Development of visual sensitivity in the fly, *Sarcophaga bullata*[1]

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Summary. Ontogeny of light response in the compound eyes of the fly, *Sarcophaga bullata* was investigated. Even though the eyes are structurally differentiated before eclosion they become electrophysiologically functional only after eclosion.

The development of function, unlike that of structure seems to be a sudden process turned on abruptly at a particular point in time during the life of an animal. Adult behavior of many insects, such as courtship in crickets[2], walking in silkmoths[3] and flight in locusts[4] are activated at the time of eclosion of the imago. It has been documented that neural circuits for these functions are already present but suppressed until adult emergence. In this investigation we examined the ontogeny of vision in the compound eyes of the fleshfly *Sarcophaga bullata* and our results reported here indicate that the retinal cells become electrophysiologically competent only after adult eclosion.

Materials and methods. The fleshfly *Sarcophaga bullata* was reared in the laboratory under constant conditions of 25 °C temperature and 16 h L/8 h D period. The adults were fed with sugar and water and the larvae were raised in fresh beef liver. Freshly formed white prepupae (less than 30 min after pupariation) were collected from the culture dish and

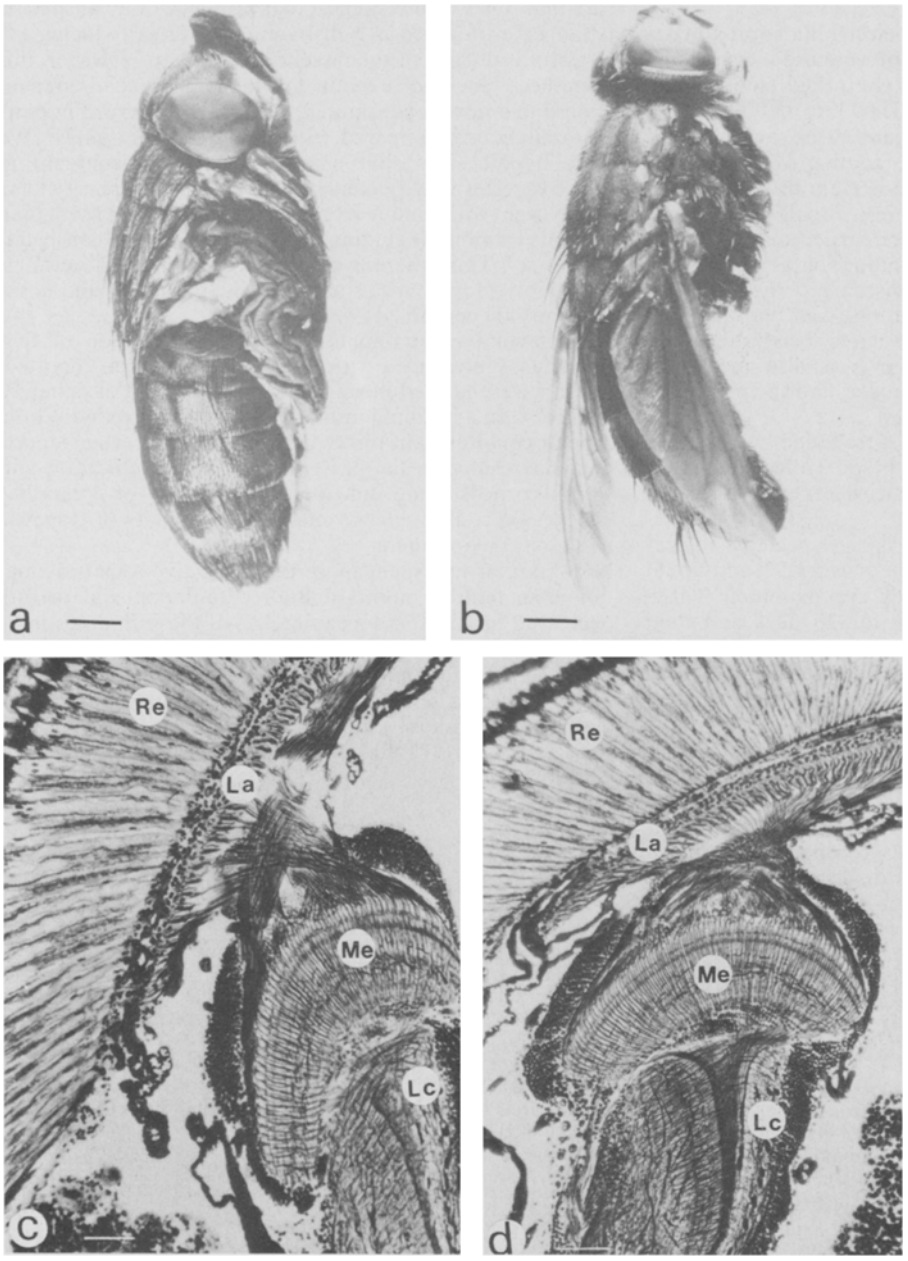

Figure 1. *a* Pharate adult. *b* Eclosed fly. *c* Optic ganglia of pharate adult. *d* Optic ganglia of eclosed fly. La, Lamina; Lc, lobula complex; Me, medulla; Re, retina. Bar = 1 mm in *a* and *b*; 60 µm in *c* and *d*.

