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Research report

Chronic electromyographic analysis of circadian locomotor activity in crayfish

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HIGHLIGHTS

- We analyzed the circadian locomotor activity of crayfish by chroic EMG recording.
- Burst latency was used to test if the activity started spontaneously or reflexively.
- In the LD condition, the latency was comparable with that of spontaneous walking.
- In the free-run condition, the burst latency was also comparable.

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ABSTRACT

Animals generally exhibit circadian rhythms of locomotor activity. They initiate locomotor behavior not only reflexively in response to external stimuli but also spontaneously in the absence of any specific stimulus. The neuronal mechanisms underlying circadian locomotor activity can, therefore, be based on the rhythmic changes in either reflexive efficacy or endogenous activity. In crayfish *Procambarus clarkii*, it can be determined by analyzing electromyographic (EMG) patterns of walking legs whether the walking behavior is initiated reflexively or spontaneously. In this study, we examined quantitatively the leg muscle activity that underlies the locomotor behavior showing circadian rhythms in crayfish. We newly developed a chronic EMG recording system that allowed the animal to freely behave under a tethered condition for more than 10 days. In the LD condition in which the animals exhibited LD entrainment, the rhythmic burst activity of leg muscles for stepping behavior was preceded by non-rhythmic tonic activation that lasted for 1323 ± 488 ms when the animal initiated walking. In DD and LL free-running conditions, the pre-burst activation lasted for 1779 ± 31 and 1517 ± 39 ms respectively. In the mechanical stimulus-evoked walking, the pre-burst activation ended within 79 ± 6 ms. These data suggest that periodic changes in the crayfish locomotor activity under the condition of LD entrainment or free-running are based on activity changes in the spontaneous initiation mechanism of walking behavior rather than those in the sensori-motor pathway connecting mechanoreceptors with leg movements.

1. Introduction

Animals show circadian rhythms in a variety of homeostatic functions and behavioral activities. These rhythms are thought to be endogenous [1–3], based on an internal clock or pacemaker that resides in a specific site in the central nervous system depending on animal species [4]. Among many kinds of behavioral rhythms, the most widely recognized in the animal kingdom is that observed in locomotor behavior including walking [5–7], swimming [8,9], and flying [10,11]. The neurophysiological mechanism underlying the circadian rhythms of locomotor behavior, however, remains largely unknown.

Crustaceans have been known to show circadian rhythms in hormonal secretion [12–15], neurosecretion [16], biogenic amine contents and actions in the central nervous system [17–19], mechanoreceptor sensitivity [20], photoreceptor sensitivity [20–36] and locomotor activity [37–43]. A previous study in the crayfish *Procambarus clarkii* showed that the circadian rhythm in leg movement disappeared when the circumesophageal commissures were surgically lesioned bilaterally, thus experimentally demonstrating the importance of descending signals from the brain to posterior ganglia [38]. Thus the rhythm of leg movements appears to be based on circadian changes in the motor commands generated endogenously in the brain.

It should be noted here that locomotor behavior could be evoked either endogenously or in response to external stimulation. In crayfish, walking behavior is initiated spontaneously in the complete absence of external stimuli that can be detected by human

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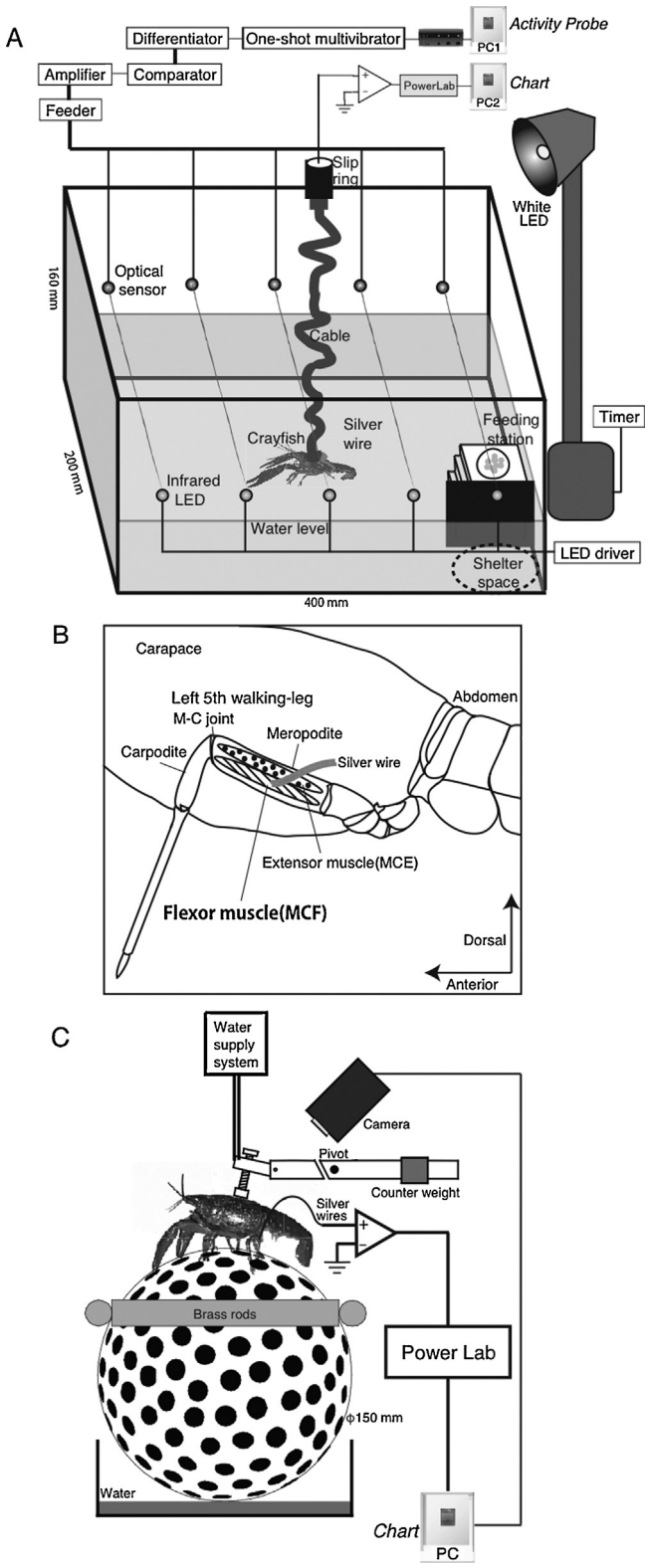

