Compound eyes as the photoperiodic receptors in the bean bug

H. Numata and T. Hidaka[1]

Department of Zoology, Faculty of Science, Kyoto University, Sakyo, Kyoto 606 (Japan), December 3, 1982

Summary. The location of photoreceptors for the photoperiodic termination of adult diapause in *Riptortus clavatus* was examined by applying phosphorescent paint on selected regions. The results showed that the compound eyes are the principal photoperiodic receptors.

The photoreceptors for insect photoperiodism in seasonal development have been studied by many researchers. These studies suggest that the 'organized' photoreceptors (compound eyes and ocelli) are not involved in the response to photoperiod[2]. It has been shown that in some species the photoreceptors for photoperiodism are in the brain[2-5].

However, Ferenz[6] concluded from cauterization experiments that the compound eyes are the photoperiodic receptors in the regulation of the adult diapause in males of *Pterostichus nigrita*. Beck[3] took this for the only example of a developmental photoperiodism in which the receptor system is apparently retinal. However, it is very difficult to destroy the retina totally without injuring the central nervous system by this method. In *Megoura viciae*, the lesion extended deeply into the optic lobes and was accompanied by a marked vacuolization of the neuropile when photoperiodic sensitivity was lost by the cauterization of compound eyes[4]. Therefore, cauterization experiments without histological observations are not sufficient to affirm or deny the involvement of a photoreceptor. Further examination of the role of the retinal receptors in developmental photoperiodism appears to be necessary.

The bean bug, *Riptortus clavatus* Thunberg (Heteroptera: Coreidae) exhibits a facultative adult diapause. The diapause can be induced by a short-day photoperiod and terminated by a long-day photoperiod reversibly[7]. The present paper describes the location of the photoreceptors for the photoperiodic control of diapause development in this species.

Materials and methods. Nymphs of *R. clavatus* were reared by the method previously reported[7] under diapause-inducing conditions, a photoperiod with a 10-h photophase and a 14-h scotophase (10 L/14 D) at 25 ± 1.5 °C. The diapausing adults were kept under the same conditions. 7-day-old female adults were used for the experiments, which were conducted at 25 ± 1.5 °C.

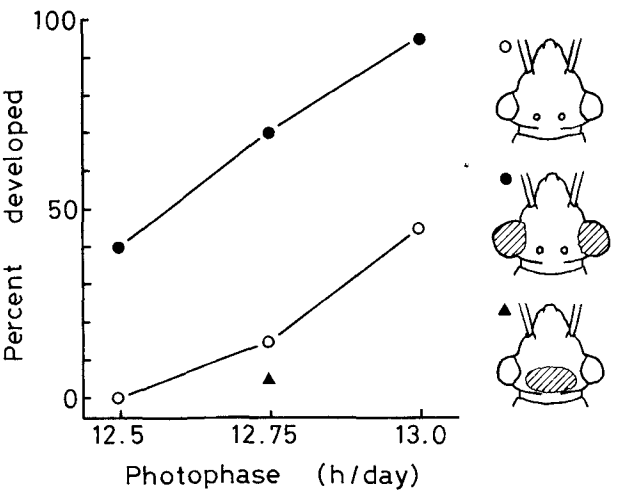
To test for photoperiodic sensitivity, the selected region was exposed to a longer photoperiod than the rest of the body surface by applying a phosphorescent paint (RMLC-G1A, Dainippon Toryo-Sinloihi). The phosphorescent pigment (LC-G1A, Sinloihi; the main component is ZnS) is a substance that absorbs light energy, and discharges its own green phosphorescence. Brightness of afterglow of this pigment decreases after arrest of illumination, promptly in the beginning but slowly afterwards: 0.1 ft-L (foot-Lambert) after 1 min, 0.01 ft-L after 5 min, 0.001 ft-L after 40 min (communicated by Sinloihi Co., Ltd).

