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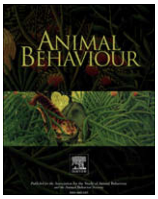

Entrainment of the circatidal activity rhythm of the mangrove cricket, *Apteronemobius asahinai*, to periodic inundations

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Adults of the mangrove cricket show a clear and persistent circatidal rhythm in their locomotor activities, with a free-running period of about 12.6 h. Individuals are active during the subjective low tide and inactive during the subjective high tide, under constant darkness. When we provided a 30 min inundation stimulus four times, at intervals of 12.4 h, the circatidal rhythm entrained to the given inundation cycles under constant darkness. After the treatment, crickets became inactive around the times when the inundation stimulus was expected. The circatidal rhythm responded to periodic inundations in a phase-dependent manner: when periodic inundations started during the first half of the active phase (i.e. the subjective low tide), a large phase delay resulted, whereas periodic stimuli that started during the second half of the subjective low tide caused a large phase advance. The shape of the phase response curve (PRC) resembled that of the Type 1 PRC of circadian rhythms, with continuous transitions between delays and advances. The similarity between the PRC patterns provides evidence of a circatidal oscillator, which has characteristics similar to a circadian oscillator.

The cycle between high and low tides (the tidal cycle) is one of the most important environmental cycles in the intertidal and sublittoral zones, apart from the cycle between day and night. Normally, the intertidal zone is submerged twice each day, with a period of approximately 12.4 h (semidiurnal tide), although some regions have a diurnal tide (one period of high tide per day) or a mixed tide (a mix of the above two tidal forms). It is important for intertidal and sublittoral organisms to synchronize their daily activities to the tidal cycle, to be active during suitable periods (e.g. limpet grazing during low tides, Gray & Hodgson 1999).

Many marine organisms show endogenous circatidal activity rhythms under constant conditions without tidal information; examples include crustaceans (Bennett et al. 1957; Naylor 1958; Barnwell 1966; Enright 1972; Akiyama 1995), the horseshoe crab *Limulus polyphemus* (Ehlinger & Tankersley 2006), gastropods (Petpiroon & Morgan 1983; Gray & Hodgson 1999) and a bivalve, *Ruditapes philippinarum* (Kim et al. 1999). This means that individuals determine the 'correct' timing for activity using an endogenous clock, which can be entrained to the tidal cycle. Many environmental cues related to tidal cycles have been reported as the zeitgeber for the entrainment of circatidal activity rhythms, such as periodic changes in water turbulence (Enright 1965; Klapow 1972; Ehlinger & Tankersley 2006), hydrostatic pressure (Morgan 1965; Reid & Naylor 1990; Northcott et al. 1991; Akiyama 2004), salinity (Taylor & Naylor 1977) and water temperature (Naylor 1963; Holmström & Morgan 1983b).

Circatidal activity rhythms and their zeitgebers have been primarily studied in marine organisms, as mentioned above. Besides marine organisms, some animals that are essentially terrestrial live in the intertidal zone, and show endogenous circatidal rhythms (e.g. terrestrial insects: Foster & Moreton 1981; Satoh et al. 2006, 2008; the mite *Bdella septentrionalis*: Ernst 1995; and the marine iguana, *Amblyrhynchus cristatus*: Wikelski & Hau 1995). These intertidal terrestrial animals are thought to synchronize their activities to the low tide using environmental cues related to the tidal cycle because of their dependence on atmospheric oxygen. However, the zeitgebers for circatidal rhythms in these animals have rarely been identified; Satoh et al. (2006) provided the only example, reporting preliminary results that cyclic submergence could be the zeitgeber for the endogenous rhythms observed in larvae of the tiger beetle, *Callytron inspecularis*. No study has

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revealed the phase responsiveness of the circatidal activity rhythm to the zeitgeber for primarily terrestrial animals.

Habitats on mangrove forest floors are influenced by tidal cycles. The mangrove cricket, a species endemic to mangrove forests, forages on the forest floor during low tides and rests on stems during high tides. We previously demonstrated that adults of this species show a clear and persistent circatidal activity rhythm under constant darkness, with a free-running period of approximately 12.6 h (Satoh et al. 2008). The endogenous rhythm consists of active phases of approximately 10 h, which coincide with expected low tide periods in the field, alternated with inactive phases of approximately 2 h, which coincide with expected high tide periods in the field. The mangrove cricket is active during the subjective low tide and inactive during the subjective high tide, under constant conditions. Until now, the mangrove cricket has been the only insect known to show a rigid circatidal activity rhythm, and the zeitgeber for this endogenous rhythm has remained elusive.

In the present study, we investigated the effects of periodic inundations on the circatidal rhythm in the mangrove cricket. The periodic change of the presence and absence of water should be the most conspicuous and reliable zeitgeber for the mangrove cricket, since it may be difficult for them to detect periodic changes in hydrostatic pressure, salinity or water temperature. We provided a 30 min inundation stimulus four times at intervals of 12.4 h and analysed the phase responsiveness of the circatidal rhythm of the mangrove cricket to the stimulus.

