

Rhythms of Locomotion Expressed by *Limulus polyphemus*, the American Horseshoe Crab: II. Relationship to Circadian Rhythms of Visual Sensitivity

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Abstract. In the laboratory, horseshoe crabs express a circadian rhythm of visual sensitivity as well as daily and circatidal rhythms of locomotion. The major goal of this investigation was to determine whether the circadian clock underlying changes in visual sensitivity also modulates locomotion. To address this question, we developed a method for simultaneously recording changes in visual sensitivity and locomotion. Although every animal (24) expressed consistent circadian rhythms of visual sensitivity, rhythms of locomotion were more variable: 44% expressed a tidal rhythm, 28% were most active at night, and the rest lacked statistically significant rhythms. When exposed to artificial tides, 8 of 16 animals expressed circatidal rhythms of locomotion that continued after tidal cycles were stopped. However, rhythms of visual sensitivity remained stable and showed no tendency to be influenced by the imposed tides or locomotor activity. These results indicate that horseshoe crabs possess at least two biological clocks: one circadian clock primarily used for modulating visual sensitivity, and one or more clocks that control patterns of locomotion. This arrangement allows horseshoe crabs to see quite well while mating during both daytime and nighttime high tides.

Introduction

The brain of the American horseshoe crab, *Limulus polyphemus*, contains a circadian clock that modulates the sensitivity of the lateral eyes (reviewed in Barlow, 1983). At night, the clock activates efferent nerve fibers that innervate the lateral eyes, and this input, using a variety of mecha-

nisms, increases the sensitivity of the eyes to light by a factor of more than 100,000 (Barlow *et al.*, 1980; Kaplan and Barlow, 1980). The present study was designed to determine whether the same clock that controls changes in eye sensitivity also influences other behaviors, such as locomotion.

One of the most dramatic and well-understood behaviors expressed by *L. polyphemus* is mating, which takes place each year between about May and July (Rudloe, 1980, 1985; Cohen and Brockmann, 1983; Shuster and Botton, 1985; Barlow *et al.*, 1986; Brockmann, 1990, 2003). Horseshoe crabs approach appropriate beaches along the east coast of North America at high tide, females deposit eggs in the sand in very shallow water near the high water mark, and then males release sperm on the eggs. In some locations there is a tendency for *L. polyphemus* to mate during both day and night high tides (Cohen and Brockmann, 1983; Schaller and Watson, unpubl. data). However, in some areas, during certain times of the year, more horseshoe crabs mate during the high tides that occur at night (Rudloe, 1980). This pattern could be the result of larger high tides at night or of modulation—by the circadian clock that enhances visual sensitivity at night—of the processes that drive them to mate during high tides (Barlow *et al.*, 1986).

Recent laboratory investigations of horseshoe crab locomotion demonstrate that, although some animals are nocturnal, many have a strong tendency to be active during the day (Chabot *et al.*, 2004, 2007). Furthermore, in the laboratory, although many adult *Limulus* (Chabot *et al.*, 2004, 2007; Chabot *et al.*, 2008) and larvae (Rudloe, 1979; Ehlinger and Tankersley, 2006) express a strong endogenous tidal rhythm of locomotion, previous investigations show no indication of a tidal component to the rhythm of visual sensitivity. These findings suggest that in *Limulus*, rhythms

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Abbreviations: ERG, electroretinogram.

of locomotion and visual sensitivity are not tightly coupled; a major goal of this investigation was to test that hypothesis.

Many marine invertebrates, especially those that inhabit nearshore and intertidal habitats, exhibit rhythms that are often synchronized to tidal cycles (Palmer, 1974, 1995a, b; DeCoursey, 1983, 2004a, b). Some of these animals are most active during low tides, when they are out of water; others are most active at high tides. Laboratory studies have demonstrated that a variety of external cues can entrain these tidal rhythms, including inundation (Holmstrom and Morgan, 1983), hydrostatic pressure changes (Naylor and Atkinson, 1972; Reid and Naylor, 1990), turbulence (Enright, 1965; Klapow, 1972; Hastings, 1981), salinity (Taylor and Naylor, 1977; Reid and Naylor, 1990), and temperature cycles (Williams and Naylor, 1969; Holmstrom and Morgan, 1983). In a recent study, we demonstrated that cyclic changes in water depth were sufficient to induce and maintain tidal rhythms of locomotion in the horseshoe crab (Chabot *et al.*, 2008). In the current study, this technique enabled us to record visual sensitivity rhythms in animals that were reliably expressing a tidal rhythm of locomotion.

Despite the abundance of literature on tidal and circadian rhythms, there are relatively few studies concerned with the expression of both rhythms by the same animal. Like *Limulus* (Chabot *et al.*, 2007), *Carcinus maenas*, the green crab, expresses very clear tidal rhythms (Naylor, 1958), with maximum activity at high tide. Moreover, the activity of green crabs is greater during nighttime *versus* daytime high tides, indicating that a diurnal element is involved in modulating the rhythm. The crabs *Sesarma haematocheir* (Saigusa, 1988), *Sesarma pictum* (Saigusa, 1992), and *Sesarma reticulatum* (Palmer, 1990) exhibit a very similar pattern of behavior that is modulated by both tidal and light input. The theoretical model suggested by Naylor (1958) is that an endogenous circatidal clock controls the locomotor activity of the green crab and a separate circadian clock modulates the expression of the tidal rhythms. This is similar to the model proposed by Barlow *et al.* (1986) to explain variations in the number of horseshoe crabs observed spawning during the mating season. However, whereas Barlow *et al.* (1986) suggested that variations in tide heights, combined with a circadian clock, might influence the number of animals mating on a given high tide, they did not speculate about the existence of an internal tidal clock.

The goal of this study was to determine if the clocks controlling rhythms of locomotion and visual sensitivity interact with each other, yielding a tendency to (1) be more active at night or (2) have enhanced visual sensitivity during bouts of locomotion. This objective was addressed by simultaneously and continuously monitoring changes in visual sensitivity and locomotion. Visual sensitivity was monitored using a technique that allowed us to record electroretinograms (ERGs) from animals that were free to move, and thus express locomotion rhythms, while we also

manipulated the light/dark (LD) cycles. Locomotion was monitored using the same running wheels described in Chabot *et al.* (2004, 2007, 2008). We found that tidal rhythms of locomotion were expressed independently of the ongoing rhythm of visual sensitivity and that there were no changes in visual sensitivity during the increases in locomotion that occurred with a tidal rhythm.

