

Comparative Studies on Dark Adaptation in the Compound Eyes of Nocturnal and Diurnal Lepidoptera

C. G. BERNHARD and D. OTTOSON

From the Department of Physiology, Karolinska Institutet, Stockholm, Sweden

ABSTRACT A comparative analysis has been carried out of the time course and range of dark adaptation in the compound eyes of some common butterflies and noctuid moths (Lepidoptera). The change in sensitivity of the eye during dark adaptation was determined by measurements of the intensity of illumination necessary to elicit an electrical response of a given magnitude of the eye. It was found that the curve for dark adaptation in the diurnal species was smooth. The range of adaptive change varied in different species but usually did not cover more than 1 to 1.5 log units. In the nocturnal species the dark adaptation was found to proceed in two phases. The first phase was usually completed in less than 10 minutes and covered a range of 1 to 1.5 log units. The second phase was more prolonged and covered a range of 2 to 3 log units. In some of the experiments on nocturnal species the second phase failed to appear. Measurements of the size of the response at different intensities showed that the intensity/amplitude relationship was the same in the light-adapted eye as in the dark-adapted eye. In the nocturnal insects the response of the eye in the light-adapted condition was about 20 per cent of that in the dark-adapted eye, while in diurnal insects it was about 60 per cent.

While there is a considerable amount of precise data concerning dark adaptation in vertebrates, very little is known about the adaptive changes in insects. Dolley (1929) observed that the sensitivity to light of the drone-fly (*Eristalis tenax*) increased about 20 times after dark adaptation for 1 hour. Wolf and Zerrahn-Wolf (1935) followed the course of dark adaptation in the honeybee by using characteristic motions of the antennae as an index of the state of adaptation. They found that the increase in sensitivity in the

The investigations were carried out at the Marum field laboratory in Björkö-Arholma. The establishment of the laboratory was made possible by support from the Office of Scientific Research of the Air Research and Development Command, United States Air Force, through its European Office (Contract AF 61(052)-21).

Received for publication, February 5, 1960.

honeybee follows a continuous course, the adaptive process being completed after about 30 minutes. Autrum (1950) studied the increase in the electrical potentials with dark adaptation in a wide variety of insects. In some insects, for instance *Calliphora*, the changes in amplitude of the electrical potentials were small, while in another group represented by *Tachycines* and *Dixippus* a marked increase in the electrical response occurred.

The present paper gives an account of a comparative investigation on dark adaptation in some common butterflies and noctuid moths. A preliminary report dealing with this work has been published earlier (Bernhard and Ottoson, 1959).

MATERIAL AND METHODS

The investigations to be described were carried out in a field laboratory north of Stockholm. The experiments were set out in the summer of 1958 and were continued and extended in the summer of 1959. In the earlier part of the studies the animals were collected and kept in a box until used. However, in the course of the investigations it was observed that many insects were in bad condition when they had been kept for a day or two. In the following studies the animals were therefore caught immediately before the experiment. The investigations were performed on lepidopteran butterflies and noctuid moths. The diurnal species chosen were *Argynnis*, *Erebia*, *Vanessa*, and *Heodes*. Among the noctuid moths *Cerapteryx*, *Rhyacia*, *Geometra* and *Hepiolus* were studied.

Extensive dissection was avoided as far as possible in order not to cause any damage that might interfere with the adaptive processes. The head was excised and divided into two parts with an incision in the midline. The antennae were then removed and a small hole made in the cornea. In some experiments the eye was excised and the optic ganglion removed by dissection. The preparation was placed on moistened cotton-wool on a platform in a metal box. Recordings of the electrical responses were made with electrodes made of chlorided silver wire. The active electrode (tip diameter 0.1 mm.) was inserted through the hole made in the cornea and the reference electrode was connected to the moistened cotton-wool. In the first part of the investigation a condenser-coupled amplifier with a time constant of 1 second was employed. In later experiments the recordings were made with a two-sided d.c. amplifier.

