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Involvement of Serotonin in the Circadian Rhythm of an Insect Visual System

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Serotonin is one of the major putative neurotransmitters active in insect optic lobes [1, 2]. Extensive documentation shows that in several insects fibers which are immunoreactive to serotonin antibody cover almost the whole area of the neuropil of the optic lobe [3–7]. Little is known about the physiological function of the serotonin-immunoreactive neurons, however, although scholars have speculated that the fibers are part of a system which modulates the overall acitvity of other optic lobe interneurons [2, 7]. Our present research has revealed that the serotonin content in the optic lobe of the cricket (*Gryllus bimaculatus*) fluctuates, depending on the time of day, and that the time course of the fluctuation is strikingly similar to that of the circadian change in the sensitivity of visual interneurons. The application of serotonin results in a reduction of the responsiveness of the visual interneurons. These results, together with the fact that the cricket optic lobe is the locus of the circadian clock [8], suggest that the serotonergic neurons are involved in the regulation of the circadian change in the visual system.

The insect optic lobe is composed of three neuropils that range from distal to proximal: lamina, medulla, and lobula [9]. In crickets the two outer neuropils, separated from the lobula by a long nerve trunk called the optic stalk [10], are the locus of the circadian clock which drives those circadian rhythms that occur in several physiological functions, such as locomotion [11], electroretinographic (ERG) amplitude [12], and optic lobe efferent neural activity [8, 13]. The long-term record of multiple unit neural activity from the optic stalk revealed that the sensitivity of visual interneurons to light pulses is also under the control of the circadian clock; the sensitivity of both light-sensitive and light-inhibited neurons decreases during the subjective day even during constant darkness (Fig. 1). Similar changes can be observed in single neuronal recording (Tomioka et al., in preparation). These results are consistent with findings that in other

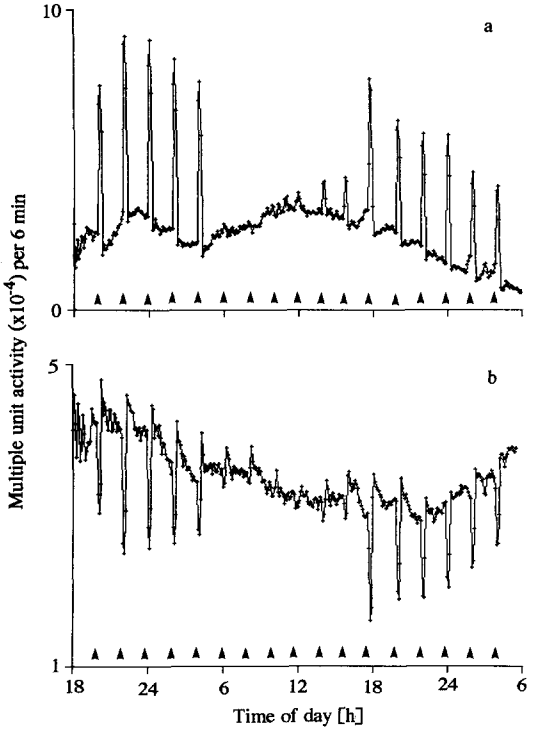

Fig. 1. Daily change in the sensitivity of the light-sensitive (a) and light-inhibited (b) visual interneurons of the lamina-medulla compound eye complex kept in constant darkness starting at 18:00 h. A suction electrode was used to record the electrical responses composed of multiple unit activity that originate in the distal cut end of the optic stalk which connects the medulla and the lobula [13]. The previous lighting regimen was 12 h light–12 h dark (dark:18:00–06:00). Responses of both neurons to light pulses (100 lx, 15 min, *arrowheads*) given every 2 h increased during the subjective night, indicating that the sensitivity to light does increase during the subjective night

insects and crayfish the sensitivity of some visual interneurons is regulated by the circadian clock [14–16].

After immunocytochemical staining with an anti-serotonin antibody, we found that the serotonergic neuronal system covered almost the whole area of the cricket's lamina-medulla region [17] as has been reported for other insect species [3–7]. An analysis carried out by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) revealed that the serotonin content in the optic lobe varies according to the time of day, being about 1.5 times higher during the nighttime than that during daytime (Fig. 2). The increase in serotonin content precedes lights-off by a few hours, which suggests that the change is endogenous and under the control of the circadian clock. The time course of this endogenous change in serotonin content is quite similar to that of the change in the sensitivity of the visual interneurons.