Materials and Methods

Animals

All 35 specimens of *Limulus polyphemus* (Linnaeus) that were used in this study were collected from the Great Bay estuary, New Hampshire, between the fall of 2005 and the fall of 2006. They were collected from shallow water during mating season in the spring of 2006 and by divers, or with lobster traps, throughout the rest of the year. Most animals were held in wire-mesh storage containers placed on the bottom of the estuary near the University of New Hampshire Jackson Estuarine Laboratory, which allowed them to continue to experience tidal cues until they were used for experiments. All animals were adults, and 33 of the 35 animals used were males, because they fit best into the running wheels used to monitor activity (our unpublished studies indicate that small females do not differ from males in their locomotor rhythms).

Electroretinograms (ERGs)

Traditionally, ERGs in *Limulus* have been recorded from stationary animals in complete darkness (Barlow, 1983). For this study it was necessary to devise a technique that allowed us to record ERGs from walking individuals of *L. polyphemus* while they were exposed to a light/dark (LD) cycle. This was achieved, in part, by placing customized stimulation/recording chambers over a single lateral eye (Fig. 1A). The inner portion of each recording chamber consisted of a clear plastic well with a silver chloride wire inside (active electrode). It was secured over the eye, filled with sterile seawater, and closed with epoxy. The reference electrode was inserted through a small hole in the lateral carapace. All wires were insulated except for the tips. After the recording chamber was in place, a cylindrical black-plastic cover was secured over it to prevent light from impinging on the eye. A green LED attached to the top of this cover was used to deliver 300-ms light pulses every 30 s. To facilitate entrainment of subjects to ambient LD cycles, the other eye was not covered.

The output of the electrodes used to record the ERG was amplified and filtered with a Grass P511 preamplifier, and signals were recorded continuously using a Powerlab data acquisition system (ADInstruments, Colorado Springs, CO). Typical ERGs are presented in Figure 2. The stimulator portion of the Powerlab system was used to drive the

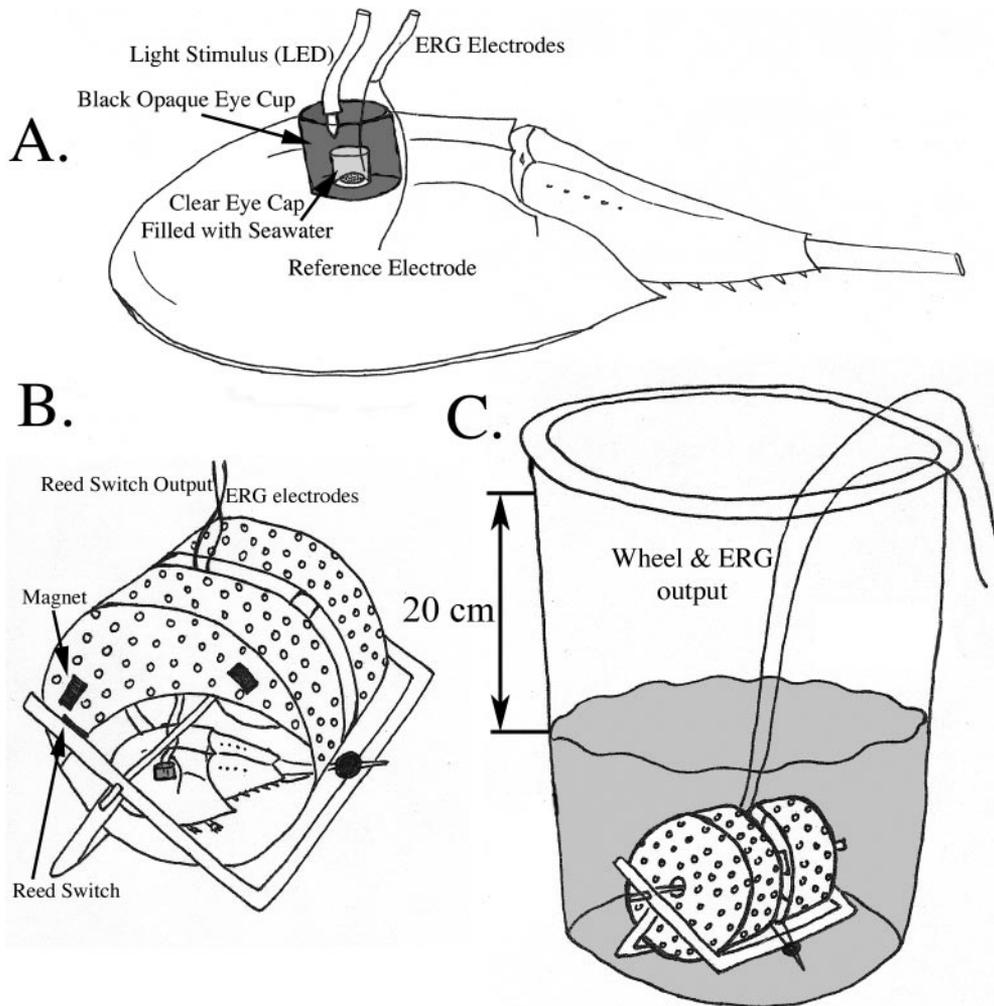


Figure 1. Apparatus used for recording electroretinograms (ERGs) and *Limulus* locomotion. (A) One lateral eye was fitted with an ERG recording electrode. The active portion of this electrode consisted of a clear plastic cup that was glued over the eye and filled with sterile seawater. A wire was inserted into this cup through a hole that was subsequently sealed with epoxy. The reference electrode was inserted into the lateral portion of the carapace. A larger black cup was sealed over the eye to keep it in constant darkness. A green LED was glued into the top of this cup and used to stimulate the eye with a 300-ms flash, every 30 s. (B) Once equipped with the ERG apparatus, horseshoe crabs were placed in a running wheel (Chabot *et al.*, 2004, 2007). The tail exited the wheel through a slit, as did the recording wires. Wheel rotations were monitored using magnets attached to the wheel and a reed switch mounted to the frame. (C) Once an animal was secured in a wheel, the wheel was placed inside a large black-plastic garbage can. When tide cycles were required, submerged pumps were used to pump water back and forth between two such recording chambers so one would become “low” while the other became “high.” The switch from one state to the other took about 30 min, with a total depth change of 20 cm (magnitude indicated with arrow).