The light stimulus was obtained with a projector bulb (Osram, 8032, 12 v., 100 w.) in a lamphousing. The image of the filament was brought to focus at the eye by a system of lenses. The intensity of illumination was varied by a neutral wedge and a series of neutral filters covering a continuous range of 5 log units. Threshold determinations were made by applying test flashes at various intervals during dark adaptation. The duration of these flashes, usually one fifth second, was controlled by a compare shutter. The light stimulus was registered by means of a photocell and marked on the lower beam of the oscilloscope. Prior to the measurements the eye was exposed to constant illumination for 3 minutes without any neutral filters inserted in the light path. For protection of the eye a heat filter was inserted in the light path during light

adaptation. After the preadapting light was turned off test flashes were applied at intervals which at first were kept short (10 to 15 seconds) and then made successively longer as the change in threshold became gradually slower. The intensities of the flashes were varied so as to give a threshold response of constant amplitude. The size of the response chosen as the threshold response usually varied from 2 to 300 μv . This corresponds to about 5 per cent of the maximum responses given by the eyes of the species that were studied. Because the setting of the neutral wedge had to be made

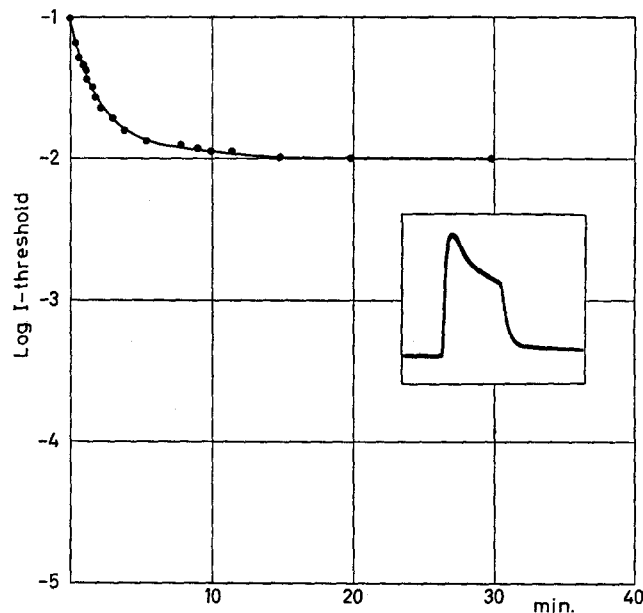


FIGURE 1. Relation between threshold intensities and time of dark adaptation for the compound eye of *Argynnis adippe*. Inset, electrical response of the eye to a one-fifth second light flash.

by trial, usually several runs had to be made in order to obtain the adequate intensities. However, in the course of the investigation it was found that the variations from one preparation to another were comparatively small in the same species. This simplified the measurements in the subsequent experiments a great deal.

RESULTS

The electrical responses of the compound eyes in insects exhibit considerable variation in shape and time course from one species to another. In the simplest form the response consists of a monophasic potential change (Hartline (1928); Bernhard (1942); Autrum (1950)). In other cases the response may have a more complicated wave form (Autrum (1950, 1958)) due to the summation of the receptor potential with the optic ganglion potentials. For the comparative analysis of the adaptive changes in different species it is essential to use

the receptor response to a threshold stimulus as a measure of the state of adaptation of the visual cells. The measurements were therefore made on preparations in which the ganglionic components of the response were absent or of insignificant amplitude. In those eyes which gave the complicated type of response the optic ganglion was removed by dissection or the eye was excluded from the measurements.

An illustrative example of the time course and range of the adaptive process in the diurnal insects is given in Fig. 1. The curve shows that the increase in

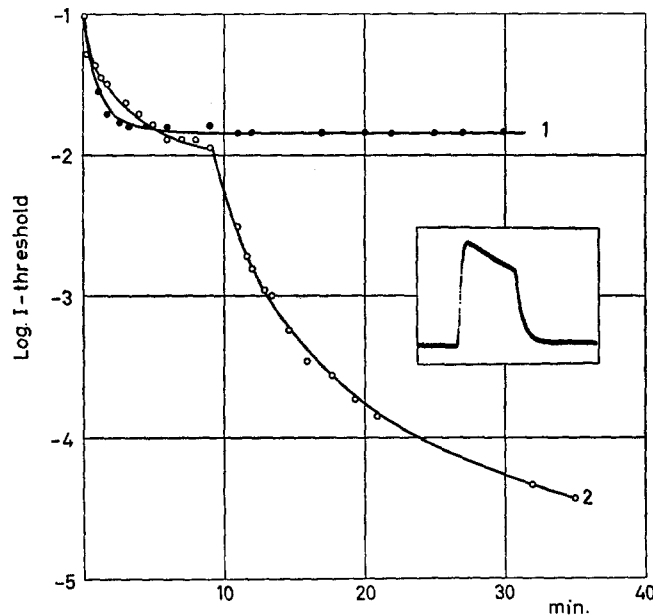


FIGURE 2. Relation between threshold intensities and time of dark adaptation for the compound eye of *Cerapteryx graminis*. Curve 2 shows the typical and curve 1 the atypical course of dark adaptation. See text. Inset, electrical response of the eye to a one-fifth second light flash.

sensitivity in terms of fall in threshold in the compound eye of *Argynnis* is very rapid during the first minutes of the dark adaptation and then becomes gradually slower. The adaptive change is completed in about 20 minutes and covers a range of about 1 log unit. About 90 per cent of this increase is attained in less than 10 minutes as a result of the rapid initial change. The curve has a perfectly smooth course and there is no indication that the process mediating the increase in sensitivity is of a dual nature.