METHODS

We collected adult male crickets from a mangrove forest in Ginoza, Okinawa Prefecture, Japan (26°30′N, 127°59′E). We housed crickets individually in recording chambers on the day of or the day after collection. The recording chambers (‘A’ in Fig. 1) were plastic petri dishes (approximately 5 cm in diameter); several slits (approximately 1 mm in width) were cut in the bottom of each dish, and each dish was installed in an identically sized hole in the bottom of a tank (‘B’ in Fig. 1). We installed six recording chambers in the tank. The tank was fixed above a water receiver (‘C’ in Fig. 1). When the receiver was filled by pumping tap water from a reservoir (‘D’ in Fig. 1), the water flowed into the chambers through the bottom slits, thereby providing an inundation stimulus. The depth of water in the chamber was maintained at approximately 5 mm during the stimulus period. Since the mangrove cricket is very small (5–7 mm in body length), a water depth of 5 mm should provide an effective stimulus. Crickets could escape from drowning by climbing the chamber wall. When the water pump was stopped, the water immediately drained back into the reservoir.

To record locomotor activity, an infrared beam (EE SPW-321; Omron, Kyoto, Japan) was passed across each chamber, and the number of times a beam was interrupted was recorded at 6 min intervals on a personal computer. We placed a small piece of dried food made from insect pellets (Oriental Yeast, Tokyo, Japan), agar, sorbic acid and propionic acid on a small Styrofoam pedestal (approximately 10 × 10 × 5 mm) at one end of each chamber and a water source at the other end; the food and water were at opposite ends of a line that was diagonal to the infrared beam. The recording of locomotor activity was conducted under constant darkness at 25 °C by housing the recording apparatus in a light-tight incubator (MIR-153 or MIR-253, Sanyo, Moriguchi, Japan). The temperature of the tap water was equilibrated to the incubator temperature (25 °C).

After the locomotor activities of mangrove crickets had been recorded over 8–12 days, we provided a 30 min inundation stimulus four times at intervals of 12.4 h; subsequent activity was recorded for 7–10 days after the stimuli. Although we provided the stimuli at the same time to each of the six crickets, the actual phases of the stimuli varied between the individuals because the free-running period of each individual varied. We determined the phase shifts produced by periodic inundations by measuring the distance between the two regression lines extrapolated to the day the first inundation was provided, that is, one was fitted by eye through the sequential points of activity onset before the inundations and the other was fitted through sequential points of activity onset after the inundations. The phase shift of the activity onset was expressed as a proportion of the free-running period for each individual.

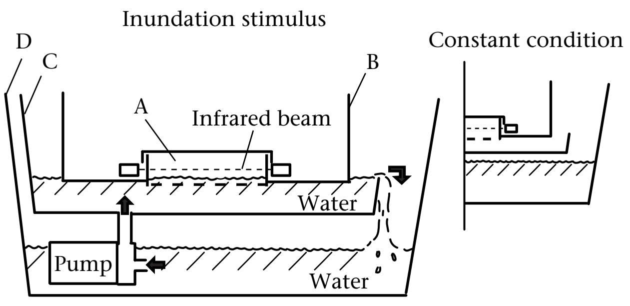

Figure 1. Simplified cross-section diagram of the recording apparatus. A: recording chamber; B: tank with six recording chambers (only one shown here); C: water receiver; D: reservoir.

RESULTS

We recorded free-running activity rhythms for 35 crickets. Of these, phase responsiveness was analysed using 15 crickets that showed a clear bimodal pattern in their daily activity during both the periods before and after inundation. Figure 2 shows four typical examples of the phase responsiveness to periodic inundations provided at various phases. When periodic inundations were initiated during the first half of the active phase, the activity onset was considerably delayed (Fig. 2a), and when periodic inundations were initiated during the second half of the active phase, the activity onset was considerably advanced (Fig. 2b). On the other hand, when periodic inundations were initiated during the middle of the active phase or during the inactive phase, the treatment had little effect (Fig. 2c, d). After the inundations, the period of the free-running rhythm ($\overline{X} \pm \mathrm{SD} = 12.59 \pm 0.17$ h, $N = 15$) showed no significant change from the preceding duration ($\overline{X} \pm \mathrm{SD} = 12.52 \pm 0.23$ h; Wilcoxon test: $T = 18$, $N = 12$, $P > 0.05$).

On the basis of this phase responsiveness, we constructed a phase response curve (PRC) for the circatidal rhythm by plotting the phase shift as a function of the phase at which the first inundation stimulus was initiated (Fig. 3). The mean onset phase of cricket activity (i.e. the beginning of the subjective low tide)±SD was $76 \pm 16°$ ($N = 15$). Only a slight phase advance was observed when the first inundation stimulus was initiated during the subjective high tide, whereas a large phase shift was observed when the first stimulus was initiated during the subjective low tide (Fig. 3). A maximum phase delay of 169° (approximately 5.9 h) occurred when the first stimulus was initiated at 229°, and a maximum phase advance of 109° (approximately 3.8 h) when the stimulus was initiated at 289°. The crossover point between the delays and advances occurred near the middle of the subjective low tide.

In 14 of the 35 crickets, a clear bimodal pattern was found in the daily activity after the periodic inundations, showing the entrainment to the cycle of the inundation stimuli, although these individuals did not show a clear circatidal rhythm before the