LEDs with 3-V, 300-ms pulses every 30 s. To facilitate analyses of these data, the DVM (digital voltmeter) feature of the Chart software, ver. 4.2, was utilized to sample the amplitude of the ERG channels only during the time when stimuli were presented. The difference between the maximum and minimum voltage recorded during each of these 10-s intervals was calculated to yield a peak-to-peak ERG value for each interval, and these values were logged for further analyses.

Monitoring Limulus locomotion

The technique used for monitoring the locomotory activity of horseshoe crabs was described previously (Chabot *et al.*, 2004, 2007, 2008). In brief, horseshoe crabs were fitted with custom-made eye-cups to record ERGs and then placed inside a plastic running wheel (Fig. 1B). The wheel had a slit in the middle for the tail to pass through, and a disk or ball was placed on the end of the tail to prevent the horse-

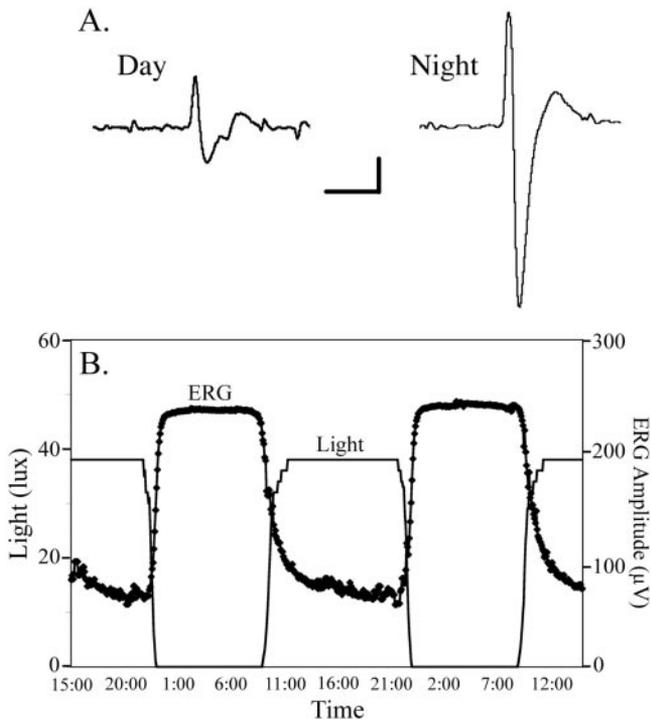


Figure 2. The amplitude of electroretinograms (ERGs) increased during the night. Typical day and night ERGs are shown in (A). Scale: 500 ms, 30 μ V. Changes in ERG amplitude during a 48-h period (data from different animal) are shown in (B). A modified light controller was used to gradually turn the lights on at dawn and off at dusk, over a period of 2 h. Note how changes in ERG amplitude anticipate dawn and dusk.

shoe crabs from pulling their tails back through the slits. Rotations of the wheel were monitored using a magnetic reed switch on the frame that was tripped each time one of two magnets secured to the wheel passed by it. The output of the reed switches was monitored with a second Powerlab data acquisition system that sampled at a much lower frequency than the system used to monitor the ERGs. Each change in voltage from a given running wheel, from 0 to 5 V and back again, indicated a one-half turn of the wheel.

Simulated tides and light

All recordings were made in a small room that was held at about 18 °C. During some of the initial studies ($n = 5$, Experiments 1 and 2 described in section entitled *Experimental protocol* below), animals were placed in aquaria that were maintained at about 15 °C, in the same room. However, as ambient water temperatures increased in the spring, it was not necessary to cool the water below 18 °C, so horseshoe crabs and wheels were placed individually in the bottom of large black-plastic garbage cans filled with seawater. Six different trials were conducted; during a typical trial we recorded from between 4 and 10 animals at the same time. Occasionally, either the electrodes or running

wheels failed to successfully record data for the entire duration of a trial, so data from those animals (11 of 35) were not used for subsequent analyses. For the remainder of the article, all values for the number of animals used in an experiment are stated in terms of the ones used for final data analyses ($n = 24$).

Containers were filled about one-half full with seawater that was diluted to a practical salinity of approximately 22, to mimic the salinity in the Great Bay estuary in the vicinity of the University of New Hampshire's Jackson Estuarine Laboratory, where most animals were collected. During some experiments, water levels were changed in a manner that simulated a tidal rhythm, similar to the method described in Chabot *et al.* (2008). Pairs of recording barrels were arranged so that, during each change in tides, water was pumped from one barrel into the other at a rate of about 600 ml/min, so that conditions for one animal changed from low to high tide while those for the other changed from high to low tide. The water level never dropped below the top of either of the running wheels because the intakes for the pumps were set at this level. Pumps were controlled by a timer that was set to advance 50 min each day. Water was bubbled continuously in each barrel, and water entered each barrel below the water level, through a pipe perforated with numerous holes. This arrangement minimized flow and sound disturbances during water level changes, making it more likely that animals were responding to water depth changes and not to other stimuli associated with the artificial tides. It took roughly 30 min to switch tides, and the total change in water level was about 20 cm. Changes in tides were monitored using a flotation system and a reed switch.

During the first four trials with 15 animals, lights were maintained on a 14:10 LD cycle using a timer and an incandescent light source that was positioned to reflect off the ceiling. During the day, light levels were between 80 and 120 lux (lumens/m²; $\sim 0.12 \mu\text{mol}/\text{m}^2/\text{s}$), and during the night it was completely dark. The light levels during the day were comparable to those in the Great Bay estuary at a depth of about 5 m. Due to the turbidity of the water in the estuary, light levels drop by almost an order of magnitude with each meter of depth. However, because the turbidity varies considerably with the season and tides and horseshoe crabs are found in depths ranging from 1 to 20 m in the estuary, these light values, obtained in May of 2006, are only an approximation of the light levels horseshoe crabs are exposed to in a typical day. When animals were held in continuous low light (LL), a 40-W light was used and light levels were comparable to the daytime levels cited above. In two trials with nine animals, a dusk/dawn system (Sun Up, Light Therapy Products, Plymouth, MA), modified by adding an additional timer, was used to simulate gradual changes in illumination during dusk and dawn transitions. This system caused a 40-W light facing the ceiling to slowly come on over a 2-h time period at dawn, and then turn off,

over the same time period, at dusk (Fig. 2). The LD cycle in these experiments was also 14:10 and roughly correlated with the ambient LD cycle.