Fig. 1. Experimental setup for chronic EMG recording. (A) Experimental apparatus. The animal was tethered to a slip-ring on the ceiling by PVC insulated cable for EMG recording. Locomotor activity was monitored by five pairs of infrared LED/phototransistor combination, each separated by an interval of 70–80 mm height from the aquarium bottom. The phototransistor signal was fed to a personal computer (CPU1) through a series of electronic devices. The EMG signal was fed into another personal computer (CPU2) for continuous recording throughout the experiment. (B) Posterior-lateral view of the fifth left leg and the target muscle for the recording. EMG recording was made from the mero-carpopodite flexor (MCF) muscle of the fifth leg on both sides using a pair of silver wires. M-C joint, mero-carpopodite joint; MCE, mero-carpopodite extensor; MCF, mero-carpopodite flexor. (C) Treadmill system. The animal was fixed to a rod that was mounted to a lever system and positioned on the top of a water-supported white Styrofoam sphere so that the animal could walk on it while fixed at the cephalothorax. The sphere (diameter 15 cm; mass 60 g) was placed in a Petri dish (150 mm; 15 mm) partly filled with water and stabilized in a constant position by four brass bars that could freely rotate along the long axis. The amount of water in the dish was adjusted so that the sphere was just in contact with those bars in order to rotate smoothly. The lever could move vertically around a pivot so that the animal held at one end of the lever could freely change its standing posture. A counterbalance was attached at the other end of the lever so that the animal was virtually free from any load in the fixed experimental condition. Since the sphere weight was larger than the animal body weight, the possibility that the moving sphere might have a momentum causing unusual feedback modification of the walking motor system could not be completely excluded. The animal was provided with water drips guided by a strip of gauze onto the dorsal part of the cephalothorax to prevent dehydration. A video camera was used to record the movement of crayfish legs. EMG recording was carried out in the same manner as mentioned above.

observers. Walking behavior of crayfish can be also initiated by slightly touching its body or putting some chemical substances in the water [44]. An electromyographic (EMG) study revealed that the temporal pattern of leg muscle activation is statistically different between spontaneously evoked and stimulus-evoked walking [44]. It was reported that the spontaneous walking was characterized by sustained, non-rhythmic activation of leg muscles that preceded their rhythmic burst activities whereas the mechanical stimulus-evoked walking was initiated abruptly following the stimulus [44]. Further studies have revealed that descending neuronal signals indicative of readiness activities, which is defined as the spike activity that transiently precedes the voluntary initiation of behavior, in the crayfish brain [45,46]. The spike activities called readiness discharge precede the onset of leg muscle activation drive spontaneous walking in crayfish [45,46]. Those descending neurons (readiness discharge neurons) identified by intracellular techniques became active >1 s before the behavioral initiation of walking and remained inactive at the onset of mechanical stimulus-evoked walking, suggesting that the central nervous mechanisms for initiating spontaneous and stimulus-evoked walking are quite different from each other [45,46].

In the present study, we addressed the following question: what physiological mechanism subserves the circadian rhythm of crayfish locomotor activity? One possible mechanism is periodic changes in the central nervous activities that are responsible for endogenous initiation of walking behavior. Alternatively, periodic changes in the functioning of sensori-motor pathways such as mechanoreceptor sensitivities and synaptic transmission efficacy might underlie the circadian rhythm observed in locomotor activity. We newly developed a chronic EMG recording system that allowed us to quantify the muscle and behavioral activities simultaneously over a long-term experimental period. Quantitative analysis of the EMG data suggests that the neurophysiological mechanisms underlying circadian locomotor activity in crayfish involve the neuronal pathways responsible for spontaneous initiation of walking.

2. Materials and methods

2.1. Animals

Adult crayfish (*P. clarkii* (Girard)) of both sexes (8.5–9.8 cm in body length from rostrum to telson) were used in this study. They were obtained commercially and maintained in laboratory tanks, fed weekly on pellet foods (Kyorin, Hyogo). Prior to the tethering of animals for electromyographic recording, they were individually kept in small aquarium and subjected to 12 h:12 h light:dark (L:D) cycle for at least 2 weeks. The animals were then equipped with EMG harnesses and acclimatized to the experimental aquarium (Fig. 1A) for 3–5 days under LD cycle conditions preceding the circadian experiments. They could freely obtain food that was placed at the feeding place with a sufficient dose for the experimental period (10 days ∼). We tested 68 animals in this study. Especially in EMG analysis of circadian locomotor activity, only those 16 crayfish that were statistically judged to exhibit circadian