The secretion was said probably to be stimulatory since the ocelli discharge continuously in the darkness. In a later paper (Harker, 1958), she said hormones were probably intermediates in the expression of rhythms in all Arthropods. There have, however, been conflicting reports. Roberts (1965a, 1965b) did not consider ocelli to be responsible for the entrainment of circadian rhythms in the American cockroach, but found the compound eyes alone to be sufficient. Some authors, however, believe that the photoperiodic induction of a daily rhythm is mediated by circa-

dian oscillations (Pittendrigh and Minis, 1964; Nishiitsutsuji-Uwo and Pittendrigh, 1968a, 1968b). They say both the compound eyes and ocelli are by-passed and light absorbed directly by the central nervous system is responsible for the circadian rhythm. In support of this theory, Lees (1964) found that direct illumination of the brain of the aphid, Megoura viciae, induced a circadian rhythm. In the cockroach Leucophaea, Nishiitsutsuji-Uwo and Pittendrigh (1968b) located the circadian clock within the optic lobes. They said that self-sustaining driving oscillations within the optic lobes caused the pars intercerebralis to secrete a substance which acted on the thoracic ganglia influencing walking speed thus effecting a circadian rhythm. Goodman (1970) concludes that at the present time ocelli are not clearly implicated in circadian rhythms.

Recently, Hinks (1970) demonstrated nerves which link directly the ocelli, compound eyes, antennae and corpora cardiaca in noctuid moths. These neural connections could provide a direct sensory input to a glandular source, the corpora cardiaca, and could be responsible for photoperiodic responses, such as the induction of diapause and circadian rhythms. Also, Brousse-Gaury (1970) described photoneuroendocrine pathways which originated in the ocelli of Gryllus domesticus, Locusta migratoria migratoriodes and Schistocerca gregaria. The ocellar impulses are conveyed to the retrocerebral complex by the nervi corporis cardiaci I and II (NCCI and NCCII) in all three cases but in different fashions: impulses from the median ocellus of Orthoptera and also the lateral ocelli of Gryllus are carried by NCCI; NCCII carries impulses

from the lateral photoreceptive organs in Locusta, Schistocerca and Gryllus. She says her findings support the assumption that light stimuli reach and control the neuroendocrine system. She also considers her results to explain the endocrine aspect of photoperiodicity and the ocellar and hormonal mechanisms in circadian rhythms.

C. Nerves Associated with Some Ocelli

Although the ocellus is in some cases thought to possibly play a role in diapause and photoperiod regulation (Harker, 1956), an effect thought by some to involve an endocrine secretion (Harker, 1958), and although in at least one case (Mimura, et al., 1970) the ocelli are thought to regulate brain excitability, no direct neural connections between ocelli, the endocrine glands, or other sensory organs were reported until recently when Hinks (1970) described a complex of nerves associated with the retrocerebral complex in noctuids. The corpora cardiaca lie above the aorta just posterior to the protocerebrum to which each is connected by a nervus corpus cardiacum I (NCCI) and a more lateral nervus corpus cardiacum II (NCCII). Two nerves connect each corpus cardiacum to its ipsilateral corpus allatum: the anterior nervus corpus cardiacum-corpus allatum I (NCCCAI) and the posterior nervus corpus cardiacum-corpus allatum II (NCCCAII). Also each corpus cardiacum is connected to the recurrent nerve by a slender nerve, the nervus corpus cardiacum-nervus oesophagus (NCCNO). Another nerve, the nervus corpus cardiacum ventralis (NCCV) arises from the corpus cardiacum ventral to the origin of NCCII. This lateral nerve passes ventrally

to innervate the various mouthparts.

Ventrally near the oesophageal connections NCCII arises then passes posteriorly to make connection with the corpus cardiacum. Where NCCII turns medially toward the corpus cardiacum a dorsal nerve arises, the nervus corpus cardiacum II dorsalis (NCCIID). NCCIID traverses the dorsal protocerebrum a short distance before dividing into four branches: nerve 1 extends dorsally to the ocellus; nerve 2 continues forward to the cephalic artery and the base of the antennal nerve; nerve 3 passes laterally and bifurcates to innervate a large seta postero-dorsal to the compound eye, and the optic lobe; and nerve 4 passes posteriorly into the thorax.

Hinks raises the question as to whether these nerves, NCCIID and NCCV are efferent and conduct neurosecretory material to the sense organs, or afferent and send sensory impulses to the corpora cardiaca. If these nerves are afferent this system would provide for direct sensory input into the corpora cardiaca and could thus play a part in photoperiod induction and diapause if they are indeed under hormonal control.

D. Electrical Characteristics of the Ocellus

Electrical responses of the ocelli of several insect species have been recorded (Parry, 1946; Hoyle, 1955, both in Locusta; Metschl, 1963, in Calliphora erythrocephala; Ruck, in several species including the dragonfly, Sympetrum rubicundulum and the cockroach, Blaberus craniifer, 1961a). The first extracellular recording from an ocellus was made by

Parry (1946) from the locust, Locusta migratoria migratoriodes. He was unable to record responses in the ocellar nerve fibers to either light or darkness. Later, Hoyle (1955) recorded three types of activity from the ocellar nerve of Locusta: an "on" discharge, an "off" discharge and continuous discharge in darkness. These pioneering studies led to the comparative studies of the electrical activity of ocelli by Ruck (1954, 1957, 1958a, 1958b, 1958c, 1961a, 1961b, 1961c, 1965).

In comparing the electrical responses to a light stimulus in the ocelli of the dragonfly, Libellula, and the cockroach, Blaberus, Ruck (1961a) was able to resolve four components of the ERG originating in either the photoreceptor cells or the ocellar nerve fibers. The first component, a depolarizing generator potential, originated in the distal end of the photoreceptor cell and evoked the second component, a depolarization of the receptor cell axons. Component 2 then evoked component 3, a hyperpolarizing inhibitory post synaptic potential originating in the dendritic terminals of the first order nerve fibers. The fourth component is composed of afferent impulses from the spontaneously active second order ocellar fibers in the dark adapted state, and is inhibited by component 3.

Light and dark adaptation processes occur in ocelli and are related to the frequency of spontaneous discharge in the ocellar fibers (Hoyle, 1955; Ruck, 1961b; Mimura, et al., 1970). Spontaneous discharges in the ocellus of the flesh fly, Boettcherisca peregrina, in the dark-adapted state (80 impulses per second) are reduced to 0 impulses per

second at the onset of illumination (Mimura, et al., 1970). A few seconds after the onset of illumination the ocellus becomes light-adapted and the ocellar nerve fibers become spontaneously active again but at a lower impulse rate (60 impulses per second). With the cessation of illumination the rate of ocellar discharges increased to a maximum (150 impulses per second) before declining to the mean dark-adapted rate (80 impulses per second). Similar results were obtained by Metschl (1963) for the ocellus of Calliphora.

Recently Mimura, et al. (1969, 1970) reported an interaction between antennal and ocellar units. The response to antennal stimulation was either facilitated or inhibited by photic stimulation of the ocelli. They say their results suggest that the ocellus is an organ concerned with the regulation of brain activity.

MATERIALS AND METHODS

I. Insects

Most moths used in this study were captured in light traps at Price's Fork, Virginia. Some diapause tobacco hornworm, Manduca sexta, pupae were obtained from A. H. Baumhover at the U.S.D.A. Tobacco Research Station in Oxford, North Carolina. These pupae were stored at 4.5°C until needed at which time they were allowed to emerge in a controlled temperature and humidity chamber.

II. Morphological Studies

The insects used for dissection purposes were decapitated and the heads fixed for several hours in either alcoholic Bouin's solution (Drake and McEwen, 1959), picroformal, or the fixative described by Chauthani and Callahan (1966). Dissections were performed with the aid of a Leitz stereo microscope. Dissection of small nerves was facilitated by using electrolytically sharpened stainless steel probes (Roeder, 1966).

III. Light Microscopy

Material used for light microscopy was fixed in alcoholic Bouin's either prior to or after dissection from the insect. Excess picric acid was removed with Lenoir's fluid prior to dehydration in graded ethanol solutions, a benzene-phenol mixture and benzene. The tissue was embedded in paraffin and sectioned with a steel knife using an American

Optical rotary microtome. Serial sections of internal ocelli were cut at 6 μ; sections of the external cuticular ocellus were made at 10 μ. The sections were mounted on a wet slide coated with albumin and stained with either Mallory's triple stain (Gray, 1964) or a modification of Holmes' silver stain (Larsen, 1960). Photomicrographs were made using a Leitz Orthomat automatic camera with a Leitz Ortholux bright field microscope or a Zeiss phase contrast microscope with an automatic camera set up.

IV. Scanning Electron Microscopy

Whole heads or pieces of cuticle containing the external cornea were dehydrated in a Denton vacuum, model DV-515, at 10^{-4} to 10^{-5} torr and coated with a 60:40 gold-platinum mixture. The specimens were examined using an Advanced Metals Research Corporation model 900-J2 scanning electron microscope and micrographs were made using a Polaroid camera set up for that purpose.

V. Transmission Electron Microscopy

Light-adapted adult moths were decapitated and their heads immersed in a glutaraldehyde fixative described by Luce (1966) and modified by Fleming and Saacke (1972). Within one minute after immersion, the vertex of the head was removed to facilitate the penetration of the fixative to the internal ocelli. Alternatively the fixative was injected directly into the head through the cervix using a hypodermic syringe while the head was still attached to the living insect. No apparent

advantage was gained by using this latter procedure.