Experimental protocol

Six separate experiments were conducted over the course of a year (28 Dec 2005–6 Nov 2006). In all experiments the

LD cycle was set to match, as closely as possible, the natural cycle at that time of year. However, because the experiments were conducted throughout the year, and we always used a 14:10 LD cycle, the match was not always tight. No attempt was made to match the natural tidal cycles, because half the animals were being exposed to high tide while the other half we being exposed to low tide. Finally,

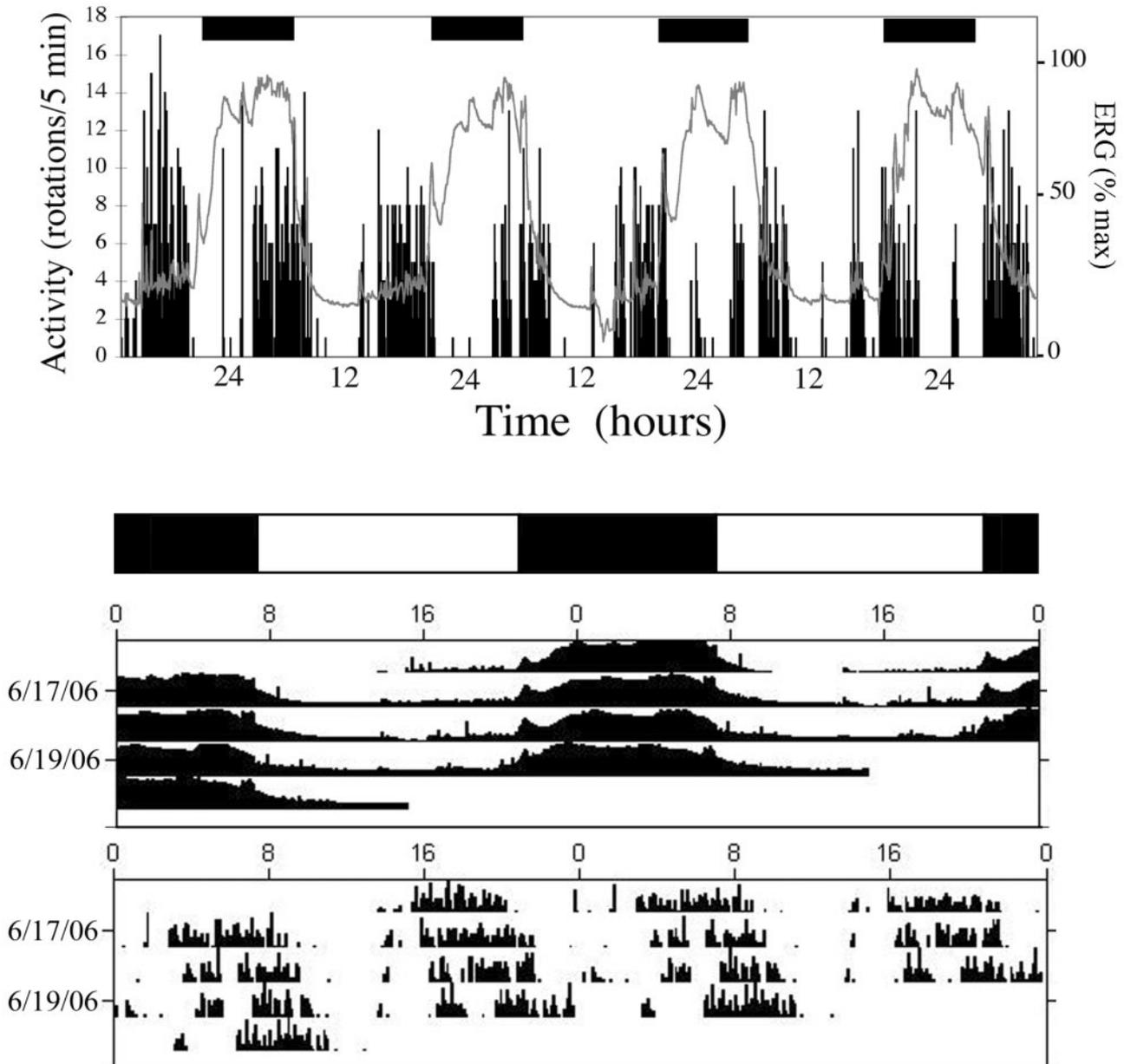


Figure 3. Tidal rhythms of locomotion and daily rhythms of eye sensitivity measured as electroretinogram (ERG) amplitude. All three panels are from the same animal, during the same time period (~4 days). The top panel is a traditional graph depicting the relationship between locomotion and ERG amplitude. The bottom two panels are double-plotted actograms of the same data, which makes it possible to visualize how the ERG rhythm is entrained to the LD cycle (top panel), while the tidal rhythm of locomotion drifts with respect to both the ERG rhythm and the LD cycle (bottom panel). The units for the horizontal axes are hours. The LD cycle is indicated by the light and dark bars.

in all the studies we omitted the first day from subsequent data analyses because the animals were adjusting to the experimental chambers.

Experiment 1 lasted 19 days (28 Dec 2005–16 Jan 2006), and the three animals were exposed to a LD cycle the entire time. Lights were turned on and off abruptly during this experiment, as well as the next three. In Experiment 2 (7 Mar 2006–9 Apr 2006), both animals were exposed to LD for 22 days and then LL for 10 days. Experiment 3 (10 May 2006–12 June 2006) was the first one that involved artificial tides, and the animals ($n = 2$) were exposed to LD throughout the study. After the initial 12 days in LD, they were exposed to tides for 9 days, and then after the tides were terminated, data was obtained for another 12 days. Experiment 4 (15 June 2006–21 July 2006) was nearly identical to Experiment 3, except we used a total of eight animals and the durations of the components of the study were slightly different (LD = 20 days, Tides = 12 days, Post Tides = 4 days). In both Experiments 5 and 6, the LD cycles were still 14:10, but LD transitions took place over a 2-h time period. In Experiment 5 (2 Aug 2006–12 Sept 2006), the four animals were treated like those in Experiments 3 and 4, except for the aforementioned difference in the LD cycle and some exposure to constant light (LL) at the end of the study. The duration of treatments were LD for 8 days, Tides for 8 days, Post Tides for 13 days, and LL for 12 days. In Experiment 6 (5 Oct 2006–6 Nov 2006), animals ($n = 5$) were immediately placed in LL conditions for 12 days before exposing them to LD for 13 days. Finally, they were exposed to artificial tides for the last 7 days of the study.

