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Circadian Rhythms in Locomotor Activity of the Hagfish, *Eptatretus burgeri* V. The Effect of Light Pulses on the Free-running Rhythm

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ABSTRACT—This study examined the effect of light pulses on the free-running rhythm of locomotor activity of the hagfish, *Eptatretus burgeri*, reared for prolonged periods under constant darkness. The free-running period (τ) of circadian locomotor activity of 55 hagfishes followed a normal distribution with mean 24.1 hr (min.: 22.8 hr; max.: 25.1 hr). After establishing the τ in constant darkness for each specimen, we examined the effect of single light pulses (intensity; 670 lx at the bottom of the aquarium, duration; 15 min) on the free-running rhythm. Light pulses in the early subjective night lengthened τ and caused a slight phase delay, whereas those in the late subjective night shortened τ and provoked a slight phase advance. Thus, the hagfish showed a distinct "period response" to short light pulse, but the "phase response" was not so clear as other organisms.

INTRODUCTION

Studies concerned with the circadian locomotor activity rhythms in aquatic animals have been both meager and controversial because of difficulties in making field observations or in providing normal healthy conditions during prolonged periods in the laboratory. Some fishes exhibit circadian rhythms under constant light and constant dark [2, 5, 8, 18, 19], whereas other fish species display arrhythmicity [3, 7, 9].

In this context, it is remarkable that the hagfish displays clear circadian rhythmic locomotor activity under laboratory conditions. The nocturnally active hagfish is active only in the first 2/3 of the dark period of a 12 hr light and 12 hr dark (12L:12D) light legime [10]. When the light-dark phases are reversed, the activity of the animal resynchronizes to the new light regime in one or two weeks [10]. In contrast, motor activity in eye-ablated animals under 12L:12D not only occurs in the dark period, but also runs into the light period to form a free-running rhythm. These facts show that one of the most important factors affecting nocturnal rhythmicity may be optically perceived peripheral lighting information [4]. By surgical operation experiments in the brain, it has been shown that the circadian pacemaker of the hagfish may be located in the preoptic nucleus in the hypothalamus [12].

There are many reports concerning the effect of light pulses on the free-running locomotor activity rhythms under DD in various animals [1, 13, 17]. In aquatic animals, however, there have been few such studies because of the difficulty in recording of activity rhythm in long time.

In the present study, we first tried to record the free-running rhythm of locomotor activity for a longer period of time than in a previous study [10]. Then we examined the effect of light pulses on the free-running rhythm of locomotor activity of the hagfish, *Eptatretus burgeri*, reared for prolonged periods under constant darkness.

MATERIALS AND METHODS

The experiments were performed at the Misaki Marine Biological Station, University of Tokyo. The hagfishes, *Eptatretus burgeri*, were collected by use of a trap containing sardines as bait. Collected hagfishes (body length, 35–50 cm) were kept in a large aquarium (1.8×1.8×0.8 m) under 12L:12D (7:00–19:00 light, 19:00–7:00 dark) and temperature of 15°C until transfer to the experimental units. Tests were carried out in recirculated-water unit (90×45×45 cm) kept at 15°C throughout the experiment. The laboratory was located in a sandstone cave to minimize external disturbance. Each unit was stocked with a single fish during photoregime manipulation. The source of the light pulse was a 15 W white fluorescent lamp placed above the top of the aquarium so as to present a light intensity of 670 lx at the bottom of the aquarium. The light intensity was the same value used in the light period in 12L:12D, and corresponded to a depth of 25 m in open seas, a common winter habitat for the hagfish.

