fluorescence staining was observed in cells that were in mid-metaphase (Fig. 1). With a staining time of 15 to 30 minutes, uniform staining with Giemsa was observed. The molarity was varied from .003 to 0.14 mole/liter by the addition of 2 to 98 ml of 0.14*M* Na_2HPO_4 buffer in a total of 100 ml of staining solution. The *p*H was held constant at 9.0, and the concentration of Giemsa was 2 ml per 100 ml of final solution in these experiments. No qualitative effect of varying the molarity was observed on the staining pattern, but the time of staining necessary to obtain the banding pattern was increased from 5 minutes to 30 minutes. Preincubation of the slide in phosphate buffer at *p*H 9.0 for 2 to 30 minutes before a 5-minute staining period did not alter the banding pattern.

On the basis of these observations we now use the following procedure. Slides are stained for 5 minutes, washed in cold running tap water for 1 minute, and air dried. The stain consists of 2 ml of Harleco Giemsa Blood Stain Azure A stock solution, 2 ml of 0.14*M* Na_2HPO_4 (5 g of $Na_2HPO_4 \cdot 12\ H_2O$ per 100 ml of H_2O) and 96 ml of H_2O. This solution is adjusted to *p*H 9.0, if necessary. The phase of contraction of the metaphase cell is particularly important. Cells which are in very early metaphase show a discrete chromomere pattern; identification of homologs, however, is difficult in these cells. Cells which are in very late metaphase are also difficult to use for homolog identification. In mid-metaphase cells, homologous pairs can be identified in the majority of cells in good preparations. We have termed this technique Giemsa 9 staining.

Fifty cells were studied in this fashion and also by QM fluorescence after the stain was removed with 70 percent ethyl alcohol; dual karyotypes were prepared as shown in Fig. 1. The important landmarks for identification of homologs were generally identical with those found with the QM fluorescence technique. That was particularly evident in group C where the same areas showed an absence of staining. A few exceptions, however, warrant comment. The distal end of the long arm of the Y chromosome fluoresces brightly, but with the Giemsa 9 technique it shows a decrease in the density of staining. The paracentric constrictions in the long arm of chromosomes A1 and C9 show only minimum QM fluorescence; Giemsa 9 produces dark staining near the centromere of both chromosomes, and only the more distal parts of the constriction regions stain lightly. Other less pronounced differences will be reported after comparison of specific regions in a larger number of cells (*3*).

The striking similarities in banding patterns obtained by Giemsa 9 and the QM fluorescence techniques suggest that both stains may be acting in a similar fashion, although the mechanism of action of neither stain is clearly understood. Since there is less staining in secondary constriction regions with both techniques, it is possible that the intensity of staining may be due in part to variations in DNA content or to the availability of the DNA. The regions where particularly bright fluorescence is often obtained, such as the centromere of A3, the satellite regions in groups D and G, and the distal end of the long arm of the Y chromosome, may be exceptions and the brightest fluorescence may result from interaction with guanine-rich areas as suggested by Caspersson *et al.* (*1*).

It is likely that this technique, which is simpler than the QM fluorescence method, will prove extremely useful in routine human cytogenetics for homolog identification, for the recognition of regions of exchange in translocations, and for the more precise characterization of certain variations in arm length. The combination of the two techniques will probably provide more information than either technique alone.

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Insect Photoreceptor: An Internal Ocellus Is Present in Sphinx Moths

Abstract. *Adult sphinx moths lack external ocelli. In* Manduca sexta *and other anocellate moths structures homologous to ocelli have been observed. Histological examination of such a structure in* Manduca sexta *has shown structural similarities to ocelli of other insects. Electrophysiological studies revealed a response to light stimuli similar to the electroretinogram of external ocelli. The evidence strongly suggests that the structures are internal ocelli.*

Ocelli are simple eyes found in adult insects. They are incapable of perceiving form but they do perceive changes in light intensity (*1, 2*). Electrophysiological studies have shown that illumination of the ocellus inhibits the continuous discharge which occurs in the ocellar nerve during darkness (*3*). Further, behaviorial studies have shown that the ocelli are important in the regulation of the daily rhythm of insects (*4*). External ocelli are absent in sphinx moths (*1, 3, 5*). This fact coupled with the presence of large superposition compound eyes in these insects has led to the suggestion that the function of the ocelli has been taken over by the compound eyes in sphinx moths (*1, 5*).

However, in a study of the morphology of the nervous system in the adult tobacco hornworm (*Manduca sexta* Johanssen) a pair of internal ocelli have now been observed. They are located in the same anatomical position as the ocelli of other insects, except that the ocellar nerve does not extend to the vertex of the head capsule (Figs. 1 and 2A). Instead the retinula cells and the ocellar nerve are enclosed in and supported by a membranous tube filled with hemolymph (Fig. 2B). This tube is in turn attached to a transverse structure which appears analogous to the transverse muscle associated with antennal circulation in cockroaches (*1*). The retinula cells thus are positioned about half way between the protocerebrum and the vertex. Examination of the cuticle of the vertex has revealed that it is evenly pigmented and without lenslike structures. Observations made on moths taken in light traps have revealed the presence of similar structures in ten different species of sphinx moths, one saturnid,

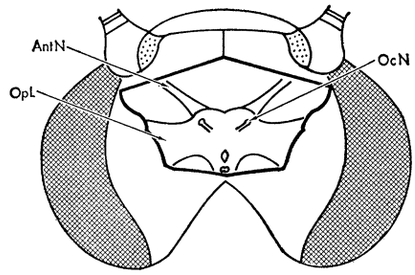

Fig. 1. Rear view of the head of *M. sexta* showing the location of the internal ocelli. *AntN*, antennal nerve; *OcN*, ocellar nerve; *OpL*, optic lobe.

and one citheronid (Table 1). A search of the literature has revealed only one description of a sphinx moth ocellus, and it was within the brain (*6*).