Data analyses

All activity and ERG data were analyzed both by visual analysis of the raw records and by using the DVM feature as described above. Data were typically averaged into 5-min bins for plotting and periodogram analyses (τ), as described in Chabot *et al.* (2004, 2007, 2008).

Results

Simultaneous ERGs and locomotor activity (wheel running) were successfully obtained from 24 out of an initial 35 individuals of *Limulus polyphemus* for more than 7 days. There was a dramatic and statistically significant increase in the peak-to-peak ERG amplitude of $311.9\% \pm 33.7\%$ ($n = 24$; mean \pm SEM; $P < 0.0001$, paired Student's *t*-test) at night, in comparison to daytime values (Fig. 2).

Simultaneous ERG and activity rhythms were recorded from 18 horseshoe crabs exposed to 14:10 cycles of light/dark (LD), practical salinities of 20–22, and no changes in water depth. All these animals had clear daily rhythms of visual sensitivity ($\tau = 24.21 \pm 0.16$ h), with maximum sensitivity at night (Fig. 2). Under an imposed LD cycle, these ERG rhythms were readily entrained, while in LL (4

animals), they free-ran from the point of entrainment, and the cycle duration increased ($\tau = 26.3 \pm 1.6$ h).

Although daily rhythms of visual sensitivity were very consistent, horseshoe crabs expressed several different patterns of locomotion. In most cases, bouts of walking were not synchronized with either the LD cycle or changes in the visual sensitivity of the eye. Rather, many animals expressed a tidal rhythm of locomotion (Fig. 3). Of the 18 animals exposed to a 14:10 LD cycle and no imposed tides, 8 expressed a tidal rhythm ($\tau = 12.96$ h \pm 0.89), 5 were most active at night ($\tau = 23.06$ h \pm 1.58), and the rest failed to exhibit any statistically significant rhythmicity.

A total of 16 horseshoe crabs were exposed to slow changes in hydrostatic pressure (water depth) that were designed to mimic the timing of natural tide cycles (12.4-h period, advancing by 50 min each day). Under these circumstances, 50% of the animals either shifted to a tidal pattern of activity or shifted their activity cycles so that they became synchronized to the imposed rhythm (Figs. 4, 5), while the rest of the animals did not express any significant rhythmicity. All 8 of the animals that were synchronized to the imposed tides were most active during the high tide portion of the tide cycle, and their bouts of activity exhibited no correlation to the LD cycle. When imposed tides were stopped, 6 of 8 animals that were still yielding good data continued to show entrained rhythms from the point of entrainment, for periods ranging from 3 to 9 days; indicating that the 12.4-h activity cycles were not simply a result of masking (Figs. 4–6).

On several occasions, apparent tidal rhythms appeared to become briefly entrained to sunset and sunrise, yielding a crepuscular pattern of activity for 2–3 cycles (Fig. 5). In the limited number of animals (Fig. 6) that expressed tidal rhythms when exposed to LL (4), none exhibited periods when activity was briefly synchronized to times when the ERG increased (“sunset”) or decreased (“sunrise”).

In 2 of the 5 animals that expressed a nocturnal pattern of activity, their ERG and activity rhythms were occasionally closely correlated (Fig. 7). Typically, periods of apparent coordination between ERGs and locomotion lasted for 2–3 days, and then the two rhythms became independent.

Discussion

The techniques used in this study allowed us to simultaneously record daily changes in visual sensitivity and in locomotion. As a result, it was possible to unequivocally demonstrate that most locomotor activity in horseshoe crabs is not synchronized with the circadian clock that controls visual sensitivity. The rhythm of eye sensitivity we recorded was very robust, it was always synchronized to imposed LD cycles, and it even maintained a circadian rhythm under conditions of continuous low light (LL) (Fig. 6). It also never varied when animals altered their pattern or degree of