The locomotor activity in the hagfish was measured by means of an infrared light-photocell system connected to an event recorder. The infrared light beam crossed the width of the aquarium (45 cm) parallel to and 5 cm below the water surface. Signals were generated when the animal swam up off the bottom of the aquarium and interrupted the beam. The period of each free-running cycle was measured as the time length from one starting point of locomotor activity to the next. The τ was determined by averaging the periods for 7–103 cycles to estimate the initial free-running period in constant dark (DD) (Experiment I), and for 19–21 cycles to assay the effect of light pulses (Experiment II). For the purpose of calculating the

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magnitude of phase shift of the activity rhythm, the onset of activity, designated as circadian time 12 was chosen as the phase reference point. Phases were determined by fitting lines by eye through activity onsets, one before and one after a light pulse. The magnitude of the phase shift was taken as the interval between the lines extrapolated to the day of the treatment. Each estimate of onset line was based on at least 10 days of data.

RESULTS

Experiment I Circadian rhythms in constant darkness

The τ of locomotor activity in DD was estimated in 55 animals. As shown in Figure 1, the τ of these animals can be plotted as a normal distribution curve with mean around 24 hr. The shortest and the longest periods observed were 22.8 and 25.1 hr, respectively; the average was 24.1 hr. Figure 2 illustrates a typical example of stable activity rhythms recorded over a long period.

Experiment II The effect of light pulses on the free-running rhythm

We then examined the motor responses to single light pulses in 13 animals.

Figure 3 shows a typical example of the record of the activity in this kind of experiment. This particular animal was subjected to 4 days of stabilization under a normal light and darkness (LD) cycle, then exposed to DD. The τ during the 13 days before the first light pulse was 23.5 hr. The τ became longer (24.4 hr) after a light pulse was given in the beginning of the active phase (circadian time [CT] 12.6) on day 18. After another 20 days, the animal was given a second light pulse near the end of the active phase (CT 22.5). The ensuing τ decreased to 24.1 hr. A third light pulse was given in the middle of the first half of the active phase (CT 16.9) after 20 days, causing the formation of a distinctly longer τ (24.7 hr). In this example, the animal showed a slight phase delay (0.8 hr) with the first and a slight phase advance (1.0 hr) with the second light pulses, respectively.

Another example is shown in Figure 4. The τ became longer (24.5 hr) than before (23.4 hr) with the first light pulse at CT 11.5. In contrast, it became shorter (24.2 hr) with a second pulse at CT 22.4. A third light pulse given in the middle of the second half of the active phase (CT 17.4) on day 60 reduced τ over the following 19 days to 23.8 hr. In this case, the animal showed phase-advances of 1.0 hr and 0.4 hr, respectively with the second and the third light pulses.

In this study, it was also observed that there was some tendency to turn back to the prior τ in the course of free-running rhythm as shown in Figures 3 and 4 after the second and the third light pulses, respectively.

Figure 5 summarizes the period response (τ change) and the phase response to the light pulses in the 13 animals. The light pulses in the early subjective night lengthened τ, whereas those in the late subjective night shortened τ. Likewise, the phases were delayed or advanced by pulses in the early subjective night or the late sbjective night, respectively.

In all cases, clear changes in locomotor activity were observed after the light pulses. In the case of pulses given at the beginning or in the first half of the active phase, the animal continued to swim actively during the pulse, briefly (1–2 hr) stopped at the end of it, then resumed activity. On the other hand, the animals also kept swimming during the pulse but stopped for the rest of the "day" when the light pulses were given in the latter half of the active phase.

DISCUSSION

Circadian activity patterns in many fishes are labile and easily fade out. Nevertheless, this study demonstrated that spontaneous rhythmical locomotor activity in hagfish can be maintained relatively stable under continuous darkness, sometimes for more than 100 cycles. Stable rhythmicity for a long period is advantageous in investigating the effect of a light pulse on the locomotor activity.