To determine whether serotonin is involved in the circadian change in sensitivity of the visual interneurons to light, we investigated the effect of serotonin on the electrical responsiveness of the neurons and recorded it as a multiple unit activity from the optic stalk. Serotonin does modulate the sensitivity of the visual interneurons in the lamina-medulla complex recorded from the optic stalk: serotonin application reduces the firing rate of the light-sensitive interneurons (Fig. 3 A) but increases that of the light-inhibited interneurons (Fig. 3 B), thus indicating that in general serotonin reduces the sensitivity of the visual interneurons. The effect is dose-dependent (Fig. 3 C). It is thus likely that the serotonergic system is a putative output pathway of the clock designed to mediate the circadian information and control the sensitivity of the visual interneurons: serotonin release is probably activated to reduce the sensitivity of the visual system during the subjective day. It is also possible that the serotonergic neurons may be involved in a system which controls light adaptation. The fact that the activity of the serotonin immunoreactive cells covers a large area of the optic neuropils [17] suggests that circadian modulation may occur at the interneuron level in both the lamina and the medulla. Our results did not show the effect of serotonin on the responsiveness of individual visual interneurons. Since there are reportedly many types of visual interneurons in the cricket optic lobe [18], careful examination of the response modulation brought about by serotonin at the single-cell level is necessary to understand more fully the functional role of serotonin in the control of visual processing.

Serotonergic modulation may also occur at the retinular cell level, since there are some varicose processes with serotonin immunoreactivity that extend toward the optic nerve from the lamina (Tomioka et al., unpublished data) and the amplitude of ERG has a clear circadian rhythm [12]. Although serotonergic control of retinal sensitivity has not yet been found in insects, extensive studies have revealed that the influence of serotonin modulates the sensitivity of the eye in other invertebrate animals, e.g., *Limulus* [19], *Aplysia* [20], and *Hermissenda* [21].

It is well known that a light pulse gives rise to the phase shifts of the circadian clock free running in constant darkness according to the phase at which the pulse is given [22]: it generally induces delay shifts, advance shifts, and no shift when

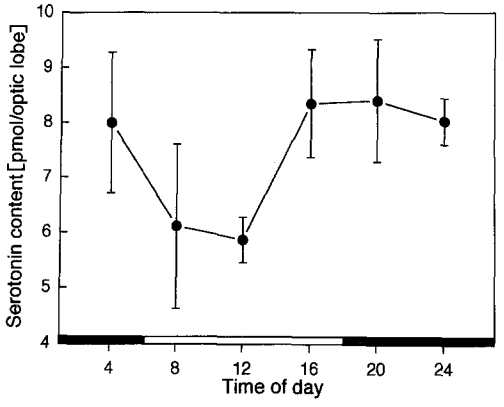

Fig. 2. Daily change in mean (± SEM) serotonin content of the optic lamina-medulla complexes from crickets exposed to a 12 h light–12 h dark cycle. Lights were turned on at 06:00 h. The oscillation of serotonin content was statistically significant (one-way ANOVA, $P < 0.01$). Six to 12 samples were used to estimate each data point. The procedures adopted for the extraction, separation, and measurement of serotonin were based on those described elsewhere [23]