Aldehyde fixation lasted about one and one-half hours. The heads were then transferred to a dissection dish containing phosphate buffer; the ocelli were removed and placed in a small Syracuse dish with buffer where they remained overnight. Post-fixation in buffered Osmium tetroxide followed.

The ocelli were then dehydrated in graded ethanols and propylene oxide. They were then infiltrated with a 1:1 mixture of Epon 812 and propylene oxide and warmed at 60°C for twenty minutes to boil off the propylene oxide. Embedding followed using Beem capsules containing Epon 812. The Epon in the capsules was polymerized in an oven at 60°C for approximately twenty hours.

Thin sections, 600-1000 Å thick, were cut using a glass knife and a Porter-Blum MT-1 ultramicrotome. The sections were picked up on 200 mesh copper grids and stained with uranyl acetate and lead citrate (Venable and Coggleshell, 1965; Watson, 1958). An RCA EMU-3H transmission electron microscope was used for viewing the grids.

For orientation purposes, adjacent thick sections, approximately 1 μ thick, were cut at intervals and stained with a buffered Azure II solution (Jeon, 1965). More details concerning the methods may be found in the appendix.

RESULTS

The term "internal ocellus" will be used to refer to the structure described by Eaton (1971). The external cornea and the cells immediately beneath will be referred to as the "external ocellus" and is reported here for the first time in anocellate Lepidoptera. The two structures together probably represent the dorsal ocelli of other insect species but have become secondarily separate.

I. The Internal Ocellus

A. Distribution

Moths (Heterocerea) collected in light traps and butterflies and skippers (Ropalocera) collected in the field were examined for the presence of internal ocelli (Table V). Internal ocelli were found in Ropalocera (Hesperiidae) for the first time. Other Ropalocera, especially the Pieridae, were found to have a flattened group of cells enclosed by a sheath on the dorsal protocerebrum where the ocelli generally would arise, but the exact nature of these cells was not ascertained (Figures I and II).

B. Structure

The overall shape of the internal ocellus is variable. In Manduca sexta a stalk, consisting primarily of nerve fibers, rises from the dorsal protocerebrum and extends approximately 75 μ to 100 μ to a bulb of retinular cells some 50 μ to 60 μ in diameter (Figure III). In

TABLE V. Lepidoptera Previously Reported to be Anocellate Having Internal Ocelli

Heterocera

Sphingidae:

- *Calasymbolus amyntor*
- *C. excaecata* (Eaton, 1970)
- *C. myops* (Eaton, 1970)
- *Darapsa myron*
- *D. pholus* (Eaton, 1970)
- *D. versicolor* (Eaton, 1970)
- *Herse cingulata*
- *Hyloicus chersis*
- *H. drupiferarum* (Eaton, 1970)
- *Lapara coniferarum*
- *Manduca quinquemaculata* (Eaton, 1970)
- *M. rustica*
- *M. sexta* (Eaton, 1970)
- *Pholus achemon* (Eaton, 1970)
- *P. pandorus* (Eaton, 1970)
- *Spectrum lineata* (Eaton, 1970)
- *Sphinx jamaiciensis*

Saturniidae:

- *Actias luna*
- *Automeris io*
- *Telea polyphemus* (Eaton, 1970)

Citheronidae:

- *Anisota rubicunda*
- *Citheronia regalis*
- *C. sepulchralis*
- *Eacles imperialis* (Eaton, 1970)

Arctiidae:

Lithosiinae

- *Hypoprepia miniata*

Ropalocera

Hesperiidae:

- *Epargyreus clarus*
- *Thorybes pylades*

other moths examined, the ocelli were either short and stumpy or elongate and thin. The ocelli of one Manduca sexta specimen were within a sheath separate from the protocerebrum but lying flat on the dorsal part of it and recognizable only as two small bumps on the brain. This is similar to the structure present in some butterflies (Figures I and II).

The insect nervous system is surrounded by a non-cellular, neurilemma and a cellular perineurium and the internal ocellus is no exception. The outer amorphous neurilemma is several microns thick. Beneath the neurilemma is the cellular perineurium, a thin layer of cells containing numerous mitochondria (Figures III and IV).

A flattened layer of glial cells surrounds each photoreceptor unit to separate it from the perineurium and other photoreceptor units in the area of the rhabdom (Figures IV and VI). Glial cell cytoplasm is scarce but mitochondria are scattered throughout. The glial cell nuclei are pleomorphic, but are often flattened (Figure IV and VI). The numerous microtubules in the glial cell cytoplasm are packed closely together (Figures IV, V and VI).

The basic structure of the photoreceptor unit is similar to other photoreceptors. Numerous microvilli arise from the surface of the retinular cells to form a rhabdom (Figures IV, V and VI). Generally the rhabdom is composed of microvilli from one retinular cell but in some cases two or three cells may contribute. The loosely organized microvilli of the rhabdom are continuous at their base with the retinular cell cytoplasm (Figure V).

The distal area of the retinula is characterized by a rhabdom

surrounding a relatively large lumen containing a homogeneous electron dense substance (Figures IV and V). In this area a peri-rhabdomeric reticulum (Curtis, 1969) is present. Numerous multivesicular bodies surround the rhabdom and in some cases open up toward the microvilli. Vesicles resembling those found in the multivesicular bodies may be seen within the microvilli (Figure V). Glial cells may penetrate the retinular cells in this area. Mitochondria, though sparse in the cytoplasm immediately surrounding the rhabdom shown here, are numerous in the retinula cell cytoplasm outside the penetrating glial cell area. Numerous ribosomes, smooth and rough endoplasmic reticulum, and myeloid bodies may occur in the cytoplasm (Figures IV and VI).

The nuclei of the retinular cells are located in the proximal or medial areas of the rhabdom (Figure VI). Here the rhabdom composed of loosely oriented microvilli is surrounded by numerous mitochondria. Multivesicular bodies are also present. The retinular cell nucleus is large and multi-lobed. The peripheral cytoplasm contains numerous ribosomes as well as both smooth and rough membranes. Glial cell membranes penetrate the cytoplasm and cell junctions occur near the base of the microvilli composing the rhabdom.

Near the base of the retinular cell below the rhabdom, short retinular cell axons, not surrounded by glia, synapse with second order neurons. The retinular cell axons are small and there is much convergence on the generally larger second order axons. Mitochondria, synaptic vesicles and microtubules characterize the pre-synaptic axons. The cytoplasm of the second order axons appears lighter than first order

axons when examined under the electron microscope (Figure VII).

The synapses are of two types: axo-axonic and axo-somatic. Axo-axonic synapses occur where pre-synaptic and post-synaptic axons meet and these are the most common. Where a pre-synaptic axon comes in contact with the soma of a post-synaptic neuron it is called an axo-somatic synapse. Axo-somatic synapses are rare but do occur (Figure VII).

C. Nerves Associated with the Internal Ocellus

Several nerve branches were associated with the internal ocelli of moths examined (Figure VIII). The largest of the nerves from each ocellus traversed the postero-dorsal part of the protocerebrum to enter the corpus cardiacum on the same side (OcN4). Another nerve branch off the ocellus, OcN1, extends to the external ocellus. Other nerve branches, OcN2 and OcN3 innervate the base of the antennae and the optic lobe, respectively.

The manner in which these nerves arise from the ocellus is variable. In some instances several nerves may arise at the ocellus but may fuse and thus innervate only one area (OcN2b). The areas most consistently innervated by ocellar nerves are: the external ocellus, the antennae, the optic lobes and the corpora cardiaca. Less frequently a connection with the tegumentary nerve is found.

Expansions along the ocellar nerve branches sometimes occur. These are thought to be nerve soma but the exact nature or function of these is not known.

II. The External Ocellus

A. Distribution

All previously described anocellate Lepidoptera studied were found to possess external ocelli. External ocelli were found in anocellate Heterocera as well as all Ropalocera examined (Table VI).

B. Structure

In all Lepidoptera reported to be lacking external ocelli, removal of the scales from the vertex of the head just posterior and mesal to the antennae revealed a pair of external ocellar lenses (Figures VIII IX, and X). These lenses measured 22 μ to 28 μ in diameter and were convex structures located in the bottom of a cuticular depression. The external ocelli of both the anocellate moths and butterflies were very similar, but the moths' ocelli were usually larger in diameter by several microns. Below each lens and surrounded by cuticle are several nerve cells (Figures XI and XII). In some moths a nerve branch from the internal ocellus may be followed to this cuticular structure (Figures VIII and XI).

TABLE VI. Lepidoptera Previously Reported to be Anocellate Having External Ocelli

Heterocera

Sphingidae:

- Calasymbolus amyntor
- C. excaecata
- C. myops
- Darapsa myron
- D. pholus
- D. versicolor
- Herse cingulata
- Hyloicus chersis
- H. drupiferarum
- Lapara coniferarum
- Manduca quinquemaculata
- M. rustica
- M. sexta
- Pholus achemon
- P. pandorus
- Spectrum lineata
- Sphinx jamaiciensis

Saturniidae:

- Actias luna
- Automeris io
- Telea polyphemus

Citheroniidae:

- Anisota rubicunda
- Citheronia regalis
- Citheronia sepulchralis
- Eacles imperialis

Arctiidae:

- Lithosiinae
  - Hypoprepia miniata

Ropalocera

Papilionidae:

- Papilio polyxenes
- P. troilus

Pieridae:

- Colias eurytheme
- C. interior

Table VI. Continued.