Available reports have demonstrated that many animals show phase response phenomena when given light pulses in

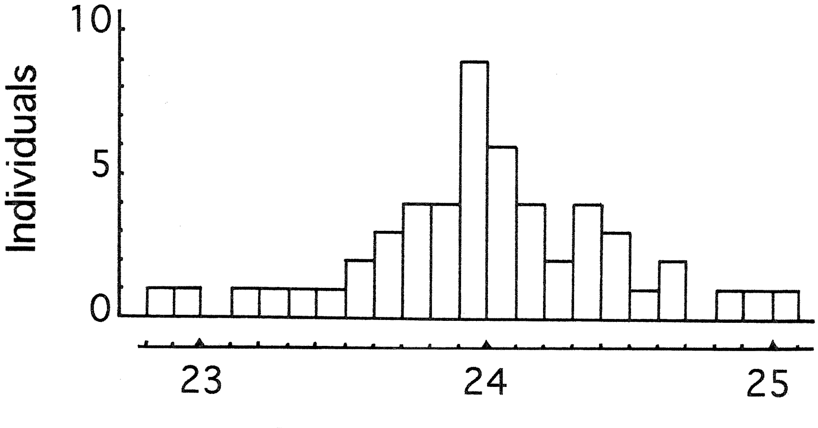

Fig. 1. The τ under DD estimated in 55 animals. The data were obtained during 1983–1988.

