MELATONIN AND CIRCADIAN RHYTHMS IN THE GREEN SHORE CRAB, *CARCINUS MAENUS*

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The vertebrate pineal hormone melatonin has recently been detected in many non-vertebrate organisms, including arthropods, plants, dinoflagellates and bacteria (Tilden, A.R. et al., *J. Insect Physiol.* 40:775-780, 1993; Dubbels, R. et al., *J. Pineal Res.* 18:28-31, 1995; Hardeland, R., *Experientia*, 49:614-622, 1993; Manchester et al., *Cell. Molec. Biol. Res. 21:307-313*, 1995). In vertebrates, one of melatonin's primary roles is the entrainment of circadian rhythms - activity patterns, metabolism, thermoregulation, and other physiological and behavioral cycles - to photoperiod (Reiter, R.J., *Experientia* 49:654-664, 1993). Melatonin is produced in response to environmental darkness, and its production is inhibited by light; this pattern of production appears to be highly conserved throughout phylogeny, from mammals to bacteria. Crustaceans, however, are an exception in that melatonin production increases during the day (Withyachumnarnkul, B. et al., *Comp. Biochem. Physiol. A* 102:703-707, 1992; Tilden, A.R. et al., *J. Pineal Res.* 23:142-147, 1997).

The role of melatonin in non-vertebrates has not been extensively studied, and a connection to other circadian functions has not been established. In a previous study (Tilden et al., 1997), we found that exogenously-administered melatonin increases the rate of limb regeneration in the fiddler crab *Uca pugilator*. Long-day photoperiods also increase the rate of regeneration; therefore, melatonin may be mediating long-day effects on regeneration and other physiological processes in crustaceans.

In the present study, we are examining the relationship between melatonin and activity cycles in crustaceans. *Carcinus maenus* was selected for its pronounced circadian activity rhythm: in laboratory conditions this species loses the tidal component of its activity pattern and becomes primarily nocturnal (Naylor, E., *J. Anim. Ecol.* 31:601-609, 1962). To establish baseline, control activity cycles, we acclimatized male crabs for two weeks to a 12L:12D photoperiod (12 hours of light and 12 hours of dark) and a constant 21 ± 1° C. Then 20 crabs were placed individually in 20 x 8 x 3 cm clear plastic boxes fitted on opposite sides with pairs of infrared emitters and sensors; the sensors were connected to a computer-interfaced system which monitored crab locomotion over 8 days.

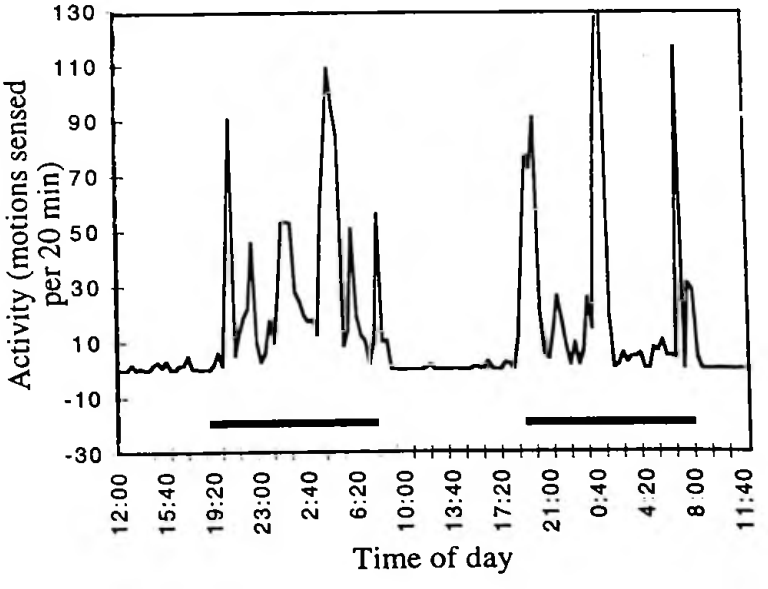

Fig. 1. Activity in *C. maenus* over a two-day period; dark bars represent darkness.

Figure 1 shows activity cycles over the last two days; these days were representative of the entire 8-day period. The data represent mean activity levels of 20 crabs as the number of times the light beam was interrupted over a 20 minute interval. The activity pattern is clearly nocturnal, with increased levels of locomotion beginning near the onset of darkness and ending near the onset of light each day. The dark bars in the figure represent darkness.

Currently we are measuring melatonin levels in the eyestalks of *C. maenus*: crabs were acclimatized to the same conditions as in the first part of the study. Eyestalks, the putative locus of melatonin in crabs (Tilden et al., 1997), were then removed every three hours over 24 hours, ten eyestalk pairs per sampling period. The eyestalks were then homogenized and melatonin was extracted with chloroform. Melatonin levels are currently being measured with HPLC and radioimmunoassay to determine the time of peak melatonin production.

Next, we will examine the influence of exogenously-administered melatonin on activity patterns in *C. maenus*: melatonin will be administered to crabs during times of low endogenous production and during various photoperiod regimes such as constant darkness or light. Their activity patterns will then be compared with those of control animals to determine whether melatonin is involved in the entrainment of circadian locomotor rhythms in this species.

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