Persistence of Circadian ERG Rhythm in the Cricket with Optic Tract Severed

K. Tomioka and Y. Chiba

Environmental Biology Laboratory, Biological Institute, Yamaguchi University, Yamaguchi, 753, Japan

We report here that the cricket *Gryllus bimaculatus* showed a circadian ERG rhythm in constant darkness (DD) and temperature (26 °C) even after the optic tracts (OT) were severed. This is the first detection, in insects, of circadian rhythm from the optic lobe (OL)-compound eye (CE) system neurally isolated from the central nervous system (CNS). The significance of this finding is not confined to the physiology of ERG rhythm but extends potentially to the physiology of locomotor rhythm.

This cricket is diurnally active in the nymphal stage but becomes nocturnal 4 to 5 days after the imaginal molt [11]. Adult males which had become fully nocturnal were obtained from a laboratory stock kept in LD 12:12 (L: 0600–1800) and at a constant temperature of 26 °C, the standard environmental condition. The crickets, whose legs were cut off, were fixed to a supporting rod and Ag-AgCl wire electrodes were chronically implanted into the immediate vicinity of the receptor layer of the compound eyes. The apparatus was so arranged that ERGs elicited by a 50 ms flash of green light at intervals of 1 h were recorded automatically. The stimulus intensities were always held below saturation for the ERG. The OT is relatively long in the cricket, connecting medulla to lobula [4]. A small square piece of head capsule was removed to make a window, through which the OT was cut with microscissors. Strict attention had to be paid to minimize damage to tracheae running into the OL. Finally, the piece of head capsule was replaced to close the window, and the wound was sealed with vaseline.

The ERG was diphasic, composed of on- and off-components. Amplitudes of the two components showed circadian rhythm in phase with each other. Hereafter, we refer to the peak-to-trough amplitude of the diphasic wave, in other words, the summation of amplitudes of on- and off-components.

In intact animals, the ERG amplitude changed synchronously with LD 12:12, peaking in the dark fraction. This rhythm freeran in the ensuing DD with a period (tau) a little shorter than 24 h. When the OT was cut unilaterally, the 4 crickets all exhibited this rhythm in both intact and operated eyes (Fig. 1). Moreover, in 2 of 3 animals in which the OT were bilaterally severed, the rhythm was obvious in both eyes under DD (Fig. 2). The remaining animal showed the rhythm only in one eye; the other eye was not rhythmic probably due to damage to tracheae during the operation. In both cases of unilateral and bilateral cuts, there was a subtle difference between taus of the two eyes obtained by the periodogram. However, since data are limited, it is premature to discuss bilateral organization of the rhythm of the two eyes as was done elegantly in the beetle [5].

Multiple physiological investigations of the compound eye have revealed circadian rhythm in arthropods held in DD [1, 2, 6, 8, 13]. These extensive studies carried out in the search for the mechanism controlling ERG rhythm in crayfish, have produced somewhat complicated results. Persistence of the ERG rhythm in brainless animals [1, 2] and isolated, organ-cultured eyestalk [8] suggests the existence of the controlling oscillator(s) inside the OL-CE system. On the other hand, OT-severance led to loss of the rhythm, which was, however, later restored in some preparations [6]. Taking these results together, it has been proposed that the rhythm is controlled by oscillations involving the OL-brain system [6].

In the cricket ERG rhythm persisted without interruption even when the OL-CE system was neurally isolated from the CNS.

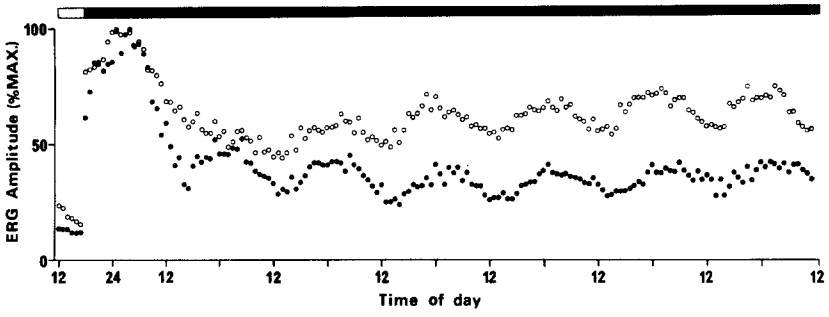

Fig. 1. ERG amplitude rhythms in a cricket after unilateral severance of OT. ○, operated (right) eye, ●, intact (left) eye, □, light, ■, darkness. Recording began at the middle (1200) of the last light fraction of LD 12:12 in which the animal had been held. Tau was 23.8 h in operated eye and 23.9 h in intact eye