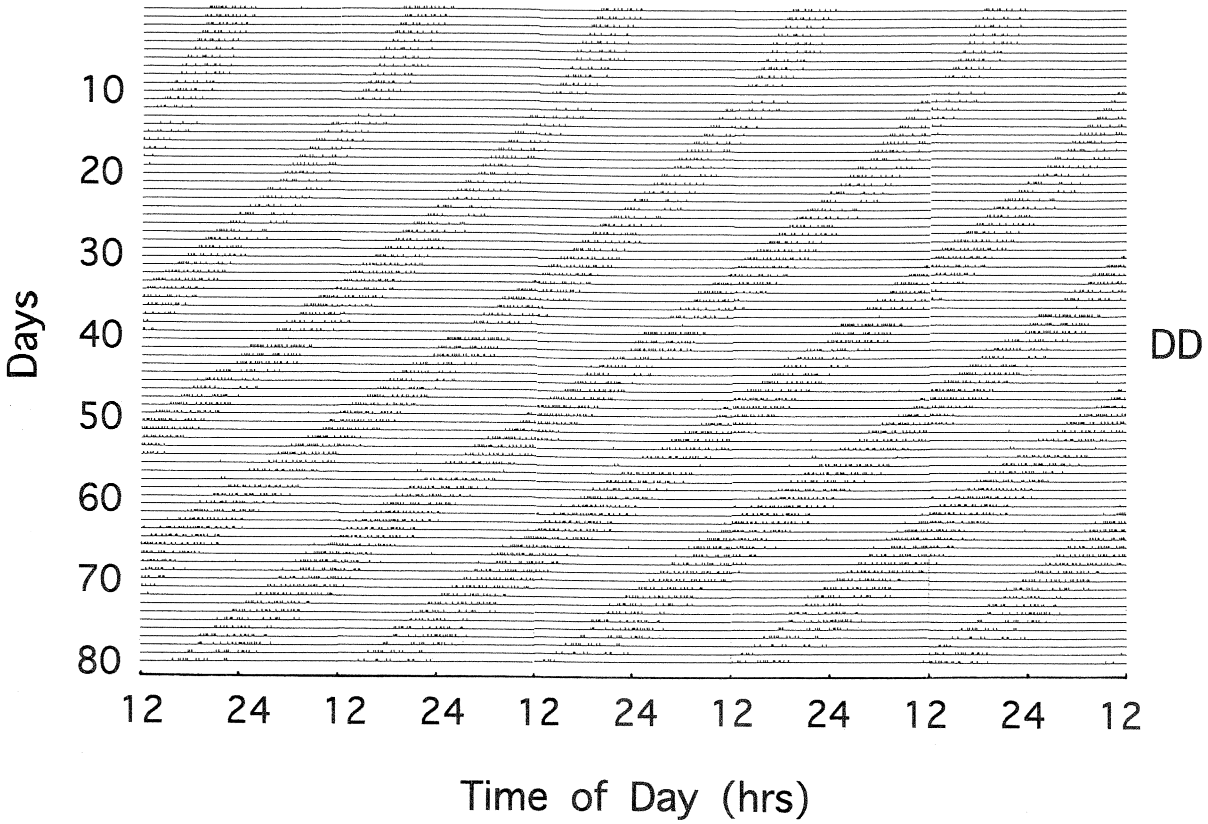

FIG. 2. Circadian rhythm of activity recorded in a hagfish kept in DD for 80 days. Original record was plotted five times. The calculated free-running period is 22.8 hr.

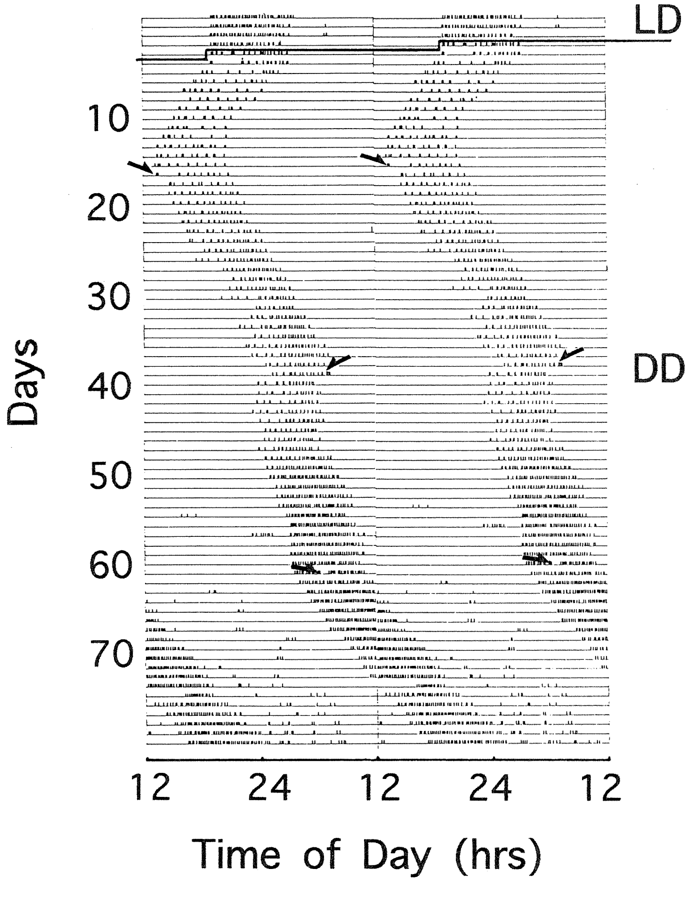

FIG. 3. The effect of light pulses on the free-running rhythm of a hagfish. The animal was subjected to 4 days of stabilization under LD (7:00–19:00 light, 19:00–7:00 dark), then exposed to DD. Arrows show the light pulses. Original record was plotted twice.