Colias philodice

Pieris rapae

Danaidae:

Danaus plexippus

Satyridae:

Cercyonis pegala

Nymphalidae:

Euphydryas phaeton

Limenitis archippus

Polygonia comma

Speyeria aphrodite

S. cybele

S. diana

S. idalia

Vanessa atlanta

Libytheidae:

Libytheana bachmanii

Lycaenidae:

Celastrina argiolus pseudargiolus

Hesperiidae:

Epargyreus clarus

Thorybes pylades

Figure I. Light micrograph of a frontal section through the head of a clouded sulfur butterfly (Ropalocera, Colias philodice) showing the left side of the protocerebrum, optic lobe and possible internal ocellus (Mallory's triple stain; section 15 μ thick; x 400). Antennal muscles, am; antennal sclerite, as; internal ocellus, io; optic lobe, ol; protocerebrum, p.

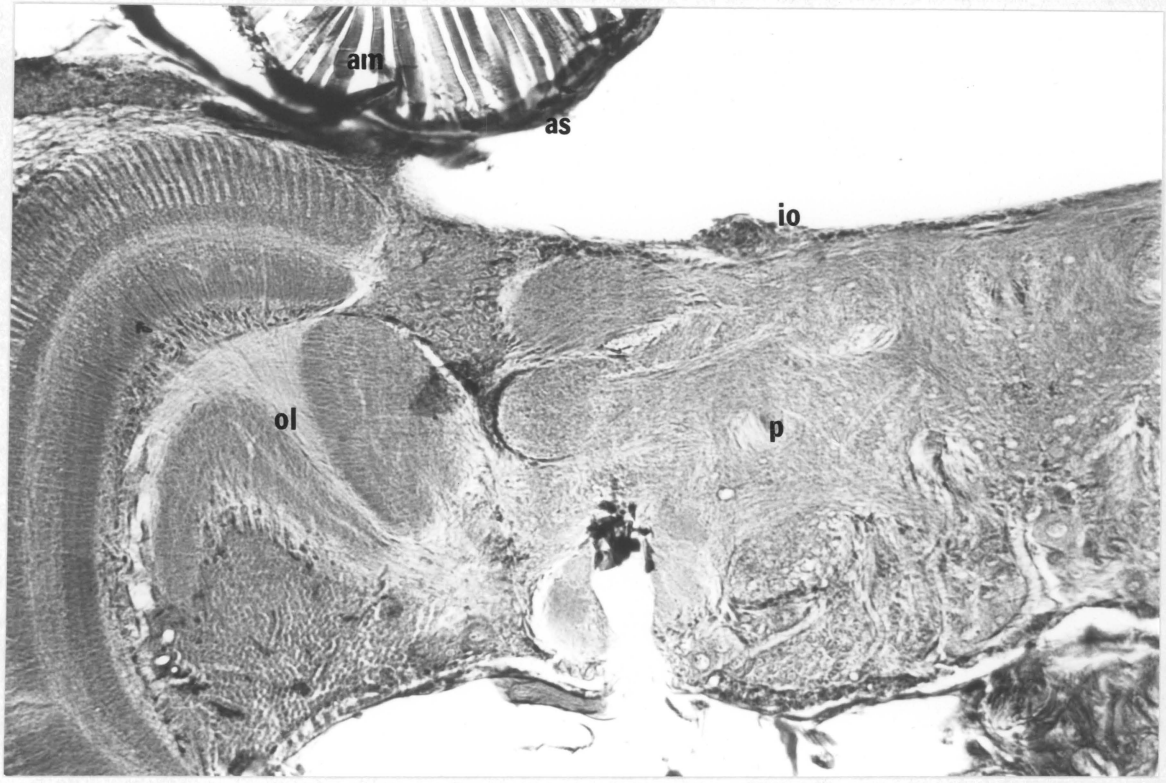

am
as
io
ol
p

Figure II. Light micrograph of possible internal ocellus of clouded sulfur butterfly seen at higher magnification (Mallory's; 15 μ; x 1600). Internal ocellus, io; protocerebrum, p.

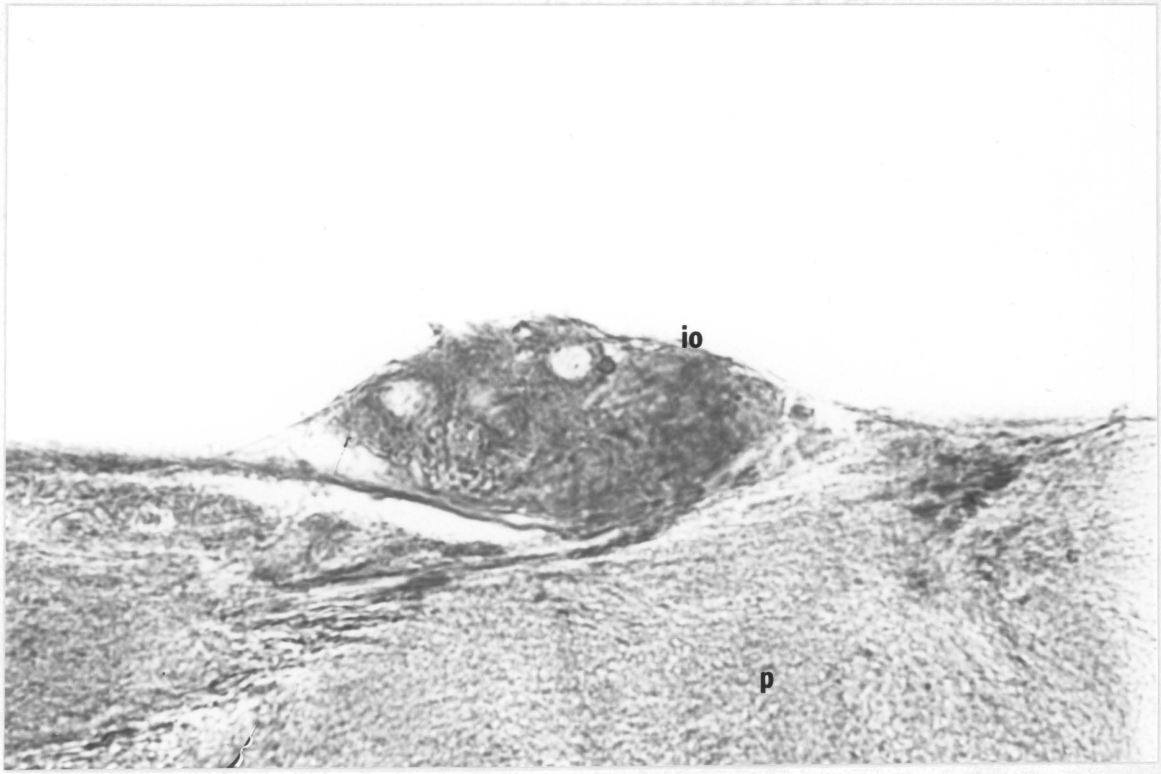
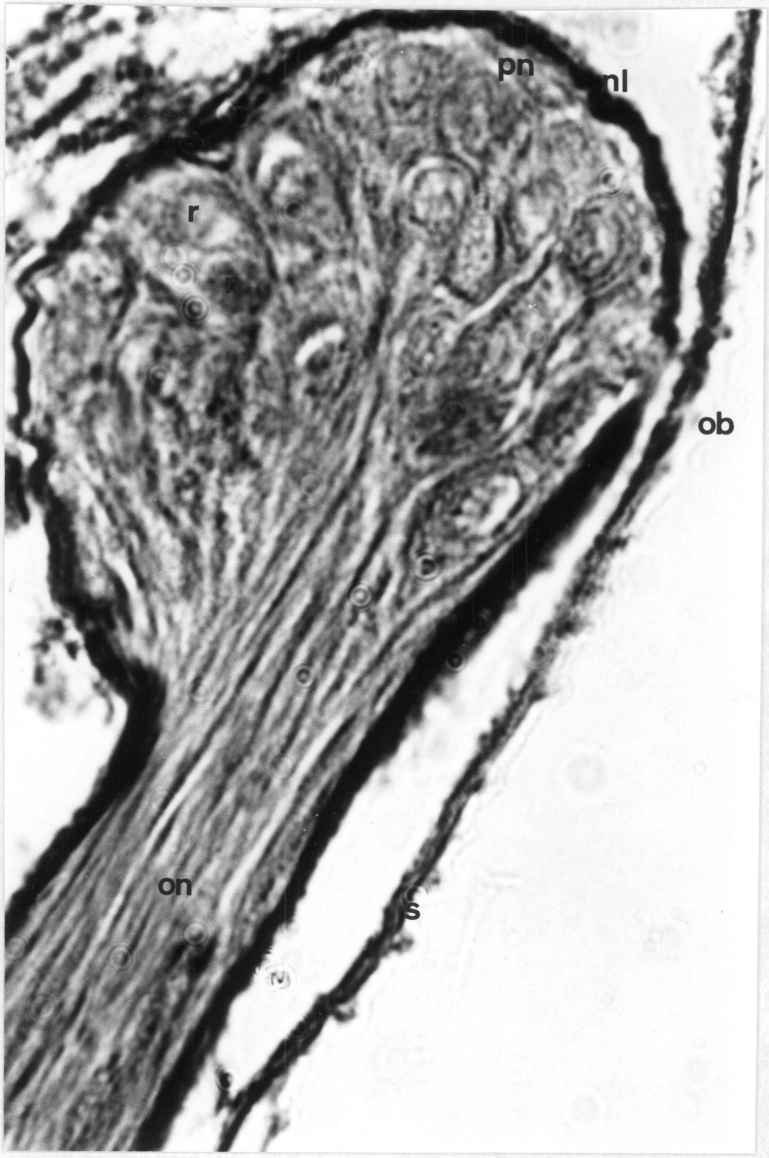

io
p

Figure III. Section through the internal ocellus of a tobacco hornworm moth (Heterocera, Sphingidae, Manduca sexta) showing ocellar nerve and bulb (Mallory's; 6 μ; x 4,000). Neurilemma, nl; nucleus, n; ocellar nerve, on; ocellar bulb, ob; perineurium, pn; rhabdom, r; sheath, s.


pn
nl
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s

Figure IV. Electron micrograph of a section through the distal end of a retinula envaginated by glia demonstrating the presence of a lumen (x 9,600). Glial cell, gl; glial cell nucleus, gln; mitochondrion, m; electron dense myeloid body, mb; microtubules, mt; neurilemma, nl; perineurium, pn; lumen, l; multivesicular body, mvb; rhabdom composed of microvilli, r; retinular cell cytoplasm, rc.

