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# Photosensitive neurogenic heart of the isopod crustacean *Ligia exotica*

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The heart of animals is regulated through the central nervous system in response to external sensory stimuli. We found, however, that the adult neurogenic heart of the isopod crustacean *Ligia exotica* has photosensitivity. The beat frequency of the isolated heart decreased in response to a light stimulus. Magnitude of the response was stimulus intensity dependent and the heartbeat frequency decreased to less than 80% of the dark value during illumination of the white light with an intensity of 6.0 mW $cm^{-2}$. The spectral sensitivity curve of the heart photoresponse peaked at a wavelength around 520 nm. In response to 530 nm monochromatic light, the relationship between light intensity and response magnitude was linear and the threshold intensity was $7.26\times10^{12}$ quanta $cm^{-2}\,s^{-1}$. Bursting activity of the cardiac ganglion, which is located in the heart and acts as the cardiac pacemaker deceased in frequency in response to illumination by white light. This fact suggests that the heart photoresponse of *L. exotica* results from the photosensitivity of the cardiac ganglion neurons. The photoresponse of the heart therefore contributes to regulation of cardiac output in addition to other regulatory systems.

**Keywords:** photosensitivity; heart; crustacea; *Ligia exotica*

## 1. INTRODUCTION

The heart of animals is a pump of haemolymph circulation and changes in the heartbeat affect directly all metabolic activities of organs and tissues. Therefore, the heartbeat is controlled neurally and hormonally by the central nervous system and regulation of the heartbeat in response to changes in animal's internal and external conditions has been reported in various kinds of invertebrates (reviewed by Maynard 1960; Prosser 1973; McMahon *et al.* 1997). For changes in environmental light conditions, cardiac responses to visual sensory inputs via neural and neurohormonal pathways of the central nervous system have been reported in crustaceans (Hara 1952; Miyazaki *et al.* 1985; Li *et al.* 2000) and insects (Campan 1972; Thon 1982). Moreover, cardiac regulation by photosensitive neurons in the central nervous system is suggested in the chelicerate *Limulus polyphemus* (Mori & Kuramoto 2004; Mori *et al.* 2004).

Thus the cardiac responses to external sensory stimuli via the central nervous system have been used as a useful model for sensory reception of the animal (cf. Larimer & Tindel 1966; Angioy *et al.* 1998). However, we found that the adult neurogenic heart of the isopod crustacean *Ligia exotica* responds directly to light stimulus by a decrease in beat frequency. There are some early reports suggesting the presence of photosensitivity in the myocardium (in the branchiopod crustacean *Daphnia magna*, Schultz 1928, cited by Maynard 1960; in the mollusc *Helix pomatia*, Arvanitaki & Chalazonitis 1947). Recently, in the cultured heart of zebrafish, photosensitivity of the myocardial cells was shown by photoentrainment of rhythmic expression of circadian clock genes (Whitmore *et al.* 2000). To our knowledge, however, there have been no reports that show physiological evidence for the presence of a photosensitive heart. The presence of the photosensitive heart implies that external light affects directly all metabolic activities of organs and tissues of the animal by changing the cardiac output aside from any possible regulation through the central nervous system.

The aims of the present study are to elucidate characteristics of the heart photoresponse and to determine the site of photoreception in the heart. Some of the results have appeared in abstract form (Miyamoto *et al.* 2003).

## 2. MATERIAL AND METHODS

### (a) *Animals and preparations*

We used adult males and females of the littoral isopod crustacean *L. exotica*, 25–35 mm in body length. They were collected at Pacific seashores Kamogawa (lat 35°1′ N, long 140°1′ E) and Shimoda (lat 34°4′ N, long 138°6′ E), Japan and kept in the laboratory at room temperature (22–26 °C). More than 100 specimens were used for the experiments.

The heart of *L. exotica* is located dorsally, extending over the posterior half of the body. The heart is tubular and the wall consists of a single layer of striated muscle cells. The cardiac ganglion composed of six neurons runs longitudinally along the midline of the ventral surface of the dorsal heart wall and gives off nerve branches to the myocardial cells. Details of the heart anatomy and the method of dissection were described previously (Yamagishi & Ebara 1985; Yamagishi & Hirose 1997).

After the ventral carapace was cut off, all the viscera and central nervous system were removed with the head.