Single ocelli have been studied histologically from paraffin sections 6 to 10 μm thick stained with Mallory's triple stain (*7*) or Holmes silver stain (*8*) (Fig. 2, B and C). Since the retinula cells are not attached to the cuticle, there is no cornea or corneal epithelium. Both the retinula cells (Fig. 2C) and the ocellar nerve are enclosed by a neutral lamella. A layer of shielding pigment cells or a tapetum is apparently present in some preparations. Axons from the retinula cells pass into the ocellar nerve where they apparently synapse with fibers from the ocellar nerve (*9*). It has not been possible to definitely identify rhabdomes in either sagittal or cross sections of ocelli examined by light microscopy.

Electroretinograms (ERG's) have been recorded from sphinx ocelli with insulated tungsten electrodes (*10*) and conventional electrophysiological techniques (*11*). The light stimulus was provided by a microscope illuminator. The duration of the stimulus was regulated by a camera shutter, and the stimulus was carried to the ocellus by a fiber optic. The fiber optic was used to restrict the illumination to the ocellus. As a precaution the optic nerve was severed and the compound eyes were removed. Figure 2D shows the response of the sphinx ocellus to light stimuli. Note that the ERG response occurs after a latency of about 0.25 second and consists of a hyperpolarization which increases in amplitude and duration with increased length of the light stimulus. Since the recording electrode was positioned closer to the ocellar nerve fibers than to the retinula cells, this response can probably be interpreted to be the inhibitory postsynaptic potential from the dendrites of the ocellar nerve fibers (*11*).

Table 1. Moths observed to have internal ocelli.

Sphingidae
Manduca sexta
Manduca quinquemaculata
Spectrum lineata
Calasymbolus excaecata
Calasymbolus myops
Darapsa pholus
Darapsa versicolor
Pholus achemon
Pholus pandorus
Hyloicus drupiferanum
Saturniidae
Telea polyphemus
Citheroniidae
Eacles imperialis

Results of both histological and electrophysical studies indicate that this sensory receptor is an ocellus and that it is capable of detecting light. It is not known at this time what function, if any, this ocellus has in the regulation of the activity of these moths.

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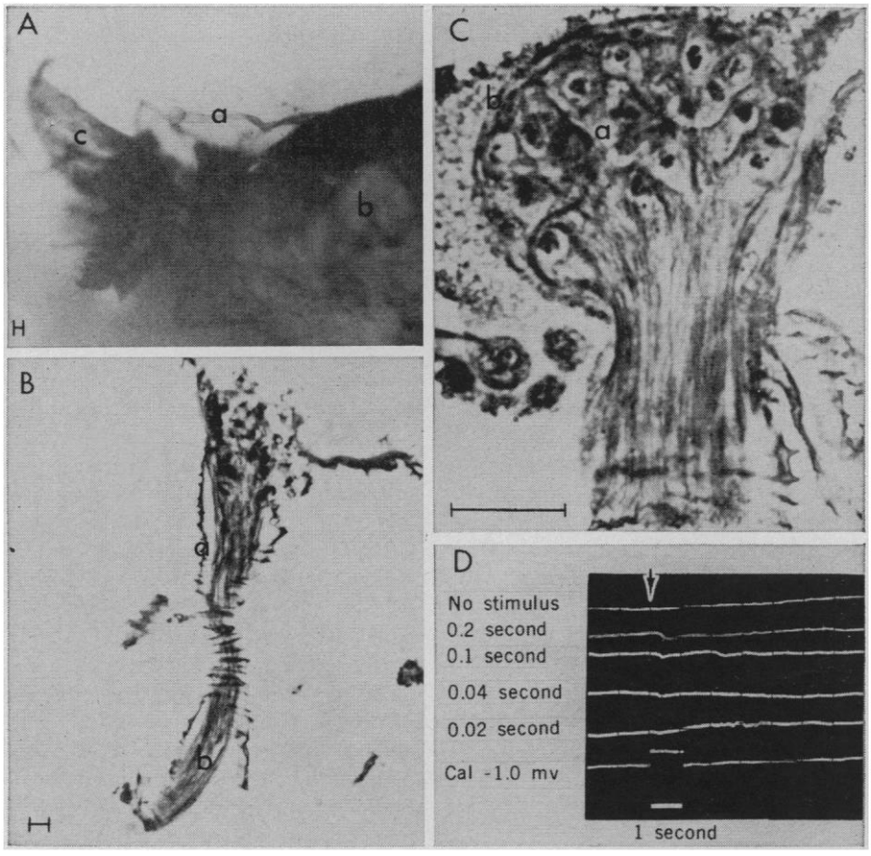

Fig. 2. (A) Rear view of the left side of the brain of *M. sexta*. The ocellar nerve (*a*) arises from the dorsal surface of the protocerebrum (*b*) and extends upward and outward parallel to the antennal nerve (*c*). (B) Longitudinal section showing the membranous sheath (*a*) which surrounds the ocellar nerve (*b*). (C) Longitudinal section of ocellus showing retinula cells (*a*) and neural lamella (*b*). Scale in A, B, and C is 20 μm. (D) Electroretinogram recorded from ocellus of *M. sexta*. The arrow indicates light on. The lengths of the stimuli are indicated to the left of each trace. The bottom trace is a —1-mv calibration pulse. The bar at the bottom is a 1-second time mark. The second trace has been retouched to improve clarity.

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