Retinal sensitivity in the fiddler crab *Uca pugilator*: evidence of a circadian rhythm and influence of melatonin

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Melatonin's influence on vertebrate circadian rhythms has been extensively studied, and recent attention has focused on the localized production and signaling of melatonin in the vertebrate retina[3]. Specifically, melatonin entrains dark-adapting responses of the retina. Our lab has previously shown that melatonin is produced in the crustacean eyestalk[2], and we suspect phylogenetically conserved roles of melatonin across phyla.

The crustacean eye responds to changes in illumination by adjusting light sensitivity through several mechanisms[1] including the migration of extra-photoreceptor shielding pigments, such that the retina is shielded during the day, and changes in sensitivity and neural activity of the photoreceptors themselves. Some of these changes are direct responses to light, similar to the vertebrate pupillary response. Other changes are the result of circadian changes in cell physiology that persist in the absence of light.

Fiddler crabs were acclimated to an ambient photoperiod of 16L:8D for two weeks and were then placed in constant darkness during the experiment. Electroretinograms (ERGs) were recorded using a chlorided silver wire in contact with the cornea through a saline bathing medium. Crabs were given four brief flashes of light (approximately 40 msec each) from a camera flash every three hours beginning three hours after they were placed in darkness at 1200h. Flashes were administered for a 72-hr period. Data were recorded using an A-M Instruments model 1700 differential AC amplifier interfaced with a PowerLab computer interface and Scope software. Data were recorded from 10 control crabs and 9 crabs that received an infusion of melatonin (5 ng/g) administered in the seawater bathing the animals.

Currently, we have analyzed two components of the data at two time-points, mid-photophase (1500h) and mid-scotophase (2400h) of the third day of recording: first, we measured the amplitude of the ERG signal from the first flash of the series of 4 flashes. Second, we measured the duration of the ERG response from the same first flash.

Table 1. Amplitude and duration of ERG response in fiddler crabs, controls and melatonin-treated, in response to a brief pulse of light. Values represent the mean $\pm$ SEM.

Time of Day	Amplitude (mV)	Duration (msec)
Control Mid-photophase	1.15 ± 0.22	61 ± 15
Control Mid-scotophase	1.92 ± 0.25	123 ± 27
Melatonin Mid-photophase	1.24 ± 0.36	69 ± 21
Melatonin Mid-scotophase	2.34 ± 0.33	$199 + 28$

Control crabs had a significantly greater response to light during subjective darkness than subjective light in both amplitude ($t = 2.61$; $P < 0.01$) and duration ($t = 3.29$; $P < 0.01$). Melatonin-treated crabs did not differ significantly from controls during mid-photophase in amplitude or duration; they did differ significantly from controls during mid-scotophase in duration ($t = 2.24$; $P < 0.01$) but not in amplitude. These preliminary analyses indicate an endogenously-driven circadian rhythm of retinal sensitivity in fiddler crabs that persists in conditions of constant darkness, and a potential role of melatonin in dark-adaptation. The lack of responsiveness to melatonin during subjective photophase suggests a circadian rhythm of sensitivity to melatonin.

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