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The heart was kept intact in the pericardial cavity and isolated together with the dorsal carapace. We mainly used heart preparations of this semi-isolated type. We also used heart preparations that were completely isolated from the body. The preparation was pinned in the experimental chamber, which was continuously perfused with aerated physiological saline solution of the following composition (mM): NaCl 557, KCl 14, $CaCl_2$ 25, $MgCl_2$ 21, $Na_2SO_4$ 4.5 and Tris 5 (Yamagishi & Ebara 1985). The pH was adjusted to 7.4 using HCl. In some experiments, 10 μM tetrodotoxin (TTX; Wako) was added to the saline.

### (b) *Electrical recordings*

Contraction of the heart (mechanogram of the heartbeat) was recorded in the semi-isolated preparation pinned dorsal side up in the experimental chamber. Part of the dorsal carapace was removed over the middle region of the heart. The dorsal suspensory ligament, which was left attached to the heart, was tied using fine thread and was connected to the mechano-electric transducer (TB-611T, Nihon Kohden). Impulse activity of the cardiac ganglion was extracellulary recorded from the ganglionic nerve branches. The anterior or posterior nerve branch of the cardiac ganglion was cut at the peripheral side and the proximal cut end of the nerve was sucked into the suction electrode. The signals were displayed on a cathode ray oscilloscope (SS-7810, Iwatsu), stored on magnetic tape with a data recorder (PC204Ax, Sony), digitalized with an analogue-digital converter (PowerLab/4SP, AD Instruments) and analysed with CHART software versions 4.2 and 5.0.2 (AD Instruments).

### (c) *Light stimulation*

White or monochromatic light from a 500 W xenon arc lamp (UXL-500D-O, Ushio) was used for illumination. A collimated beam of light produced with quartz lenses was passed through a heat-absorbing filter (IRA-25S, Toshiba). Monochromatic light was produced by passing the light beam through one of a set of nine narrow-band interference colour filters (MIF-S, Optical Coatings Japan; half-band width less than 13 nm). The light-emitting end of the light-guide (diameter 5 mm) was positioned approximately 3 cm above the preparation. The light intensity was altered with quartz neutral density filters (Optical Coatings Japan), with which the monochromatic light was adjusted to contain an equal number of photons. The intensity of white light was measured with a power metre (TPM-310, Gentec Electro-Optics, Inc) and that of monochromatic light was measured with a silicon photodiode calibrated with a photoelectric tube (S1227-1010BR, Hamamatsu Photonics). The maximum intensity of white light was 6.0 mW $cm^{-2}$ and the intensity range of monochromatic light was between $5.87\times10^{12}$ and $7.03\times10^{14}$ quanta $cm^{-2}$ $s^{-1}$ at the surface of the preparation. The timing and duration of light stimulation were determined by an electro-magnetic shutter (LS6, Uniblitz) controlled by an electronic stimulator (SEN-7103, Nihon Kohden).

## 3. RESULTS

### (a) *Photoresponse of the heart*

We examined the effect of light on the semi-isolated heart by recording a mechanogram of the heartbeat. In constant darkness, the heart beat regularly at a frequency of 148.0 ± 19.6 beats $min^{-1}$ (mean ± s.d., $n=65$). When white light illuminated the heart, the heartbeat frequency decreased immediately and remained low during illumination (figure 1). In this case, illumination of 6.0 mW $cm^{-2}$ white light for 20 s caused a decrease in the heartbeat frequency from 169 to 129 beats $min^{-1}$. When illumination ended, the heartbeat frequency recovered to the control dark value quickly. Sometimes, the heartbeat frequency became a little faster transiently than the control value just after the end of strong light illumination (cf. figure 1). There was little change in the amplitude of the heartbeat in response to light stimulus.

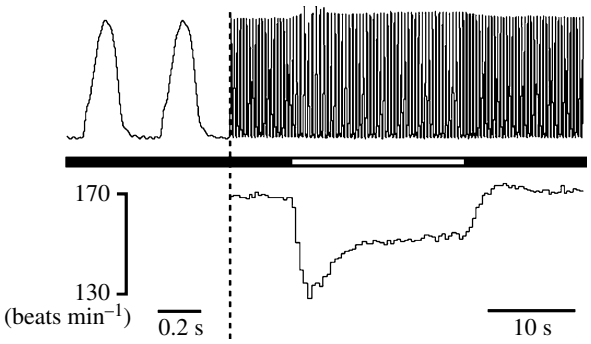


Figure 1. An example of a photoresponse of a semi-isolated heart. Mechanogram of heartbeat (upper trace) and heartbeat frequency (beats $min^{-1}$, lower trace) are shown. Black and white parts of the horizontal bar indicate the periods of constant darkness and illumination, respectively. White light of 6.0 mW $cm^{-2}$ was applied during for 20 s. Note the different time-scale in the left portion separated by the vertical dashed line.