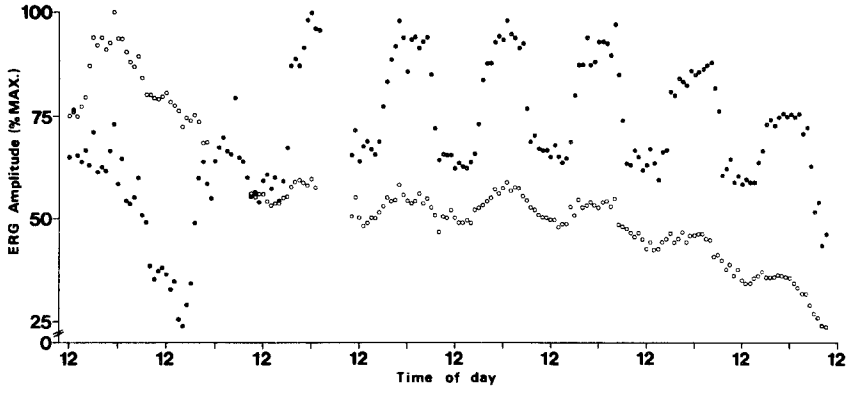

Fig. 2. ERG amplitude rhythms in a cricket after bilateral severance of OT. ○, right eye, ●, left eye. Record was taken in DD. Taus of right and left eyes were 23.2 and 23.4 h, respectively. Data were partly missing on days 2 and 3 due to technical failure

In general ERG rhythm may be regarded as a secondary phenomenon induced by pigment migration rhythm in the CE. However, hormones have not been shown so far to cause pigment migration in insects [3]. Thus a more plausible possibility is that the ERG rhythm is driven neurally by an oscillator in the OL-CE system.

OT-severance or micro-lesioning of parts of the OL resulted in disappearance of circadian rhythm in the cockroach [7, 9] and the cricket [10]. From these facts, the authors proposed that a crucial mechanism for the locomotor rhythm is located in OL. This proposition would apply to our cricket which also lost locomotor rhythm after bilateral OL-ectomy [12]. Nevertheless there is no direct evidence proving that a self-sustaining oscillator exists in OL. For this reason it can not be disregarded that the oscillation even in ERG was found in the distal side of the severed OT. Most interesting is the problem of whether ERG and locomotor rhythms share the oscillating center with each other.

We wish to thank Prof. T. Yamaguchi of Okayama University for his consultation. This work was supported by the Ministry of Education, Science and Culture (Grant-in-Aid to Y.C. for Special Project Research on Mechanisms of Animal Behavior).

Received April 6, 1982

1. Barrera-Mera, B.: Physiol. Behav. *17*, 59 (1976)
2. Barrera-Mera, B.: Comp. Biochem. Physiol. *56A*, 601 (1978)
3. Goldsmith, T.H., Bernard, G.D.: Physiology of Insects, Vol. II, p. 165 (M. Rockstein, ed.). New York-London: Academic Press 1974
4. Honegger, H.-W., Schürmann, F.W.: Cell Tiss. Res. *159*, 213 (1975)
5. Koehler, W.K., Fleissner, G.: Nature *274*, 708 (1978)
6. Larimer, J.L., Smith, J.T.F.: J. Comp. Physiol. *136*, 313 (1980)
7. Nishiitsutsuji-Uwo, J., Pittendrigh, C.S.: Z. vergl. Physiol. *58*, 14 (1968)
8. Sanchez, J.A., Fuentes-Pardo, B.: Comp. Biochem. Physiol. *56A*, 601 (1977)
9. Sokolove, P.G.: Brain Res. *87*, 13 (1975)
10. Sokolove, P.G., Loher, W.: J. Insect Physiol. *21*, 785 (1975)
11. Tomioka, K., Chiba, Y.: J. Comp. Physiol. (in press)
12. Tomioka, K., Chiba, Y.: in preparation
13. Wada, S., Schneider, G.: Z. vergl. Physiol. *58*, 395 (1968)