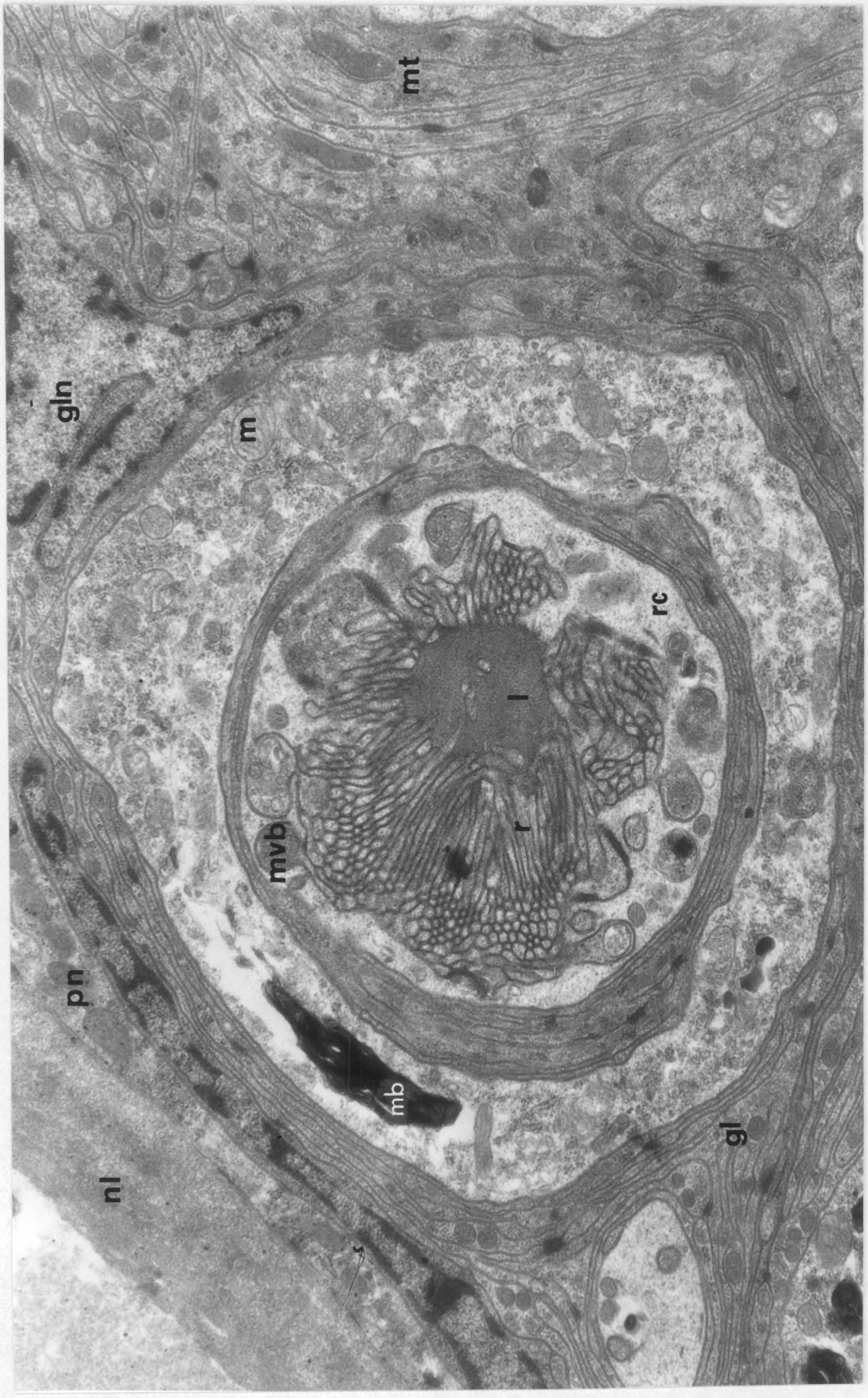

mt
gln
m
rc
l
r
mvb
mb
pn
nl
gl

Figure V. Higher magnification of rhabdom of Figure IV showing more clearly a peri-rhabdomeric reticulum, multivesicular bodies and vesicles within the microvilli (x 13,100). Glial cell, gl; mitochondrion, m; electron dense myeloid body, mb; microtubules, mt; lumen, l; microvilli, mv; multivesicular body, mvb; vesicles within the microvilli, v.

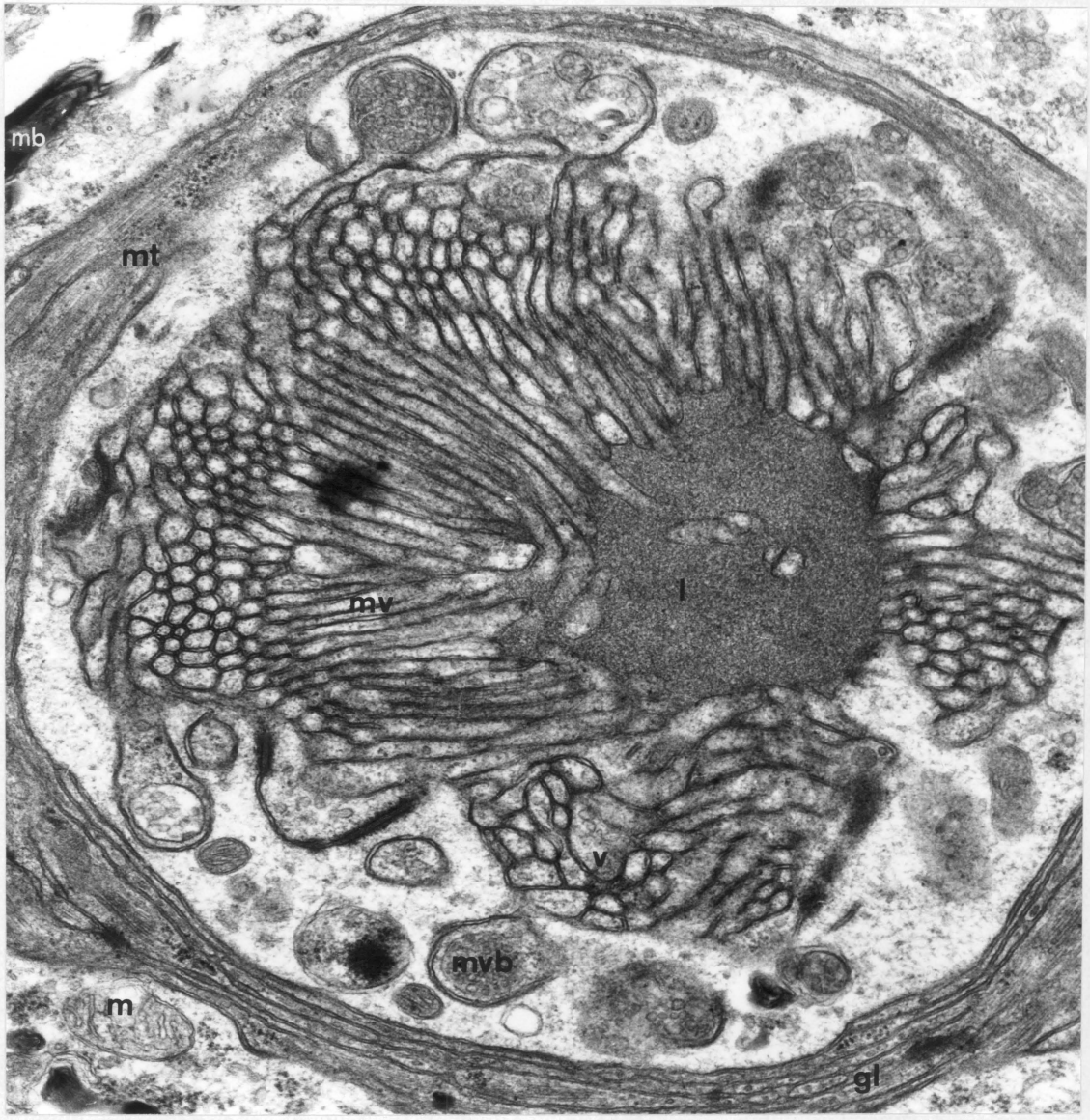

mb
mt
mv
l
v
mvb
m
gl

Figure VI. Electron micrograph of a section through proximal end of rhabdom in area of nucleus (x 8,550). Cell junction, cj; glial cell, gl; glial cell nucleus, gln; mitochrondrion, m; multivesicular body, mvb; microtubules, mt; microvillar rhabdom, r; multilobed retinular cell nucleus, rn.