We also measured beat frequency changes in response to light stimulus in the heart isolated completely from the body by visual observations or electrical recordings of the myocardial activity. We found no noticeable differences in the light response of the heart (decrease in beat frequency) between semi-isolated and completely isolated heart preparations. In addition, we examined the effects of indirect light illumination to estimate the magnitude of the heart photoresponse in living animals. When white light was applied through the dorsal carapace, the magnitude of the heart photoresponse decreased to approximately 80% of the response to direct illumination (not shown).

To examine characteristics of the heart photoresponse, we applied white light of various intensities to the heart. The magnitude of the response to white light increased with increasing light intensity (figure 2). Under weak light, the beat frequency decreased monotonically to a steady-state level (figure 2*a*(i),(ii)). As the light intensity was increased, the response became biphasic with an initial trough phase and a subsequent steady phase (figure 2*a*(iii),(iv)). To determine the relationships between light intensity and response magnitude (% change in the heartbeat frequency) in the trough and steady phases, the control heartbeat frequency was defined as the mean frequency during 5 s period just before the onset of light stimulus. The heartbeat frequency of the steady phase was defined as the mean frequency during 5 s period between 12 and 17 s after the onset of light stimulus, because the duration of the trough phase was almost constant (cf. figure 2*c*). The response magnitude in the trough phase increased as the light intensity was gradually increased to 6.0 mW $cm^{-2}$, at which point the heartbeat frequency had decreased to approximately 80% of the dark value (figure 2*b*). The response magnitude in the steady phase was also increased

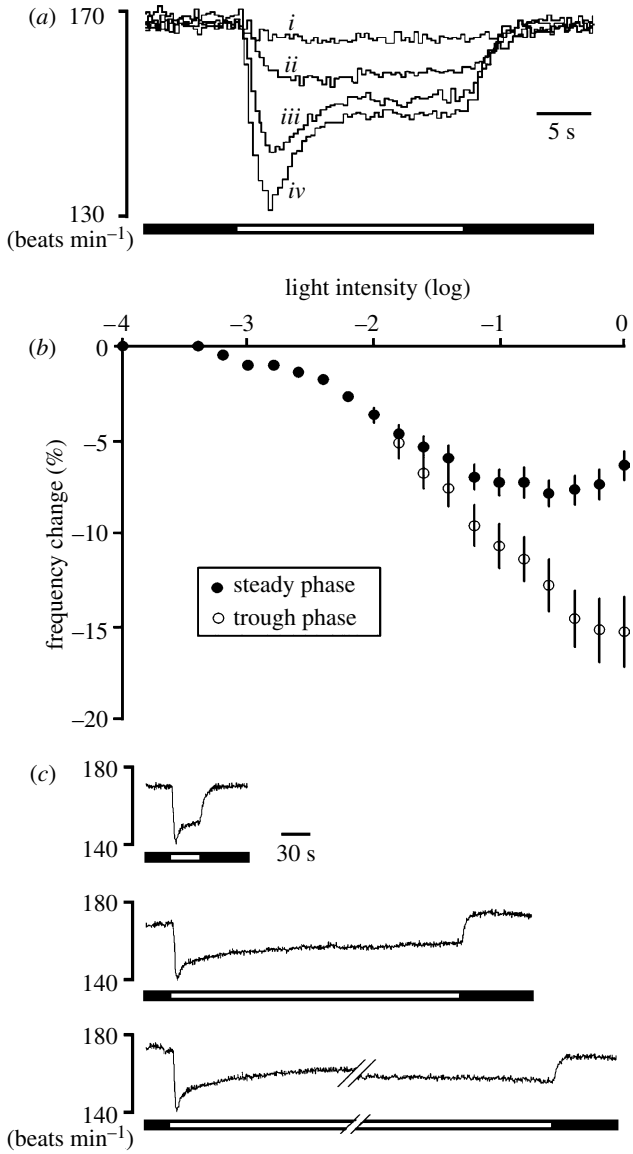


Figure 2. Characteristics of the heart photoresponse. (*a*) Photoresponses to white light of four different intensities are superimposed. Light intensities in trace *i*, *ii*, *iii* and *iv* are $9.5\times10^{-3}$, $9.5\times10^{-2}$, $9.5\times10^{-1}$ and 3.8 mW cm$^{-2}$, respectively. Black and white parts of the horizontal bar indicate the periods of constant darkness and illumination, respectively. White light was applied for 20 s. (*b*) Relationships between light intensity and magnitude of photoresponse in the steady phase (closed circle) and in the trough phase (open circle). White light was applied at eighteen different intensities and the maximum intensity (log 0) was 6.0 mW cm$^{-2}$. Each data point shows mean$\pm$s.e. ($n=4$). (*c*) Photoresponses to white light of three different durations. Light intensity was 6.0 mW cm$^{-2}$. Black and white parts of the horizontal bar indicate the periods of constant darkness and illumination, respectively. Durations of illumination were 30 s (upper trace), 5 min (middle trace) and 63 min (bottom trace).