Three photoperiodic regimes were employed: 12.5 L/11.5 D, 12.75L/11.25D and 13L/11D, because the critical daylength for the termination of diapause in this species was a little longer than 13 h[7]. Each photoperiod was provided by a 10-W 'daylight' type fluorescent lamp (Toshiba) controlled by a time switch. The light intensity of photophase was kept between 5 and 10 lx by covering the lamp with black semitransparent polyethylene sheets, in order to assure the best use of the effect of phosphorescent paint.

Two individuals were reared in each 200-ml plastic cup with 10 grains of soybean and water. After 21-day exposure to those photoperiods, the insects were dissected and their ovarian stages were classified as follows: (−) no yolk was deposited in oocytes; (+) light blue yolk was deposited in oocytes; (++) mature eggs were ovulated into the oviduct. Individuals which had ovaries in stage (+) or (++) were considered to be diapause-terminated because light-blue yolk deposition never occurred in diapausing females[7].

Results and discussion. The results are summarized in the figure. Under 12.75L/11.25D, 11 out of 14 diapause-terminated individuals in the group in which the compound eyes were painted had ovaries in stage (++). No significant difference was observed in the proportion of (++) individuals among the diapause-terminated ones between any groups. Most of (++) individuals had oviposited.

The percentage of diapause-terminated individuals was higher in the experimental group, in which the compound eyes were painted, than in the control, untreated group under each regime ($p < 0.01$ by Fisher's exact probability test). Phosphorescent paint on compound eyes shortened the critical daylength by about 0.5 h. Although the visual sensitivity of this insect is unclear, the phosphorescence visible to human eyes without dark adaptation lasted about 0.6 h after the light was switched off, which is nearly coincident with the shortening of the ciritical daylength. On the contrary, phosphorescent paint on the ocelli and vertex, beneath which lies the pars intercerebralis, had no effect on diapause termination ($p = 0.60$). Thus, the compound eyes are the principal, if not the only, photoperiodic receptors in the control of diapause development in *R. clavatus*.



Effect on the termination of adult diapause of exposing the selected region to a longer photoperiod than the rest of the body surface by applying phosphorescent paint in *Riptortus clavatus*. Hatching indicates the painted area. ○, Untreated; ●, compound eyes painted; ▲, ocelli and vertex painted. Results were obtained from the ovarian stages after 21-day exposure to 12.5L/11.5D, 12.75L/11.25D or 13L/11D at 25 ± 1.5 °C. 20 animals were used to obtain each point.

Truman[8] proposed to divide animal clocks into 2 categories based on the difference in the mode of action of light. Type I clocks are stopped in continuous light and thus must have a 'dark process'. Type II clocks can free-run in continuous light. This ability of the latter is due to the fact that photoreceptors are external to the clock mechanism. In insects, the compound eyes or other 'organized' photoreceptors are not involved in type I clocks, associated with developmental rhythms such as of eclosion, hatching or brain hormone release, the photoperiodic receptors lying in the brain itself. In type II clocks, such as those controlling locomotor activity rhythm, the compound eyes are the principal and sometimes the only photoreceptors involved and the light information is transmitted synaptically to the clock. He suggested that photoperiodic clocks be classed as type I.

This suggestion has been supported by many studies[2], among which the study of Williams[9] in *Antheraea pernyi* and that of Steel and Lees[10] in *M. viciae* are especially excellent. They localized both the photoperiodic clock and the receptor to a small region of the protocerebral lobes of the brain.

Nevertheless, the results of our present experiments demonstrated an example of developmental photoperiodism in which compound eyes are the principal photoreceptors. The role of compound eyes as photoreceptors in insect developmental photoperiodism should be more carefully examined before it is generally denied.