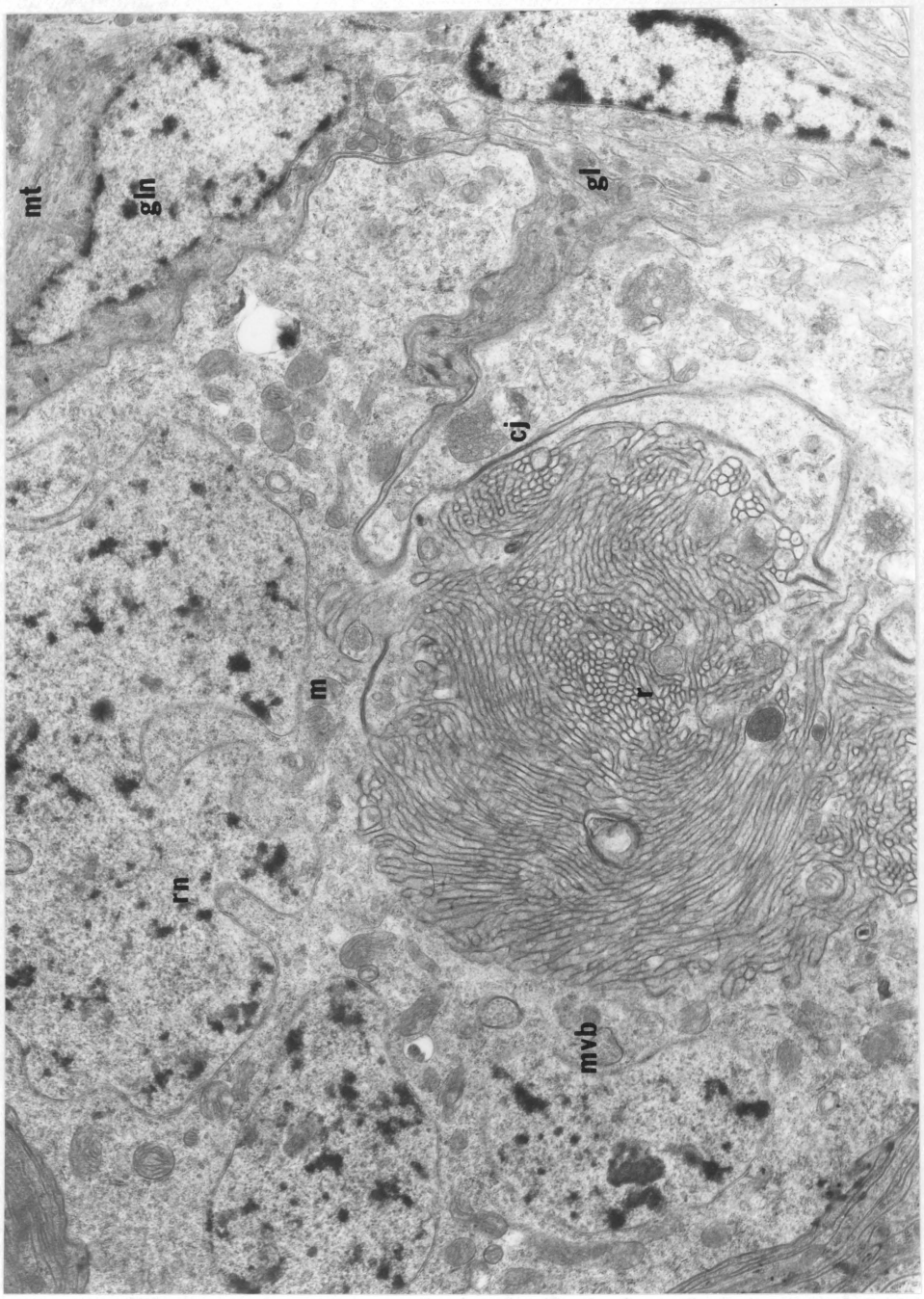

mt
gln
gl
cj
m
t
rn
mvb

Figure VII. Electron micrograph of synapse of retinular cell axons with second order neurons (x 47,500). E-type synaptic vesicle, e; I-type synaptic vesicle, i; mitochondrion, m; retinular cell axon, rx; second order axon, sx; synapse, s.

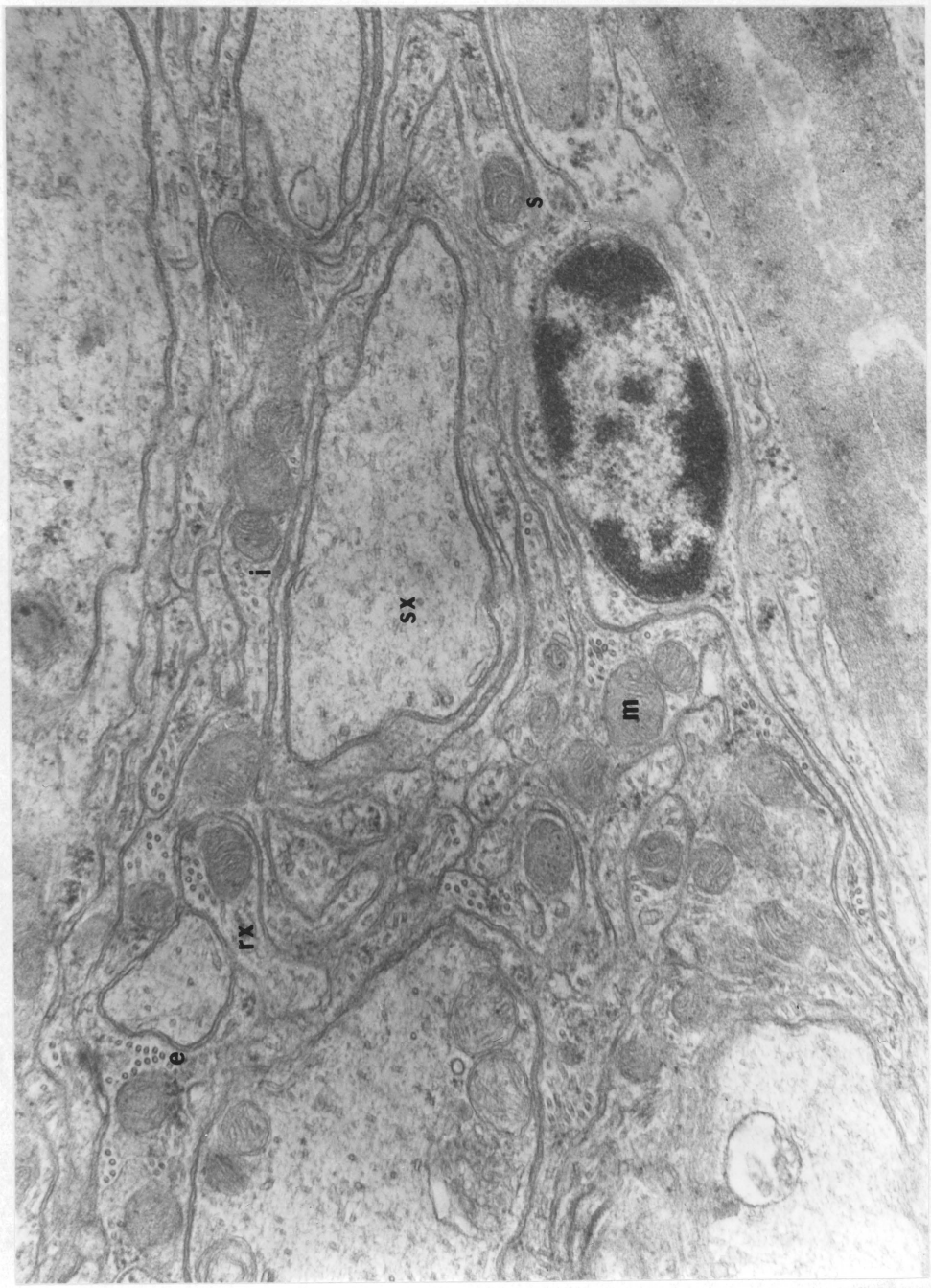

s
i
sx
m
rx
e

Figure VIII. Sketch of nerves (not to scale) associated with the internal ocellus of Manduca sexta. Antennal nerve, an; corpus cardiacum, cc; corpus allatum, ca; internal ocellus, io; external corneal lens, el; nerve branches associated with the internal ocellus: 1, branch to the external ocellus; 2a and 2b, branch to the antennal nerve; 3, branch to the optic lobe; 4, branch to the corpus cardiacum.