depending on the light intensity, but it began to saturate to strong light stimuli. We further examined the heart photoresponse to strong white light with various durations. Regardless of the duration of illumination, the trough phase of the response always lasted 7–8 s in duration at the beginning of the following steady phase (figure 2*c*). In contrast, the steady phase of the response prolonged with increasing the duration of illumination and persisted during illumination for more than 1 h (figure 2*c*, bottom trace).

### (b) *Spectral sensitivity of the heart photoresponse*

To obtain a spectral sensitivity curve of the heart photoresponse, nine monochromatic lights each of which has a wavelength between 430 and 590 nm were tested on the heart. These monochromatic lights were applied at various intensities in the range between $5.87\times10^{12}$ and $7.03\times10^{14}$ quanta cm$^{-2}$ s$^{-1}$. To all the monochromatic lights of this intensity range, the heart photoresponse was monophasic and no trough phase appeared. For the five monochromatic lights tested (450, 470, 530, 570 and 590 nm), each response magnitude was plotted against each light intensity (logarithmic unit). The relationships between response magnitude and light intensity of these monochromatic lights were almost linear and parallel with each other (figure 3*a*). Similar results were obtained for the other four monochromatic lights tested (430, 490, 510 and 550 nm; not shown).

Based on the above results, we determined a spectral sensitivity curve of the heart photoresponse. The spectral sensitivity was defined as a reciprocal of the light intensity at which each of the nine monochromatic lights evokes a response with equal magnitude. Then sensitivity at each monochromatic light was normalized against that at 530 nm monochromatic light and plotted as a function of wavelength. The spectral sensitivity curve obtained peaked at a wavelength around 520 nm (510–530 nm; figure 3*b*). The minimum intensity of 530 nm monochromatic light to induce the heart photoresponse, the threshold light intensity, was $7.26\times10^{12}$ quanta cm$^{-2}$ s$^{-1}$. In addition, selective light adaptation experiments in which 380 or 610 nm monochromatic light was applied for 10 min showed that magnitude of the responses decreased but no spectral response shift occurred (not shown).

### (c) *Photoreceptive site in the heart*

The heart of adult *L. exotica* is neurogenic and the cardiac ganglion acts as a pacemaker of the heartbeat (Yamagishi & Ebara 1985; Yamagishi & Hirose 1997). To determine a photoreceptive site in the heart, we examined the effect of strong white light on cardiac ganglion activity. The frequency of periodic bursting activity of the cardiac ganglion decreased in response to the light stimulus (figure 4*a*). The photoresponse of the cardiac ganglion was similar to that of the heartbeat with trough and steady phases. Moreover, simultaneous recording of the heartbeat and the cardiac ganglion activity showed that the heartbeat frequency decreased in association with the frequency decrease of the cardiac ganglion activity (figure 4*b*). These results suggest that the heart photoresponse results from the photosensitivity of the cardiac ganglion neurons.

In the heart of adult *L. exotica*, the myocardium also acts as a secondary pacemaker and the heartbeat changes reversibly from neurogenic to myogenic upon application of TTX which suppresses the cardiac ganglion activity (Yamagishi & Hirose 1997). To confirm the neural origin of the heart photoresponse, we examined the effect of white light on the myogenic heartbeat induced by TTX. The heart photoresponse observed in the neurogenic heartbeat abolished in the myogenic heartbeat induced by 10 μM TTX and recovered after washout of TTX (figure 5).