1 We thank I. Shimizu and J. Nishiitsutsuji-Uwo for their helpful advice.

2 Saunders, D.S., Insect Clocks, 2nd edn. Pergamon Press, Oxford 1982.
3 Beck, S.D., Insect Photoperiodism, 2nd edn. Academic Press, New York 1980.
4 Lees, A.D., J. exp. Biol. *41* (1964) 119.
5 Williams, C.M., and Adkisson, P.L., Biol. Bull. *127* (1964) 511.
6 Ferenz, H.-J., J. Insect Physiol. *21* (1975) 331.
7 Numata, H., and Hidaka, T., Appl. Ent. Zool. *17* (1982) 530.
8 Truman, J.W., in: Circadian Rhythmicity, p. 111. Proceedings of the Symposium on Circadian Rhythmicity, Wageningen 1971, Ed. J.F. Bierhuizen. Centre for Agricultural Publishing and Documentation, Wageningen 1972.
9 Williams, C.M., Symp. Soc. exp. Biol. *23* (1969) 285.
10 Steel, C.G.H., and Lees, A.D., J. exp. Biol. *67* (1977) 117.

0014-4754/83/080868-02$1.50 + 0.20/0

Neural connections between antrum and duodenum

K. Kraglund, H.D. Schrøder and H. Stødkilde-Jørgensen

Institute of Experimental Clinical Research, Institute of Anatomy B, University of Aarhus, DK-8000 Aarhus (Denmark), and Department of Surgical Gastroenterology L, Municipal Hospital, DK-8000 Aarhus (Denmark), November 15, 1982

Summary. Postprandial coordination of antroduodenal motility partly takes place via intrinsic mural pathways. The nature and origin of these nerve fibers have not yet been clarified. In this investigation using fluorochromic substances injected into the antrum and duodenum it was demonstrated that common central neurons for the antroduodenal area exist in the vagal nucleus.

The interdigestive motor activity of the stomach and the duodenum is cyclic with a quiescent phase (phase I), a phase of random activity (phase II), a phase of regular intense activity (phase III) and finally a transition phase between phases III and I (phase IV)[1-4]. Feeding interrupts the interdigestive cyclus immediately and induces rhythmic coordinated antegrade activity, the digestive phase. This fast response to feeding suggests a reflex phenomenon rather than a direct muscular reaction to food[5,6]. It seems to depend upon vagal integrity. Thus, when the vagal nerves are intact, the digestive pattern overrides the interdigestive pattern[7].

Reports have shown that nerve fibers run from the stomach to the duodenum[8,9]. From such studies one can hypothesize that coordinated antroduodenal motility takes place at least in part via intrinsic mural pathways. Such studies provide evidence for a hypothesis that coordination of motor accents in the distal stomach with those in the proximal duodenum is accomplished in part via intrinsic neural pathways. The nature and origin of these intramural nerve fibers are yet unexplained.

The use of nerve tracers has allowed better clarification of nervous pathways[10,11]. It was the aim of the present study to investigate the origin of antroduodenal nerves, employing this new technique.

Methods. In 8 rats (weight 150-250 g) the anterior aspect of the antrum and duodenum was exposed under pentobarbital anesthesia. Bisbenzimid 10% and True Blue 5% were injected randomly on the exposed sites. Both chemicals are transported in a retrograde fashion to the cell body of the nerve. Bisbenzimid provides a yellowish green fluorescence and True Blue a dark blue fluorescence. Bisbenzimid stains the nucleus and True Blue the cytoplasm and the nucleus. 2-3 days after injection the rats were anesthetized again and sacrificed by transcardial perfusion with 0.8% sucrose, 0.4% glucose and 0.8% sodium chloride, followed by formaldehyde 4%, tannic acid 1% and magnesium sulphate 4%. Specimens from antrum, duodenum and brain stem were examined.

Results. When the antrum was examined, neurons stained with a neuro-tracer injected into the duodenum could be observed in the antrum and vice versa (figs 1 and 2). No diffusion into the pyloric region was observed. When examining the vagal nucleus, it was noticed that staining was found almost exclusively in the left part of the vagal nucleus. Staining was particularly pronounced in the ventral aspect. In all animals, double stained neurons were found in the vagal nucleus (fig. 3) suggesting partly a common vagal innervation of the antrum and the proximal duodenum.

Discussion. This study provides morphological data supporting the existence of both local and central regulatory mechanisms. The findings clearly show individual central neurons innervating both antrum and duodenum in the rat. This central common control mechanism, which appears to be situated in the ventral aspects of the vagal nucleus,