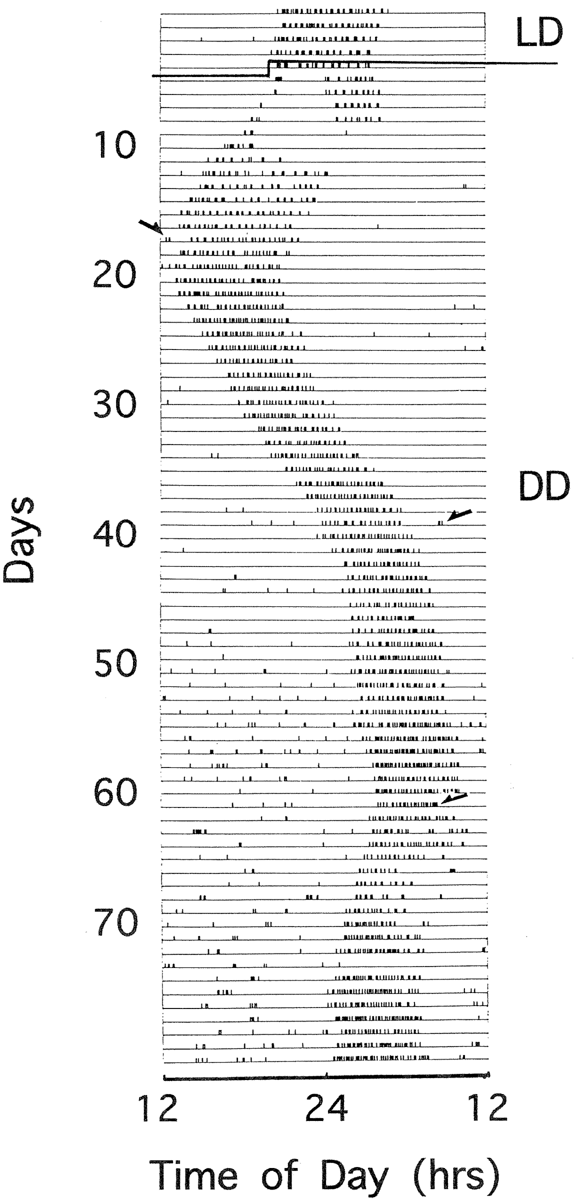

Fig. 4. The effect of light pulses on the free-running rhythm of a hagfish. Arrows show the light pulses.

the course of free-running activity rhythm. The direction of the shift depends on the timing of the light pulses. Generally, the phase is advanced when light pulses are given in the late subjective night, while it is delayed when light pulses are given in the early subjective night. This phenomenon called "phase response" was first demonstrated for the pupal eclosion rhythm of *Drosophila* [13]. Similar experimental results have been obtained for other organisms [1, 15, 16].

In this study, it was found that a single short light pulse distinctly changed the τ in addition to producing a small phase shift. The magnitude and the direction of the change in τ depended on the circadian time at which the light pulses were given (Fig. 5).

Pittendrigh [14] reported that when a long light pulse (e.g. 12 hr) imposes a major advance on the free-running activity rhythm of a hamster, the new steady state is characterized by a shorter τ than prior to the phase shift, while a phase delay is usually associated with a longer τ. He described this phenomenon as an "after effect" of the pulse. Pittendrigh and Daan [16] also mentioned that τ changes were associated with phase shifts caused by light pulses in four species of nocturnal rodents (*Mesocricetus auratus, Peromyscus leucopus, P. maniculatus* and *Mus musculus*).

Pohl [14] reported relatively small but significant changes in τ following light pulses in three diurnal rodents, *Ammospermophilus leucurus, Tamias striatus* and *Tamiasciurus hudsonicus*. These changes depend on the direction and/ or amount of phase shifts, but they can also occur without any phase shift. He described this phenomenon as "period response".

Thus, the results of the present study are similar to those of rodents in that τ is lengthened and the phase is delayed by pulses in the early subjective night, whereas τ is shortened and the phase is advanced by the pulses in the late subjective night. However, the response of *Eptatretus burgeri* differs in that the main reaction to the light pulse is the "period response" (change of τ), whereas rodents show mainly "phase response". Both "phase response" and "period response" are compensatory, and may be part of a mechanism adjusting τ to the external light period. For example, if the circadian clock is repeatedly delayed by light pulses in the environment, then lengthening the period will diminish the magnitude of phase delay required each day, thereby ensuing that the clock stays within the limit of entrainment.

The fact that the hagfish shows mainly "period response" to light pulses indicates that the clock of hagfish may be flexible about the τ. Even in the "phase response", a change in τ also occurs during transient term and the τ returns to its "original" value within a short term. On the contrary, the altered τ in "period response" remains for a long term. This suggests a weak "inertia" to return back to the previous length.