The course of dark adaptation in the other diurnal butterflies studied was essentially the same as that in *Argynnis*. There were minor differences from one species to another particularly in the range of the fall in threshold. It is well known that some butterflies are only active in bright sunlight while

others are active also in dim light. It is likely that the characteristic behavior of different species depends on differences in visual adaptive capacities. It is therefore not unreasonable to assume that the observed differences in adaptation reflect typical features of the particular species studied, but the material is too small to permit any definite inference on this point.

In the nocturnal Lepidoptera studied in the present investigation the course of dark adaptation was entirely different, as may be exemplified by the curve obtained in an experiment on *Cerapteryx graminis*. This is a common noctuid moth that is active in the late evening or at night and hides in dark places during the light hours of the day. It has a positive phototactic reaction

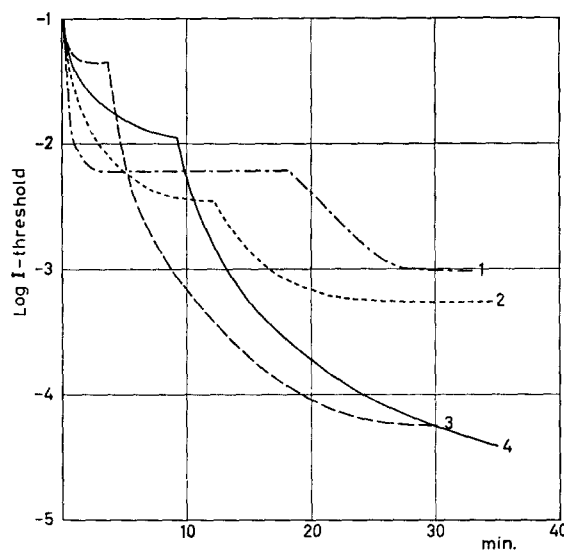


FIGURE 3. Dark adaptation curves for *Hepiolus hectus* (1), *Rhyacia augur* (2), *Geometra papilionaria* (3), and *Cerapteryx graminis* (4).

and is therefore easy to catch at night. Curve 2 in Fig. 2 demonstrates that *Cerapteryx graminis* possesses a considerably greater adaptive range than the diurnal insects. The interesting point is that the increase in sensitivity during dark adaptation occurs in two stages, as revealed by the distinct break in the curve. The first phase of adaptation lasts only for about 10 minutes and involves a fall in threshold over a range of about 1 log unit. The second phase covers an intensity range of 2.5 log units and is not complete until after more than 30 minutes.

The adaptation of the other nocturnal moths followed a similar dual course. The curves in Fig. 3 show that there are certain differences in range and rate of the changes during the two stages. Thus, in *Hepiolus* and *Rhyacia* the second phase starts later than in *Cerapteryx*, whereas in *Geometra* the transition took place less than 5 minutes after the preadapting light had been turned off. The

increase in sensitivity attained during the two phases appears to be different for different species, as indicated by the curves.

In some of the experiments on nocturnal insects the second step of adaptive change did not appear. Curve 1 in Fig. 2 is from an experiment in which only the first phase of adaptation was present in the eye of *Cerapteryx graminis*. Similar curves were also obtained in *Rhyacia* and *Geometra*. The reason why the eyes of the nocturnal insects sometimes lacked the second phase is not clear. The most probable explanation appears to be that the preparations