Cellular Polarity in Root Epidermis of *Gibasis geniculata*

S. Nakazawa and Y. Yamazaki

Department of Biology, Yamagata University, Yamagata, 990 Japan

Prior to root hair formation in root epidermal cells of monocotyledons, the cytoplasm is locally concentrated on the side near the root apex. The cell then divides into two different daughter cells, the larger containing less condensed cytoplasm on the basal side of the mother cell, and the smaller containing more of it on the apical side. The smaller cell forms the root hair [1]. The research described here is concerned with the question of whether this is also the case in the present material or not, as well as whether the site of root hair formation is determined by gravity or formation occurs even in simulated weightlessness under rotation as reported on cytoplasmic localization in *Lepidium* root cells [2].

Adventitious roots regenerated from shoot cuttings of *Gibasis geniculata* were used. Cuttings including two nodes were cultured (a) in Petri dishes placed still, and (b) in glass tube-bottles fixed on a vertical disk rotating at a rate of 24 cycles/min by means of the same apparatus as reported formerly [3]. (c) Some cuttings forming adventitious roots in a still culture were centrifuged at 2500 *g* for 10 min so as to move the cytoplasm of the root epidermal cells toward the basal side of the cell, and then cultured again in still Petri dishes. A piece of wet blotting paper was set in the culture vessel to retain the moisture. The culture was grown under diffuse laboratory light, and the temperature was 20 to 25 °C. In culture (a), one to three adventitious roots were formed at each of the nodes in two days. Microscopic observation revealed that the root hair inception appeared at the apical end of cells near the root apex. Later, the cells elongated without forming a septum. In other words, the root hair formed without differential cleavage of the mother cell, which is in contradiction to classical knowledge [1] (Fig. 1). Localized higher concentration of cytoplasm was sometimes seen, but rather than always being on the side of root hair formation, it varied. The site of the nucleus also differed from cell to cell. Sometimes it was at the tip, base, or middle of the root hair, and sometimes in the base, apex or middle of the epidermal cell.

In rotary culture, the result was the same. The root hair always formed at the apical end of the cell, and the localized higher concentration of cytoplasm was not always in coincidence with the site of root hair formation. This strongly implies that in this species the site of root hair inception in the cell is independent of gravity, but is determined by the intrinsic polarity of the cell.

In centrifugation, the cytoplasm was mechanically concentrated on the centrifugal side of the cell and the vacuole was moved to the centripetal side. No matter what

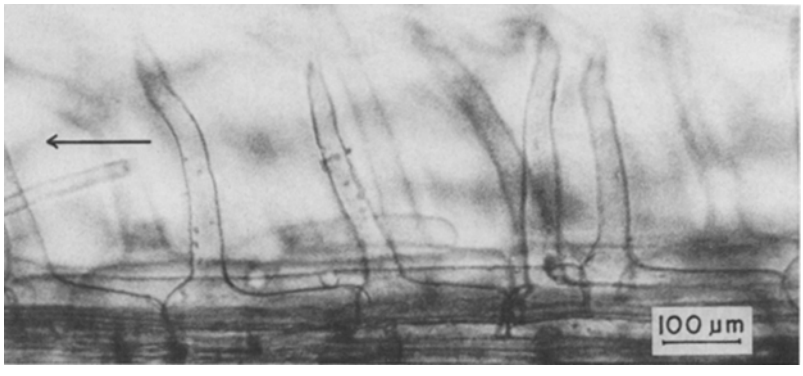

Fig. 1. Root hair formation at the apical end of root epidermal cells without division in *Gibasis geniculata*. Arrow indicates direction of root apex