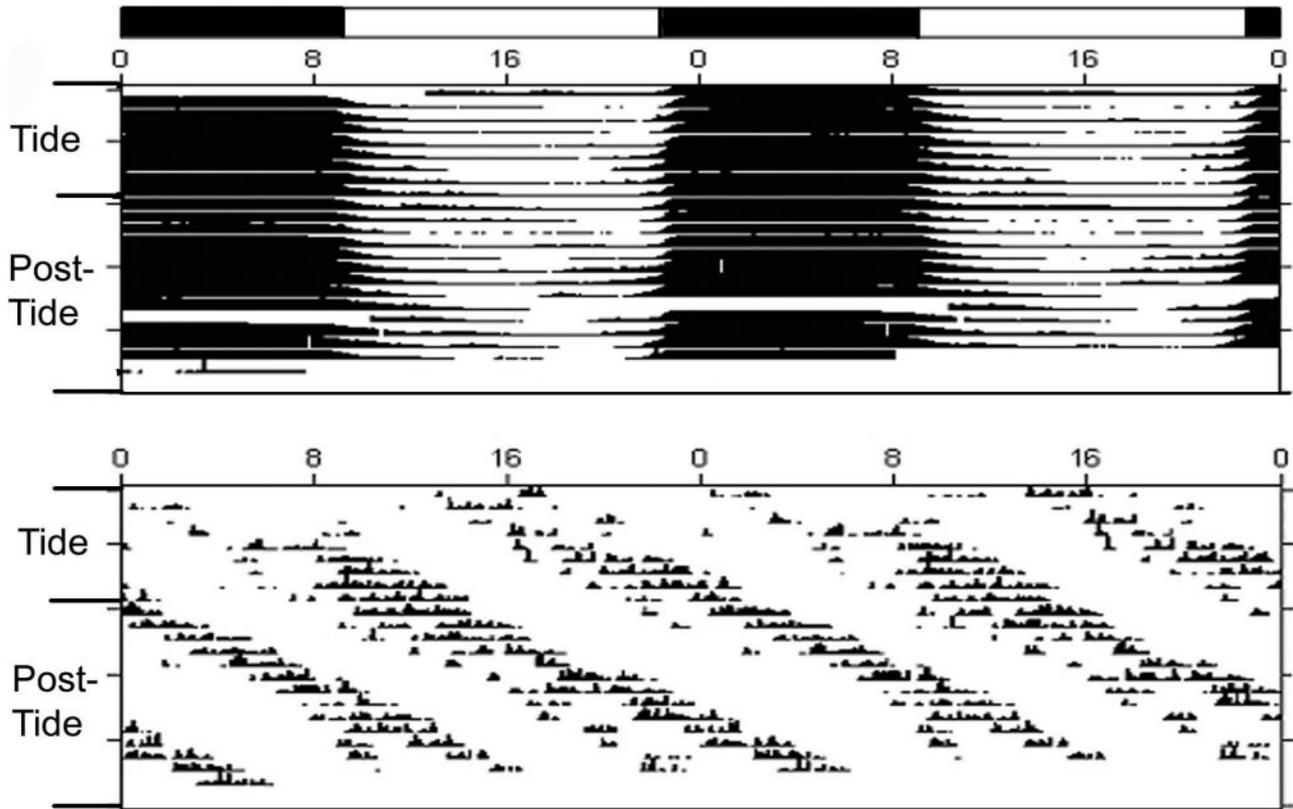


Figure 4. Locomotory activity rhythms can be synchronized to artificial tides and are independent of daily rhythms of visual sensitivity. This animal was first exposed to changing cycles of water depth with a period of 12.4 h, for 8 days; then the imposed tides were turned off and the water level was kept at a constant intermediate depth for an additional 13 days. Although locomotion became entrained to the imposed tides (bottom panel), the electroretinogram rhythm remained entrained to the LD cycle (top panel). The units for the horizontal axes are hours. The LD cycle is indicated by the light and dark bars.

locomotion. This indicates that the clock controlling eye sensitivity most likely does not receive input from the clock and other neural networks controlling locomotion or from the receptors involved in sensing the changes in water depth that helped entrain tidal rhythms of locomotion in this study.

Many of the horseshoe crabs used in this study expressed spontaneous tidal rhythms of locomotion in the laboratory that were clearly independent of the circadian rhythms of visual sensitivity. Furthermore, many of the animals that were not spontaneously expressing tidal rhythms of locomotion did so after being exposed to changes in water depth every 12.4 h. The changes in water depth provided, on the order of 20 cm (equivalent to 2 kPa, 20 mbar, or 0.28 psi), fell within the hydrostatic pressure detection range (0.5–50 mbar) previously documented for a number of other marine species (Morgan, 1984). Although recent studies suggest that some marine invertebrates have receptors that are capable of detecting small changes in water depth (Fraser, 2006), no candidate pressure receptors have ever, to our knowledge, been identified in *Limulus*. In the Great Bay estuary, the water depth changes by approximately 2–3 m

with each tide cycle, yielding fluctuations of about 250 mbar, which should be more than sufficient to synchronize rhythms of locomotion in the field, especially if the horseshoe crabs are in relatively shallow water (Chabot and Watson, unpubl. data). Thus, during mating season, we suggest that changes in water depth, and perhaps other tidally associated cues such as surge and changes in current strength and direction (Rudloe and Herrnkind, 1976, 1980), serve to entrain the population to tidal cycles and synchronize mating activity. Whether the horseshoe crabs continue to express tidal rhythms during the remainder of the year remains to be determined. Our laboratory studies demonstrate that they are capable of expressing tidal rhythms all year (Chabot *et al.*, 2004, 2007), but more field work is necessary to determine if they actually express tidal rhythms during times of the year when they are not mating and are often in deeper water.

There was considerable variability in the types of rhythms expressed by the *L. polyphemus* in this study and, at least in our hands, this is not unusual. In each laboratory study we have conducted, whether animals were under