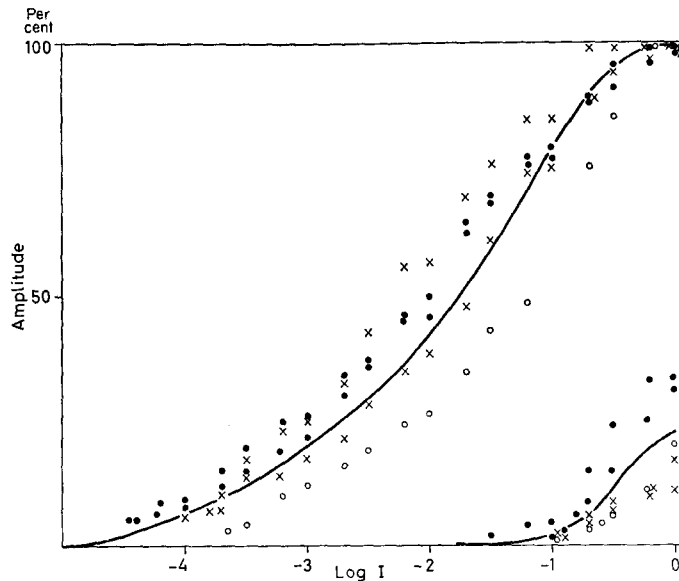


FIGURE 4. Relation between amplitude of response and intensity of illumination in the dark-adapted (left-hand curve) and light-adapted (right-hand curve) eyes of *Rhyacia augur* (O), *Cerapteryx graminis* (●), and *Geometra papilionaria* (X).

were in bad condition either because the animals had been kept too long before the experiments or because the eyes had been injured during the dissection procedure.

In connection with the studies on changes in threshold during dark adaptation measurements were also made on the amplitude of the response at various intensities of illumination. It has been established by several investigators (see *e.g.* Hartline and McDonald (1947)) that the amplitude of the response at a given intensity and duration of stimulation is a function of the state of adaptation and the number of active elements. Considering the many uncertainties in recordings from the whole eye and the structural differences of the eyes in different species, data on the *absolute* magnitude of the response are of little interest in evaluating the comparative functional

characteristics. In Figs. 4 and 5 the amplitude values are therefore given in percentages of the maximum response after 30 minutes of dark adaptation.

The curves for the nocturnal insects are shown in Fig. 4. In the dark-adapted eyes the responses extend over a range of about 4.5 log units while in the light-adapted state the capacity of the eye is reduced to less than 1.5 log unit. The curves further demonstrate that in the light-adapted eyes the responses only amount to about 20 per cent of the maximum dark-adapted response. It is to be noted, furthermore, that the intensity/amplitude re-

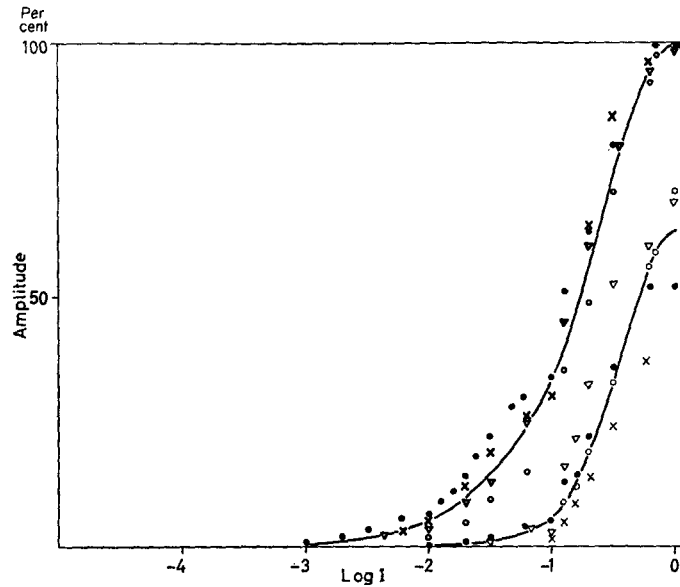


FIGURE 5. Relation between amplitude of response and intensity of illumination in the dark-adapted (left-hand curve) and light-adapted (right-hand curve) eyes of *Erebia ligea* (○), *Argynnis adippe* (●), *Vanessa urticae* (×), and *Heodes virgaurea* (▽).

lationship is essentially the same in the light-adapted eyes as in the dark-adapted ones. The fact that the curves for the dark-adapted eyes run parallel with the curves for the light-adapted eyes may be regarded as evidence of a constant intensity amplitude relationship in the dark- and light-adapted states. As a result of the decreased sensitivity of the eyes in the light-adapted condition the curve is shifted to the right (*cf.* Hartline and McDonald (1947)). The same holds true for the curves of the diurnal insect eyes in Fig. 5. In the light-adapted state the eyes of diurnal insects retain a comparatively greater capacity to react to light, as shown by the fact that the maximum response in the light-adapted eye amounts to about 60 per cent of that in the dark-adapted eye.

DISCUSSION

There exists a well established body of experimental data relating the increase in sensitivity in the vertebrate eye during dark adaptation primarily to the biochemical events in the visual cells. In addition to the resynthesis of photopigments, nervous interaction and photomechanical changes are involved to a different extent in different species.