However, the flexibility of the τ in the hagfish is not infinite [11]. When the hagfishes are subjected to light regime gradually departing from 12L : 12D (24 hr), the animals show stable entrainment only within 24±1.2 hr. In periods longer and shorter than 24±1.2 hr "day", the animals show "relative coordination". These results show that the changes of the τ of the hagfish are limited in certain ranges. Recently, we reported the effect of light pulses on the free-running rhythm of the locomotor activity in another hagfish species, *Paramyxine atami*. The "period response" is observed in this species in which the "phase response" also occurs [6].

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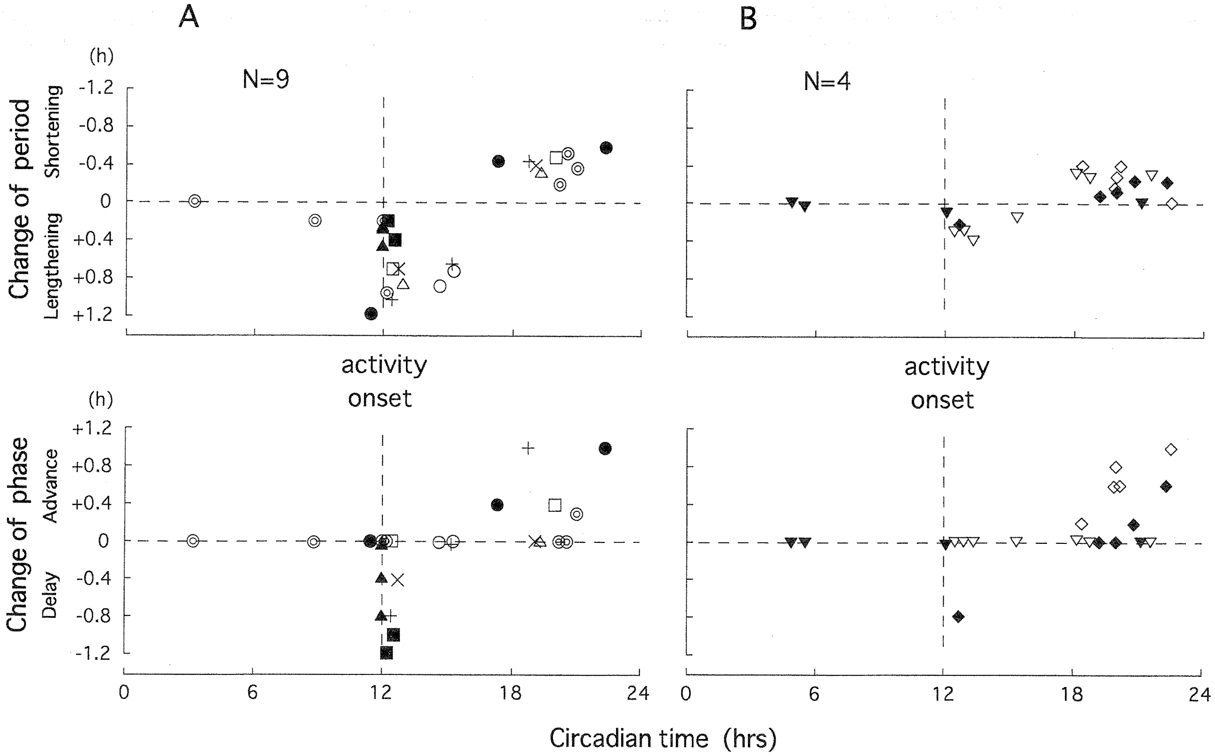

FIG. 5. Period and phase responses plotted as functions of circadian time at which the light pulse was given in the hagfish. Different symbols refer to different individuals. Circadian time 12=activity onset. A: animals whose initial τ was shorter than 24 hr. B: animals whose initial τ was longer than 24 hr.

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