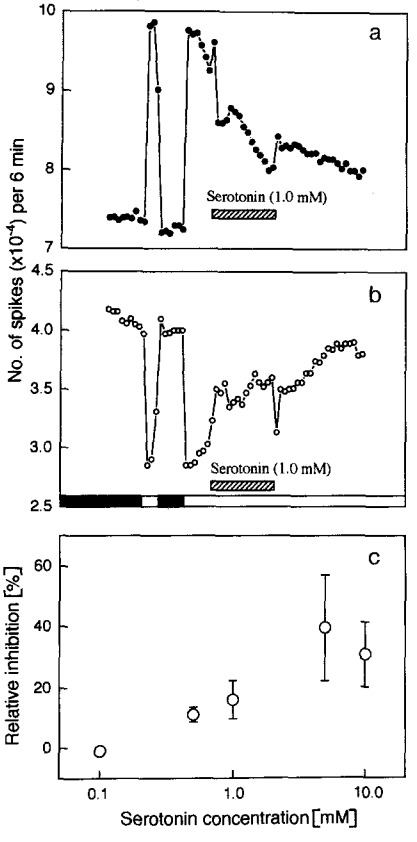

Fig. 3. Effects of serotonin on the light-sensitive (a) and light-inhibited (b) efferent neuronal activity of the optic lamina-medulla compound eye complex. Illustrated are the firing rates per 6 min. The lighting regimen is indicated by *white* (lights-on) and *black* bars (lights-off). Serotonin (1.0 m*M*) was applied for 90 min, 30 min after the second lights-on. Responses of light-sensitive and light-inhibited neurons decreased and increased, respectively, during the serotonin application, suggesting that the responsiveness of neurons to light was reduced. c) Dose-response relationships between serotonin concentrations and the magnitude of their inductive effects on light-induced responses of light-sensitive neurons. The data illustrate the mean ± SEM of three to five in sects. The effect of serotonin on the firing frequency was quantitatively evaluated by comparing the average firing frequency during the application of the chemical with that during 30 min of the pre- and posttreatment periods. All experiments were performed during the night (18:00–01:00). The relatively high concentrations of serotonin needed to modulate the sensitivity of the interneurons are probably due to the well-developed blood-brain barrier of insects [24]

given, respectively, in the early subjective night, in the late subjective night, and in the subjective day. Since the compound eye and the optic lobe visual system form the only pathway for the conveyance of photic information of the clock [11], the serotonergic system may consequently regulate the magnitude of the phase-shifting ability of the clock by changing the sensitivity of the visual system. If we consider these facts together, we suppose that the serotonergic system may be involved in both the input and the output pathways of the circadian clock.

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Phototaxis in an "Eyespot"-Exposing Ciliate

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Motile microorganisms have developed three basically different strategies with which they respond to physical or chemical stimuli: kinetic, phobic, and taxic responses [1, 2]. Taxis denotes a movement oriented with respect to the stimulus direction. If the orientation is towards the stimulus source, the taxis is "positive"; if away from it, "negative". Taxic responses to light have been described for a variety of unicellular pro- and eukaryotes [3, 4], the majority of ciliates being unexplored. Photosynthetic flagellates such as *Chlamydomonas* and *Euglena* are best studied. These organisms usually carry a red-orange "eyespot" or "stigma", a cluster of carotenoid droplets [5], which is assumed to have some function in phototactic orientation [4].

Here, we describe a newly discovered phenomenon of a pronounced light-induced taxic orientation of a *ciliated protozoan.* Cells of a not yet identified species of *Chlamydodon* [6–9], which are colorless except for their numerous bluish food vacuoles, show a precise negative phototaxis in unilateral, constant white light when they are well-fed on cyanobacteria. An accentuated reversal of this response occurs in slightly underfed individuals which have accumulated several hundred orange vesicles at their anterior left side, forming an "eyespot"-like structure [6] (Fig. 1). Such cells temporarily tend to show positive phototaxis at identical illumination.

Chlamydodon sp. was isolated from a brackish pond near Carolinensiel on the coast of Germany. Cells were cultivated in a mixture of North Sea water and soil medium [10], diluted 1:1 with *aqua*

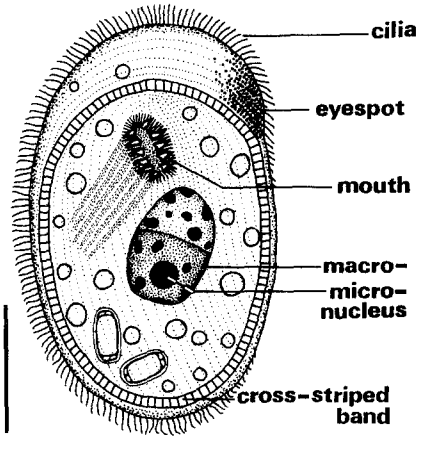

Fig. 1. Schematic diagram of a slightly underfed cell of *Chlamydodon* sp.: Small orange vesicles at the anterior left cell side form the "eyespot-like structure" which is characteristic for this stage. The cross-striped band is an obscure organelle encircling the margin of the dorsoventrally flattened cells. *Scale bar* 25 μm