kept for experiments. At 25 °C adult flies eclose about 11 days after pupariation. Two stages of flies were used in these studies, namely, pharate adults which had completed development and were waiting for their photoperiodic gate for eclosion (fig. 1,a) and freshly emerged adults (fig. 1,b). Electroretinograms (ERG's) of the compound eyes were recorded with a subcorneally placed NaCl-filled microelectrode as described in a previous communication[5]. The ERG measures all light-elicited responses in the electrode's proximity; it includes, in addition to a negative wave from receptor depolarization, on- and off-transient potentials shown to originate in the first synaptic neuropile, the lamina[5,6]. Receptor function was also evaluated by the pupillary response, the light-elicited migration of screening pigments within the retinula cells. In the fly, one observes an increase in reflection from the optical image of the photoreceptors called the deep pseudopupil[7]. We used a 575-nm stimulus which is near the peak reflection of the screening pigment granules. A beam splitting pellicle under a long working distance photo objective allowed measurement of the eye's reflection of our stimulus using a Leitz microspectrophotometer. For structural examination the flies were fixed in alcohol: acetic acid: formalin (85:5:10) fixative, sectioned in paraffin and silver stained by Bodian method[8].

Results and discussion. At least 20 flies from each stage were used for recordings. The freshly emerged flies exhibited normal electroretinogram response with sustained corneal negativity and pronounced on- and off-transients. However, in the case of pharate adults taken out of their pupal cases only one showed any response at all and even this lacked the on-off-transients (fig. 2). Our results confirm an earlier report on butterfly eye development[9] wherein the electrical competance of the eye develops gradually; first the red receptor becomes sensitive to light followed by shorter wave length receptors. Our pharate adults were virtually of the same chronological age as the eclosed flies. However, since they missed their photoperiodic gate (dawn) they did not emerge and were waiting for their next gate to open. They had completed their development and even exhibited feeble ptilinial movements when their pupal cases were removed. At the light microscope level as seen in silver stained preparations (fig. 1,c and d) there was very little difference in the structural organization of the visual centers between the pharate adults and eclosed flies. As mentioned above, the transients originate in the neuropile, presumably from functioning synapses. Thus although neural circuits involved in the perception of light seem to be present in the pharate adults but somehow there is no response upon stimulation. The circuits presumably become functional only after eclosion. Pharate adults do show a different kind of light-evoked response, namely the pupillary response with nearly the same magnitude and rate as normally eclosed flies (fig. 2). The pupillary response is believed to be a non-invasive means of monitoring the receptors' function[7], although the electrical and pupillary responses may not be directly coupled[10]. Our results substantiate this latter notion since the screening pigments within retinular cells are able to migrate upon light stimulation before the development of electrical competence. The latter process must await eclosion even though the neural circuits are apparently fully formed before eclosion.

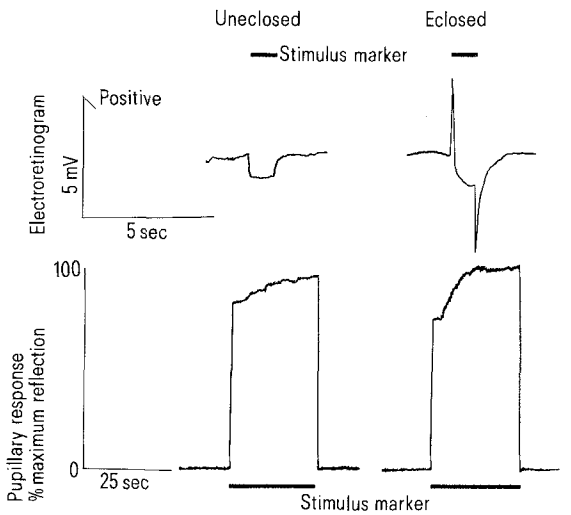

Figure 2. Reaction of the compound eye to a light stimulus before and after eclosion. *Top* ERG and *bottom* pupillary response. In the top, stimulus markers show the timing of bright 600-nm stimuli which elicit ERGs. Both flies shown have a negative-going wave, but the uneclosed fly, the only one which showed any response, lacks the conspicuous on and off transients clearly present in all eclosed flies. The bottom stimulus markers show the timing of bright 575-nm stimuli which elicit pupillary responses. These are observed as increases in reflection over time as labeled and are present in both stages of flies.

In several groups of insects certain types of motor programmes that are characteristic of adults are turned on at the final molt. A few of the well documented cases are the courtship song in crickets[2], locust flight[4,11] and walking behavior in silkmoths[3]. It has been demonstrated that neural circuits and synapses that control these activities are preformed but somehow suppressed until the final molt. Bentley and Hoy[2] produced adult song patterns in cricket nymphs after making heat lesions in the mushroom bodies of the brain. A similar neural inhibition of walking activity in the peeled pharate moths was removed by injection of either picrotoxin or eclosion hormone[3] suggesting that the eclosion hormone released at the time of adult emergence turns off neural inhibition associated with walking behavior. What activates the visual function at eclosion in the fleshfly is highly speculative at this time. However, in view of the fact that eclosion hormone activity is present in the central nervous system of Diptera[12] it is tempting to suggest that the ERG response but not the pupillary response to light in the compound eyes of the fleshfly *Sarcophaga bullata* is activated by the eclosion hormone at the time of adult emergence.

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