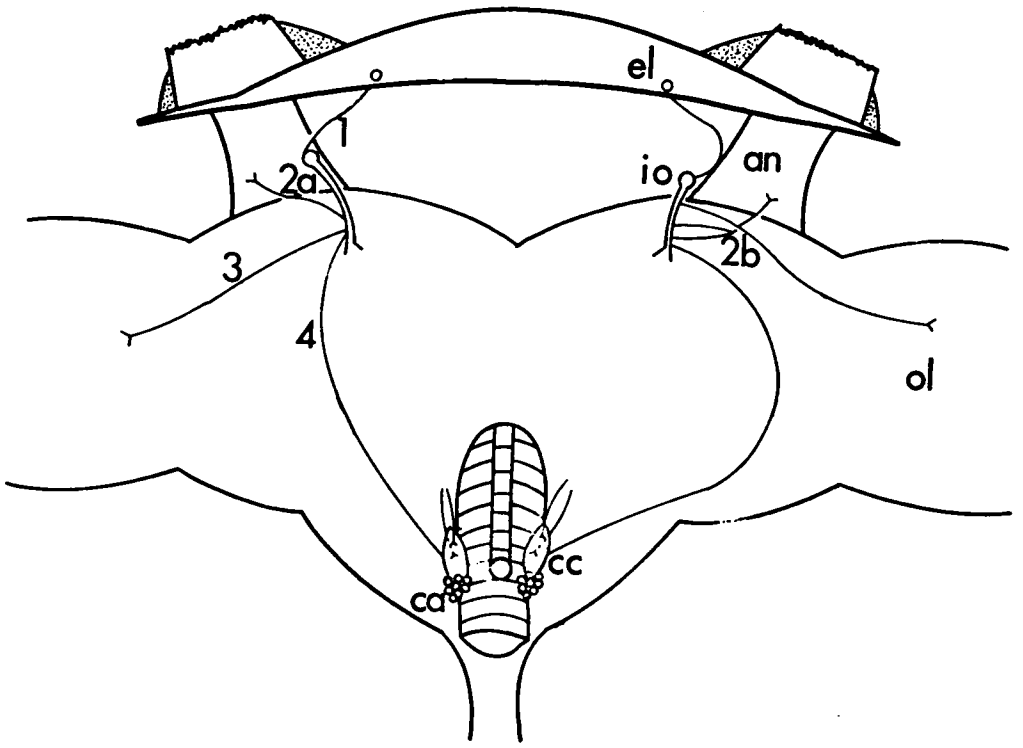

el
1
2a
io
an
2b
3
4
ol
cc
ca

Figure IX. Scanning electron micrograph of the external cornea of the tobacco hornworm moth, Manduca sexta (x 2,700). Cornea, c.

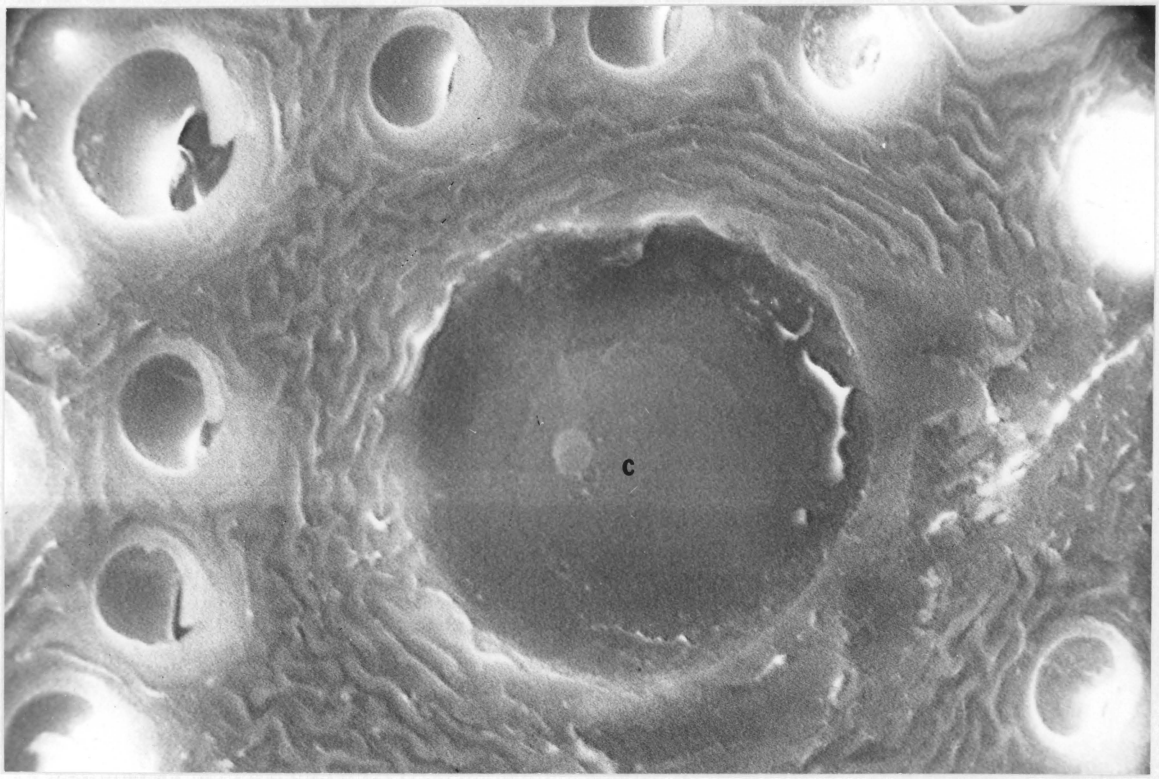

c

Figure X. Scanning electron micrograph of the external cornea of the butterfly, Colias sp. (x 6,750). Cornea, c.

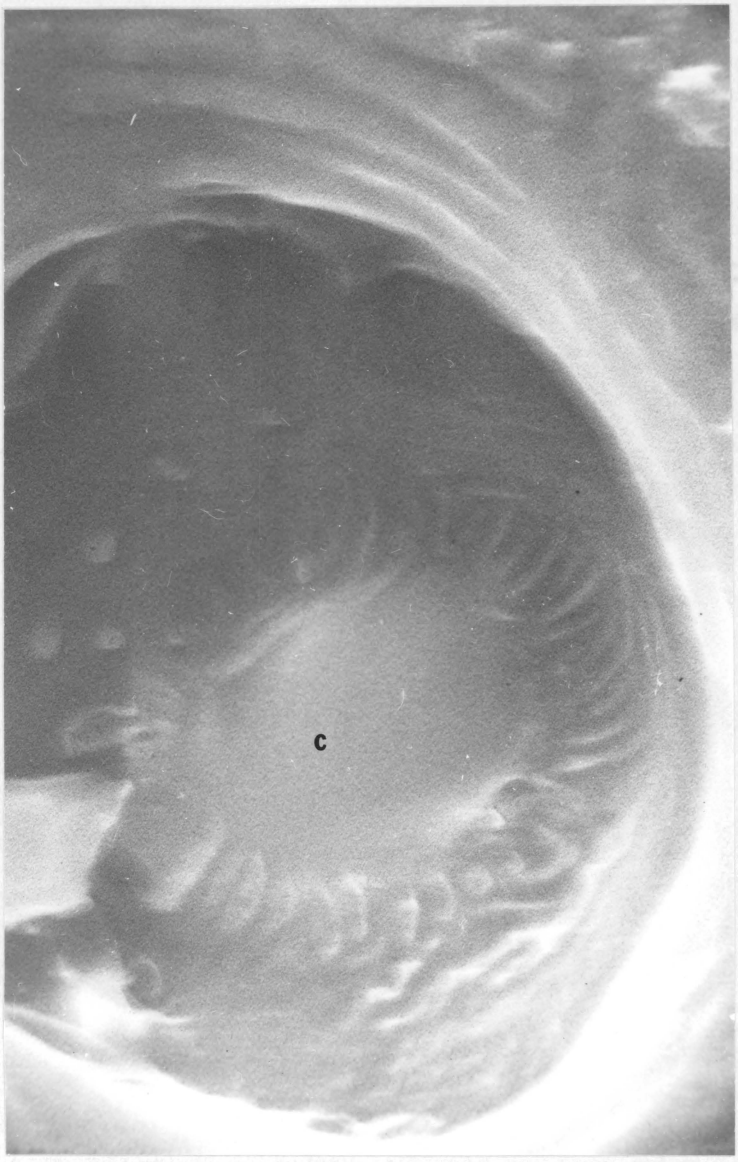
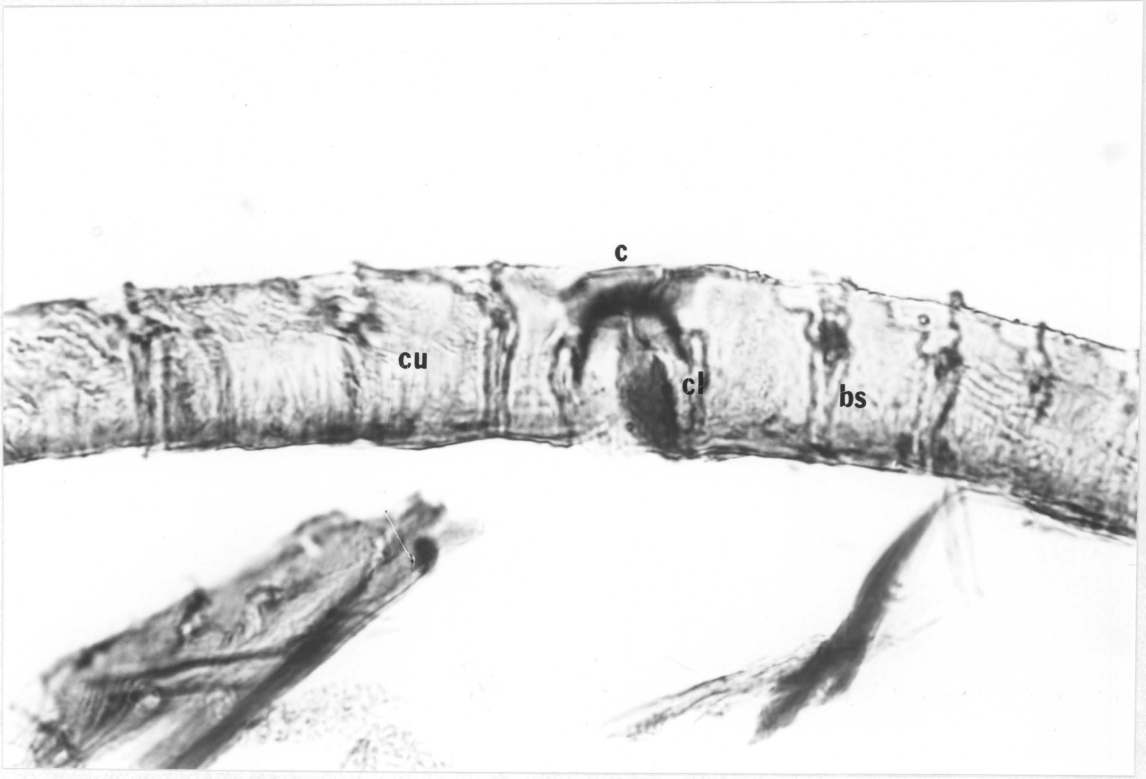

c

Figure XI. Light micrograph of a longitudinal section through the external ocellus of Calasymbolous excaecata showing the cornea and a nerve extending from the cells beneath the cornea (Mallory's; 10 μ; x 1600). Cornea, c; cells beneath cornea, cl; cuticle, cu; nerve leading to cells beneath cornea, nc.