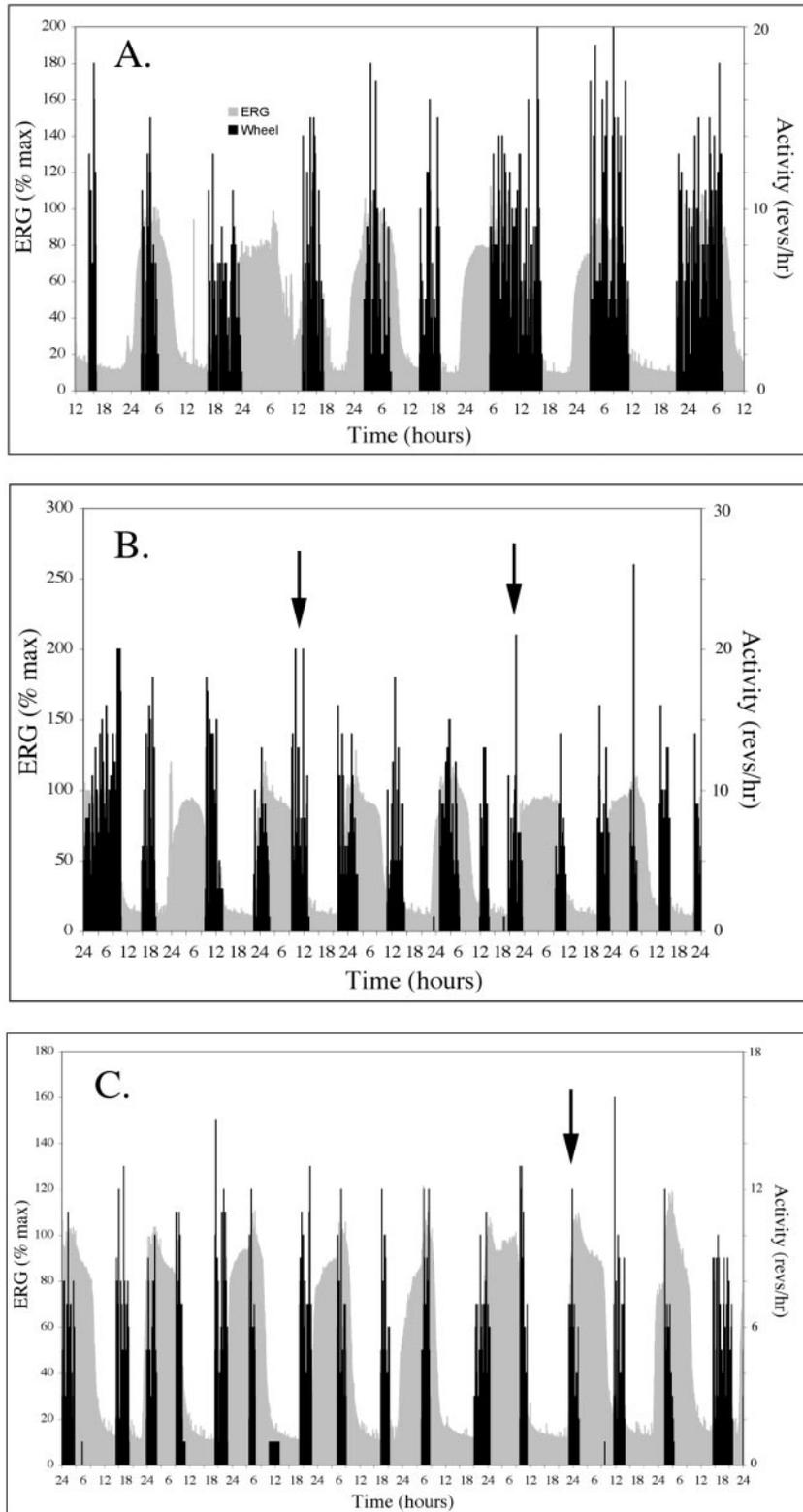


Figure 5. Locomotor rhythms can be entrained to an imposed tidal cycle and are occasionally sensitive to changes in light intensity. These data were obtained from an animal (Run 5, animal 2) put directly into the recording apparatus from the Great Bay estuary in August 2006. The top panel (A) shows spontaneous locomotion and electroretinogram activity in a 14:10 LD cycle. The animal was then exposed to an artificial tidal cycle (B), and activity became entrained to the imposed tides. Finally, when the tidal cues were stopped (C), the tidal rhythm continued. However, in both B and C it also appears as if there were times when bouts of locomotor activity were either advanced or delayed by sunset or sunrise (three examples indicated by arrows).

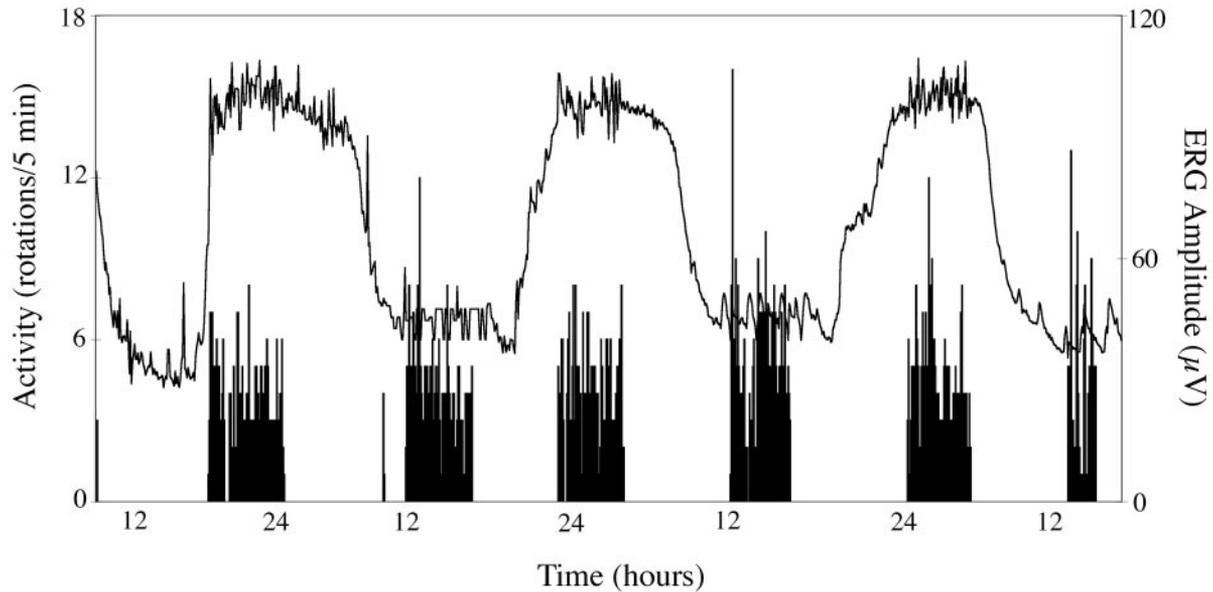


Figure 6. Independence of tidal rhythms of locomotion and circadian rhythms of visual sensitivity under conditions of constant light (LL). This animal was exposed to tidal cycles in LD conditions, and then tides were turned off and the lights were kept on continuously.

artificial or natural lighting conditions, inside or outside, freely moving or in running wheels, we found the same level of variability expressed by the horseshoe crabs in this study. Some animals tend to be nocturnal, some diurnal, some tidal, and some arrhythmic. Moreover, in this study, only about 50% of the animals became entrained to the artificial tides. There are several explanations for this degree of variability. First, the animals we used were all collected from the same basic region, but at different depths and different times of year, so they may have entered the laboratory more or less synchronized to the natural tides. Second, the changes in water depth we provided were quite small, and may have been close to the threshold level that the animals could sense. Finally, when exposed to artificial tides, half the animals were at low tide, while half were at high tide; therefore we did not attempt to coordinate the artificial tides to those they experienced before entering the laboratory. As a result, entrainment might have been difficult because, for a portion of the animals, tidal cues may not have been present at the optimal time in the tidal rhythm cycle.

In general, the influence of LD cycles or circadian clocks on the expression of tidal rhythms has received little attention (Palmer, 1995b). Horseshoe crabs, along with two intertidal crabs (*Carcinus maenas* and *Sesarma intermedia*), appear to be exceptions. In addition, fiddler crabs express both tidal and daily patterns of activity (Honegger, 1973a, b), and some crabs release larvae with a pattern indicative of both solar and tidal influences (Saigusa, 1988, 1992). Although the dominant finding of the present study is that changes in eye sensitivity were not synchronized with rhythms of activity, the two rhythms were loosely coordi-

nated in several instances. For example, for several days several animals expressed nocturnal patterns of locomotion that were closely linked with changes in visual sensitivity (Fig. 7). Unfortunately, our results do not allow us to determine whether, during these periods of time, the circadian clock controlling visual sensitivity was modulating locomotion, or whether the locomotor centers of the brain were being influenced by light input. However, in LL we never saw any indication that the two rhythms could influence one another. Thus, our current working hypothesis is that the circadian clock controlling eye sensitivity never influences locomotion, but light cycles can impact both rhythms.