Concerning the adaptive processes in insects there is very little precise information. Furthermore the interpretation of the few available data on the course of adaptation is greatly hampered by the paucity of knowledge of the photochemistry of the compound insect eye. Until quite recently all attempts to demonstrate the presence of photopigments in the insect eye have been unsuccessful. In 1958 it was shown by Goldsmith that extracts from heads of the honeybee contained *retinene*, which apparently is linked with a protein in the visual cells to form a photosensitive pigment with a λ_{max} at about 440 $m\mu$. This finding provides strong evidence that the photochemical characteristics of the insect eye are similar to those of the vertebrate eye. If this is so it is conceivable that the adaptive changes in the insect eye may be interpreted in accordance with the current theories of dark adaptation in the vertebrate eye.

It is a well established fact that the first phase in the dark adaptation curve for the human eye is mediated by the cones and the second phase by the rods. In view of the similarity between the curve for the nocturnal insects (Figs. 2 and 3) and that for the human eye it is tempting to assume that the two phases in the insect curve are attributable to two photochemical processes operating at different rates and possibly linked to two distinct types of receptors corresponding to the rods and cones. Some few experimental data lend support to this view. Thus it has been demonstrated by Cajal and Sanchez (1915) that some insects possess two types of visual cells reminiscent of the rods and cones in the vertebrate eye. Similar observations have been made by Hanström (1927) in studies on the eye of *Munida*. However, the notion that insects in general have two types of receptor cells does not seem to be widely accepted. Even if there is a certain structural similarity between some cells in the insect eye and the rods and cones in the vertebrate eye this is no evidence of a functional similarity.

The observation that the second phase of adaptation in the nocturnal species sometimes failed to appear is of particular interest for the interpretation of the adaptive processes. The identification of the simple curve in Fig. 2 with the first phase in the composite curve is based on their similarity in range and time course. In no case was a simple curve obtained that could be interpreted as an isolated second phase of the composite curve. It is well known that in the human eye a pure first phase of the adaptation curve can be obtained by selective stimulation of the cones. There is very little likelihood

that the appearance of the first phase only in the nocturnal insects is produced by selective stimulation of one hypothetical set of photoreceptors. A more probable explanation appears to be that the atypical behavior of the eye is the result of a failure of the mechanism by which the second stage of adaptation is mediated. The fact that the diurnal insects never showed the discontinuous type of dark adaptation curves indicates that they normally lack this mechanism. The presence of a second phase in the typical dark adaptation curve of the nocturnal insects must therefore be explained by some basic functional property which is lacking in the eye of the diurnal insects. This problem will be further discussed in a following paper.

REFERENCES

1. AUTRUM, H., Die Belichtungspotentiale und das Sehen der Insekten, (Untersuchungen an *Calliphora* und *Dixippus*), *Z. vergleich. Physiol.*, 1950, **32**, 176.
2. AUTRUM, H., Electrophysiological analysis of the visual systems in insects, *Exp. Cell Research*, 1958, suppl. 5, 426.
3. BERNHARD, C. G., Isolation of retinal and optical ganglion response in the eye of *Dytiscus*, *J. Neurophysiol.*, 1942, **5**, 32.
4. BERNHARD, C. G., and OTTOSON, D., On the mechanisms of dark adaptation in day- and night-insects, *Acta Physiol. Scand.*, 1959, **47**, 383.
5. CAJAL, S. R., and SANCHEZ, D., Contribucion al conocimiento de los centros nerviosos de los insectos, *Trab. lab. inv. biol. Univ. Madrid*, 1915, **13**, 1.
6. DOLLEY, W. L., Dark adaptation in the eye of *Eristalis tenax*, *Physiol. Zool.*, 1929, **2**, 483.
7. GOLDSMITH, T. H., The visual system of the honeybee, *Proc. Nat. Acad. Sc.*, 1958, **44**, 123.
8. HANSTRÖM, B., Über die Frage ob funktionell verschiedene, Zapfen- und Stäbchenartige Sehzellen im Komplexauge der Arthropoden vorkommen, *Z. vergleich. Physiol.*, 1927, **6**, 566.
9. HARTLINE, H. K., A quantitative and descriptive study of the electrical response to illumination of the arthropod eye, *Am. J. Physiol.*, 1928, **83**, 466.
10. HARTLINE, H. K., and McDONALD, P. R., Light and dark adaptation of single photoreceptor elements in the eye of *Limulus*, *J. Cell. and Comp. Physiol.*, 1947, **30**, 225.
11. WOLF, E., and ZERRAHN-WOLF, G., The dark adaptation of the eye of the honey bee, *J. Gen. Physiol.*, 1935, **19**, 229.