cu
c
cl
nc

Figure XII. Light micrograph of a nearly longitudinal section through the external ocellus of the tobacco hornworm moth, Manduca sexta (Mallory's; 10 μ; x 1600). Base of cuticular scale, bs; cornea, c; cells beneath the cornea, cl; cuticle, cu.


c
cu
cl
bs

DISCUSSION

I. The Internal Ocellus

A. Distribution

The distribution of internal ocelli in anocellate Lepidoptera has been significantly expanded. Eaton (1971) described the presence of internal ocelli in several species of sphingids (10), one saturniid and one citheroniid. The present study expands this list to include 17 sphingids, 3 saturniids, 4 citheroniids, an arctiid (Lithosiinae) and for the first time two butterflies (Ropalocera, Hesperiidae). The possibility of other Ropalocera having ocelli but in a somewhat different form (Figure I) was illustrated in a pierid, the clouded sulfur butterfly (Colias philodice).

B. Structure

The surface structure of the internal ocellus is similar to other ocelli: a slender stalk arising from the dorsal protocerebrum leads to a bulbous expansion of cell bodies. A major difference in the structure is the ocellus does not extend to the vertex of the head (Eaton, 1971) where it might lie beneath a corneal lens (Wigglesworth, 1965). Since the brain including the ocelli is enclosed in a membraneous sheath above which is a large air space, possibly the internal ocellus is degenerate. The presence of the large air space above the protocerebrum would apparently serve to effect more efficient circulation by reducing the volume of hemolymph, but it also might lighten the

insect and thus facilitate locomotion.

That ocelli in these moths and butterflies are internal and appear degenerate due to their size and structure does not mean they are any less important in the functioning of these insects. The absolute level of light intensity as well as changes in light intensity could very well be detected by them (Dufay, 1964; Cassier, 1965). The fact that they might act as stimulatory organs (Wolsky, 1933) or even regulate the overall excitability of the brain (Mimura, et al., 1969, 1970) may not be ruled out. The loosely organized structure of the rhabdom and the fact that it is usually composed of only one retinular cell does, however, rule out the possibility of the detection of the plane of polarized light, since an orderly linear arrangement of the microvilli of several cells composing a rhabdom is prerequisite to the detection of the plane of polarized light (Toh, et al., 1970; Waterman and Horch, 1966).

Eaton (1971) claimed it not possible to definitely identify rhabdoms in sections of sphinx ocelli stained with either Mallory's triple stain or Holmes' silver stain. That membranes are argentophilic is well-known (Ruck, 1957; Samuel, 1953). Paraffin sections of the bulb of the internal ocellus stained with Holmes' silver stain revealed dark circular argentophilic spots 6 μ in diameter which were obviously the rhabdoms. With Mallory's triple stain the rhabdoms did not stain well at all.

Light and electron microscopy have also shown the internal ocellus to lack pigment cells or a tapetum as Eaton (1971) suggested. He said

that he was referring to portions of the neurilemma and the perinenium which surround the entire ocellus.

The structure of the rhabdom of the internal ocellus is unique among insect photoreceptors. Neither the apparent unicellular retinula structure nor the circular rhabdom have ever been reported in insect photoreceptors. Also the sometimes irregular packing together of the microvilli has never been reported for an Arthropod photoreceptor. The only similar rhabdom structure I am aware of was reported by Jones, et al. (1971) for the median ocellus of Limulus. They refer to circular self-rhabdoms in which both layers of microvilli arise from a single photoreceptor cell.

The ocelli used in this study were removed and fixed from light-adapted moths. Both Horridge and Barnard (1965) in the compound eye of Locusta, and Goodman (1970) in the ocellus of Schistocerca, reported a zone of mitochondria surrounding the rhabdom of the light-adapted photoreceptor. They found a zone of vacuoles surrounding the rhabdom in the dark-adapted eye. They proposed the low refractive index of the vacuoles surrounding the rhabdom in the dark-adapted eye allowed for a greater light acceptance by the individual cells. No studies of dark-adapted internal ocelli have been done, but mitochondria do surround the rhabdom in the light-adapted internal ocellus (Figure VI) except in the distal end where a peri-rhabdomeric reticulum (Curtis, 1969) is well-developed (Figures IV and V).

Curtis (1969) described a peri-rhabdomeric reticulum composed of tubular and vesicular elements in the compound eye of the spider, Mito-

pus morio. He also noted the presence of vesicles within the microvilli. These vesicles were demonstrated to have acetylcholinesterase activity and were assumed to be involved in the acetylcholine-acetylcholinesterase system and possibly played a part in the production of the receptor potential. In some cases, especially at the distal end of the rhabdom where glia often penetrate the receptor cell, a well-defined peri-rhabdomeric reticulum is found in the internal ocellus (Figures IV and V). Vesicles similar to those within multivesicular bodies may also be found within the microvilli (Figure V).

Photic stimulation of the internal ocellus produces a hyperpolarizing response which Eaton (1971) refers to as an inhibitory post-synaptic potential. Electron microscopy of the synapse, however, reveals numerous E-type round synaptic vesicles characteristic of an excitatory synapse, but some I-type vesicles (Uchizono, 1965; Atwood and Lang, 1972) are also present (Figure VII). The presence of the flattened I-type vesicles may be due to changes in round E-type vesicles, or vice versa, due to fixation (Valdivia, 1971). Goodman (1970) noted in the ocellus of Schistocerca the second order neuron in some cases appeared to be the pre-synaptic unit. This apparently also occurs in the internal ocellus.

Numerous tubular and vesicular structures occur throughout the cytoplasm of both apparent first and second order neurons in the internal ocellus. These are sometimes indistinguishable from synaptic vesicles. Early workers thought these neurotubules were involved in the conduction of nervous impulses, but this theory has mostly been aban-

doned (Bullock and Horridge, 1965).

Membrane bound vesicles 200 Å to 500 Å in diameter are generally characteristic of a synapse, but their presence is not universal (Bullock and Horridge, 1965). Also close apposition of the membranes of pre- and post-synaptic fibers generally occurs (Boistel, 1968). Most synapses in the insect nervous system are formed where axons of two nerve cells come close together and these are referred to as axo-axonic synapses (Boistel, 1968; Bullock and Horridge, 1965). These are by far the most common type of synapses found in the internal ocellus, but these are not the only kind. An axo-somatic synapse, where the pre-synaptic axon synapses with the soma of the post-synaptic unit, was reported in the ganglion of the walking stick, Carausius morosus (Leghissa, 1942), but Bullock and Horridge (1965) question the presence of these types of synapses in insects. Although apparently rare, what appears to be an axo-somatic synapse occurs in the internal ocellus (Figure VII). To my knowledge this is the first time such a synapse has been reported in an insect photoreceptor.

C. Nerves Associated with the Internal Ocellus

The nerves associated with internal ocelli in the moths studied are similar to those described by Hinks (1970) for noctuid moths. The one major difference between the two nerve complexes is the point at which branches occur to the various sensory organs. Hinks (1970) describes nerve branches to the antennae, ocelli and compound eyes which occur over the dorsal protocerebrum from the nervus corporis cardiaci II dor-

salis (NCCIID). In the sphingids observed, generally the nerve branches to the sensory organs extended from the internal ocellus (Figure VIII).

The possible significance of this nerve complex is considerable. The activity of the endocrine glands in insects is widely recognized to play a role in the coordination of the insect's activity with its environment. Direct sensory input to the endocrine glands from the sense organs could function to regulate activity periods, such as diapause and circadian rhythm. The direct connection between ocelli and other sense organs could also provide an anatomical basis for the possibility of the ocellar regulation of brain excitability in these insects, as found in the flesh fly by Mimura, et al. (1969). Brousse-Gaury (1970) reported photoneuroendocrine pathways in several Orthopterans from the ocelli to the corpora cardiaca via the corpora cardiaca nerves, NCCI and NCCII. It would be of interest to find whether or not these pathways exist in Lepidoptera where ocelli already have a direct link with the corpora cardiaca.

II. The External Ocellus

This is the first report of external ocelli (Figures VIII through XII) in anocellate Lepidoptera. Although Berlese (1909) and Eaton (1971) reported the presence of internal ocelli in some moths, no report has ever been made of external ocelli in anocellate moths or butterflies. Eaton (1971) said removal of the scales on the vertex of certain sphingids, saturniids and citheroniids revealed an evenly pigmented cuticle with no lens-like structure present. The present study indicates that

in all anocellate Lepidoptera examined, including both moths and butterflies, small external ocelli are present (Table VI).