In several cases we observed apparent tidal rhythms that drifted into synchronization with dawn and dusk, became locked to the LD rhythm for a couple of cycles, and then drifted out of synchronization (Fig. 5b, c). During our initial experiments in this study, lights were simply turned on and off on a 14:10 cycle, which could possibly cause masking and give rise to this type of pattern. In the last two experiments, however, transitions from light to dark took place over a period of 2 h, decreasing the likelihood that masking was a factor. Our working hypothesis is that, in *L. polyphemus*, light has a weak influence on the tidal clock. Similar sensitivity of a tidal rhythm to light has been reported previously in at least two species (Honegger, 1973a, b), and it may exist in other species as well. For horseshoe crabs, one wonders why it might be advantageous to have a tidal rhythm with sensitivity to light. One possibility is that in bays and estuaries, characterized by turbid water that limits the penetration of light, light levels fluctuate with each tide and thus provide a redundant tidal zeitgeber.

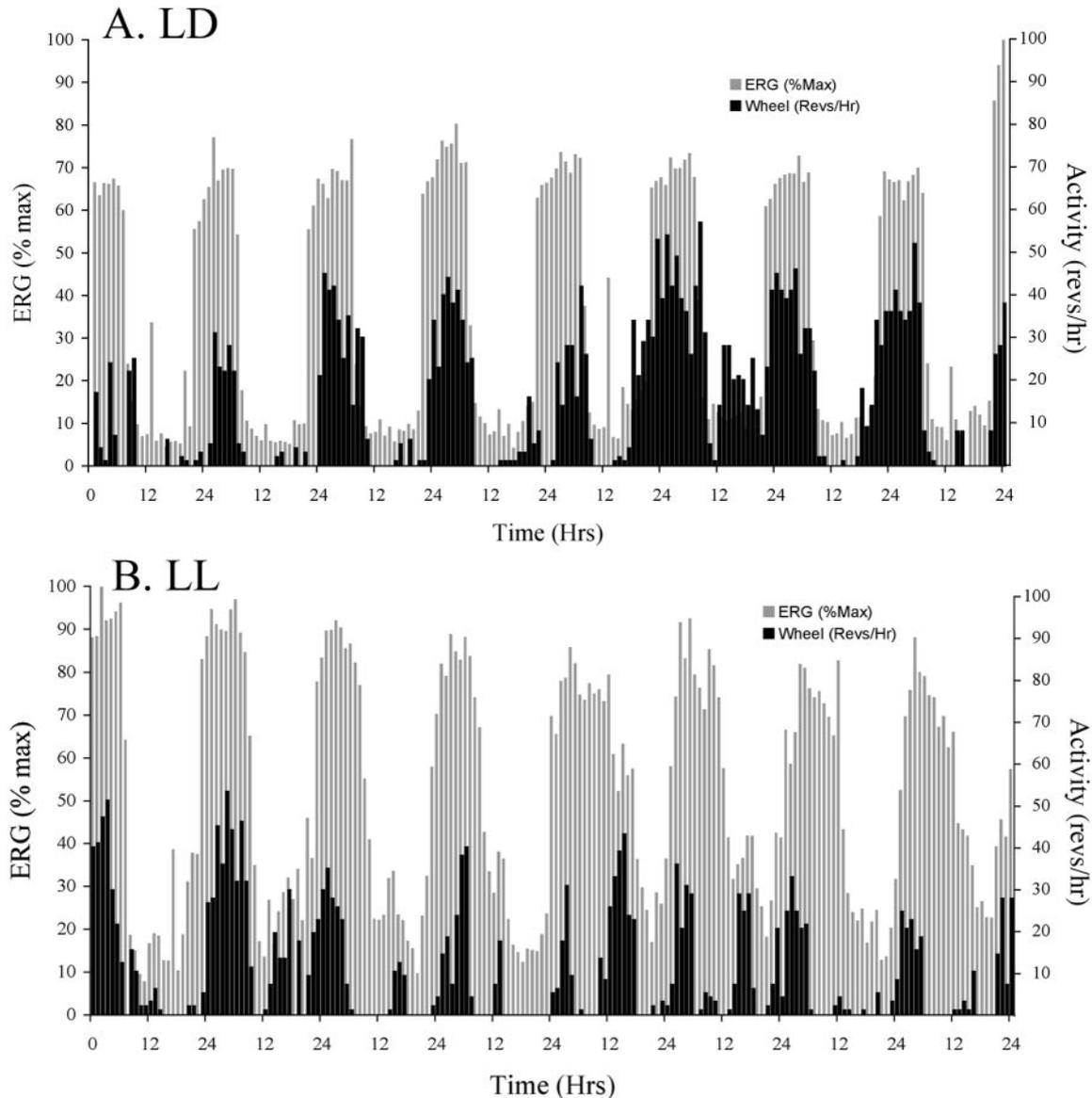


Figure 7. Coordination of visual sensitivity rhythm (electroretinogram) and locomotion (running wheel rotations). (A) This animal (Run 2, animal 2) was on a 14:10 LD cycle, and changes in visual sensitivity from 22 March 2006 to 29 March 2006 were closely associated with locomotion. (B) When exposed to constant light (LL), the degree of coordination gradually declined.

For many years, it was generally accepted that horseshoe crab mating is most robust during the full and new moons and more animals mate at night than during the day (Rudloe, 1980, 1985; Shuster, 1982; Cohen and Brockmann, 1983). The discovery of a clear circadian rhythm of visual sensitivity (Barlow, 1983) that provides greatly improved visual capabilities at night reinforced the view that horseshoe crabs are somewhat nocturnal and thus more likely to mate during nighttime high tides. However, recent data indicate that, at least in New England waters, large numbers of animals mate during both the daytime and nighttime high tides (Barlow *et al.*, 1986; Schaller and Watson, unpubl. data), and in the

laboratory a significant proportion of the horseshoe crabs prefer to be most active during the day (Chabot *et al.*, 2007). Therefore, our current view is that a circadian rhythm of visual sensitivity evolved to allow horseshoe crabs to see equally well during all high tides, whether they occur during the day or night.

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