In some moths a nerve branch from the internal ocellus could be traced to the external ocellus (Figures VIII and XI). The presence of this nerve branch indicates: the presence of synapses of sense cells of the external ocellus with second order neurons whose somata lie in the brain; the unlikely extension of sense cell fibers whose somata lie in the external ocellus; or possibly, but unlikely, internuncial neurons may be present between sense cell fibers from the external ocellus and fibers from somata within the brain.

The possibility that the nerve branch to the external ocellus might act to guide light to the internal ocellus has been considered. Due to the torturous route taken by this nerve much light would be lost, so the idea was rejected.

SUMMARY

The study of internal ocelli in anocellate Lepidoptera yielded the following results:

1. The distribution of internal ocelli within anocellate Lepidoptera was significantly expanded to include 17 sphingids, 3 saturniids, 4 citheroniids, 1 arctiid (Lithosiinae) and for the first time two butterflies (Ropalocera, Hesperiidae).

2. A possible internal ocellus with a somewhat different form was found in other butterflies (Ropalocera).

3. A unique circular rhabdom apparently in some cases formed by only one retinular cell is present in the internal ocellus.

4. Nerve branches connecting the internal ocelli, optic lobes, antennal nerves, and corpora cardiaca are reported in moths having internal ocelli.

5. An external ocellus composed of a corneal lens with cells beneath was reported for the first time in anocellate Lepidoptera. A nerve branch from the internal ocellus was found to extend to the external ocellus. Thus the possibility of a two-part ocellus exists.

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APPENDIX

HISTOLOGICAL TECHNIQUES

I. Morphological studies

A. Fixation

1. Alcoholic Bouin's solution (After Drake and McEwen, 1959)

a. Formalin 25 ml

b. Glacial acetic acid 5 ml

c. 2% Picric acid in 70% ethanol 75 ml

2. Picroformal--Duboscq-Brasil (Pantin, 1960)

a. Formalin 60 ml

b. Glacial acetic acid 15 ml

c. Picric acid 1 g

d. 80% ethanol 150 ml

3. Chauthani and Callahan (1966)

a. 40% Formaldehyde 2.5 ml

b. Glacial acetic acid 1.25 ml

c. Chloral hydrate 10 g

d. Add water to make 100 ml

B. Sharpening of dissecting probes (Roeder, 1966)

1. Steel cleaning wires provided with hypodermic needles were used.

2. Tapering--With one electrode in 3 M HCl solution saturated with KCl dip wire attached to other electrode from 6 volt A.C. source dipped rapidly up and down in the solution.

II. Light microscopy

A. Fixation--Alcoholic Bouin's > 4 hrs

1. Formalin 25 ml
2. Glacial acetic acid 5 ml
3. 2% picric acid in 70% ethanol 75 ml

B. Removal of excess picric acid--Lenoir's fluid > 1 hr

1. Distilled water 70 ml
2. 95% ethanol 30 ml
3. Ammonium acetate 10 g

C. Dehydration--Graded ethanols--each > 10 min

1. 15% ethanol
2. 35% ethanol
3. 50% ethanol
4. 70% ethanol
5. 85% ethanol
6. 95% ethanol
7. absolute ethanol

D. Cleaning

1. Carbol-benzene 20 min
   a. Benzene 70 ml
   b. Phenol 30 g
2. Benzene 20 min

E. Embedding--Paraplast Plus

F. Sectioning--AO rotary microtome with a steel knife

G. Re-hydration

1. Xylene-removes paraffin - 2 changes each 1 min
2. Graded ethanols and distilled water - each > 1 min
   a. absolute ethanol
   b. 95% ethanol
   c. 85% ethanol
   d. 70% ethanol
   e. 50% ethanol
   f. 35% ethanol
   g. 15% ethanol
   h. distilled water

H. Staining

1. Mallory's triple stain (Gray, 1966)
   a. First staining solution - 1% acid fuchsin - 2 min
   b. Thorough rinse in distilled water
   c. Differentiating and mordanting solution - 2 min
      1% phosphotungstic acid
   d. Quick rinse in distilled water 5 sec
   e. Second staining solution 15 min
      (1) Water 100 ml
      (2) Aniline blue 0.5 g
      (3) Orange G 2 g
      (4) Oxalic acid 2 g
   f. Thorough washing in water
   g. Differentiate in absolute ethanol

h. Xylene

2. Holmes' silver stain (Larsen, 1960)

a. Silver nitrate 20% aqueous at 25°C in darkness 1 hr

b. Distilled water, 3 changes 3 min each

c. Second silvering solution at 37°C 24 hr

(1) Boric acid 12.4 g/1 distilled water 5.5 ml

(2) Borax ($Na_2B_4O_7 \cdot 10H_2O$) 19g/1 distilled water 4.5 ml

(3) 1% silver nitrate 1.0 ml

(4) 10% Pyridine 5.0 ml

(5) Distilled water 49.4 ml

d. Reducing solution 2 min

(1) Hydroquinone 1 g

(2) Sodium sulfite ($Na_2SO_3 \cdot 7H_2O$) 10 g

(3) Distilled water 100 ml

e. Wash in tap water followed by rinse in distilled water

f. Toning solution - 0.2% gold chloride 3 min

g. Distilled water rinse

h. Second reducing solution - 2% oxalic acid 3-10 min

i. Distilled water rinse

j. Sodium thiosulfate ($Na_2S_2O_3$) 5% 5 min

k. Dehydration in graded ethanols (see II.C. above)

I. Mounting - Permount

III. Transmission electron microscopy

A. Fixation - composition of fixative

1. Buffer

a. Stock solutions

(1) Solution A - 0.2 M $HaH_2PO_4 \cdot H_2O$ 27.6 g/l

(2) Solution B - 0.2 M $NaHPO_4$ 28.4 g/l

b. Buffer stock (0.1 M)

(1) Solution A 23 ml

(2) Solution B 77 ml

(3) Distilled water 100 ml

(4) 1% $CaCl_2$ 20 drops or 1 ml

2. Fixatives

a. Glutaraldehyde (3%) fixative - pH 7.2 - 7.6 1.5 hrs

(1) 50% Glutaraldehyde 6 ml

(2) 0.1 M Phosphate buffer 94 ml

b. Osmium fixative - pH 7.2 - 7.6 1 hr

(1) Osmium tetroxide .25 g

(2) Distilled water 12.5 ml

(3) 0.1 M Phosphate buffer 12.5 ml

(4) Sucrose 1.125 g

B. Dehydration

1. Graded ethanols - each 2 min

a. 30% ethanol

b. 50% ethanol

c. 70% ethanol

d. 95% ethanol

3. 100% ethanol

2. Propylene oxide - 2 min

C. Embedding

1. Composition of embedding medium (Ladd, W. P. E., 1960)

a. Mixture A

(1) Dodecenylsuccinic anhydride 93 g

(2) Epon 812 80 g

b. Mixture B

(1) Nadic methyl anhydride 78 g

(2) Epon 812 100 g

c. Final Embedding mixture

(1) Mixture A 3 g

(2) Mixture B 7 g

(3) Accelerator - 2,4,6-tri(dimethyl-aminoethyl) phenol 0.14 ml

2. Embedding procedure

a. Warm tissue in 1:1 embedding mixture - propylene oxide to allow some propylene oxide to boil off 20 min

b. Place tissue in Beem capsule containing the embedding mixture and position

c. Place capsules in oven at 60°C overnight. Allow capsules to set at room temperature several days before sectioning.

D. Staining sections on copper grids (Venable and Coggleshell, 1965; Watson, 1958)

1. Uranyl acetate stain - 3-4 min

   a. Uranyl acetate 0.9 g

   b. Double distilled water 30 ml

2. Lead citrate stain - 5-10 min

   a. Lead citrate 0.09 g

   b. Double distilled water 30 ml

   c. Sodium hydroxide 10 N 0.3 ml

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DISTRIBUTION AND STRUCTURE OF OCELLI IN LEPIDOPTERA PREVIOUSLY REPORTED TO BE ANOCELLATE AND MORPHOLOGY OF A NERVE COMPLEX ASSOCIATED WITH THE OCELLI

by

Joseph Clifton Dickens

(ABSTRACT)

The distribution of internal ocelli has been extended to include not only additional anocellate moths but also two species of butterflies. The possible presence of internal ocelli of a different external structure in other Ropalocera is also examined.

The structure of internal ocelli is examined by light and electron microscopy. A unique circular rhabdom apparently composed of a single retinular cell was observed in the internal ocelli of sphingids. Pigment cells were found to be absent. Observations were also made on the synapse between first and second order neurons where an apparent axosomatic synapse was found.

A nerve complex was found to be associated with the internal ocelli of moths. Nerve branches were found to connect the ocelli to the antennal nerve, optic lobe, corpus cardiacum and sometimes the tegumentary nerve as well as the external ocellus.

Adult moths and butterflies reported to lack external ocelli were found to possess a pair of small external ocelli located on the vertex posterior to the antennae and dorso-medial to the compound eyes. Scan-

ning electron microscopy and histological studies showed the external ocellus to consist of a corneal lens about 26 μ in diameter with cells beneath it. A nerve branch was found to extend from these cells to the internal ocellus in moths. Thus the possibility of a two-part ocellus exists.