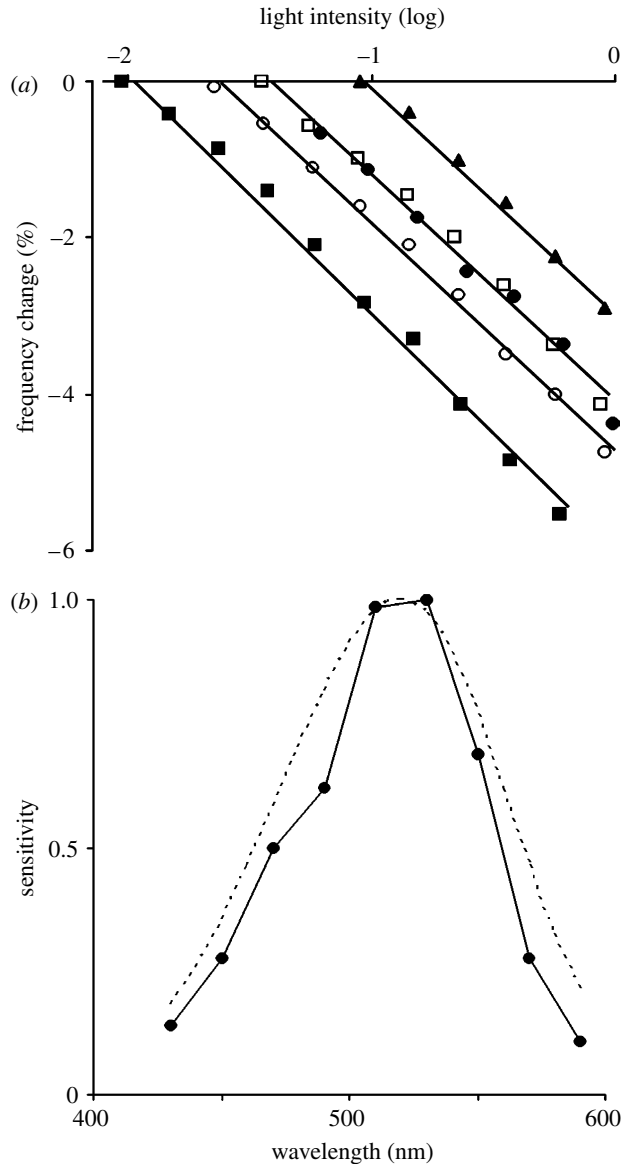


Figure 3. Heart photoresponse to various monochromatic lights. (*a*) Relationship between intensity of monochromatic light and magnitude of photoresponse. Five monochromatic lights of various intensities were successively applied. The maximum intensity (log 0) was $7.03\times10^{14}$ quanta $cm^{-2}$ $s^{-1}$. Each data point shows the mean of six specimens. Closed circle, 450 nm; open circle, 470 nm; closed square, 530 nm; open square, 570 nm; closed triangle, 590 nm. (*b*) Spectral sensitivity curve of the heart photoresponse. Each data point shows the mean of six specimens. The dashed line shows the vitamin A1-based visual pigment absorption curve obtained from the template by Govardovskii *et al.* (2000).

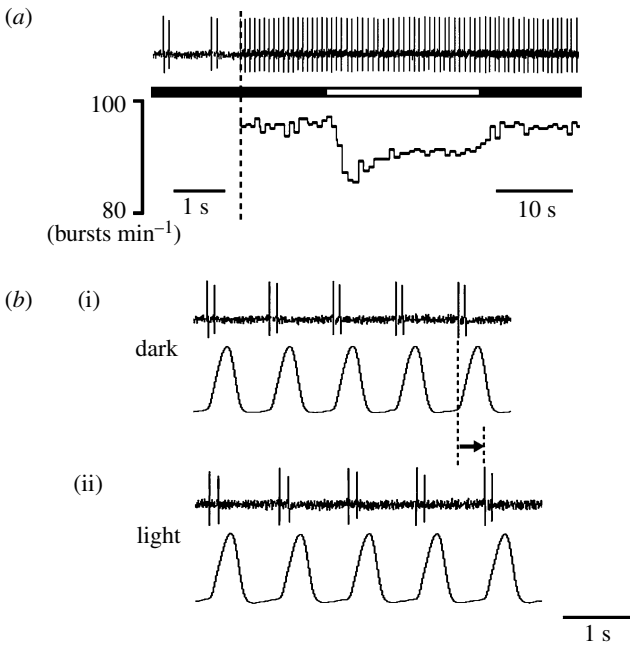


Figure 4. Photoresponses of the cardiac ganglion. (*a*) Spontaneous burst discharge of the cardiac ganglion (upper trace) and frequency of the burst discharge (lower trace) are shown. Black and white parts of the horizontal bar indicate the periods of constant darkness and illumination, respectively. White light of 6.0 mW $cm^{-2}$ was applied for 20 s. Note the different time-scale in the left portion separated by the vertical dashed line. (*b*) Burst discharge of the cardiac ganglion (upper trace) and mechanogram of the heartbeat (lower trace) recorded simultaneously in (i) constant darkness and (ii) during illumination with white light at intensity of 6.0 mW $cm^{-2}$. The timing of the fifth burst discharge was delayed during illumination (dashed lines and an arrow).

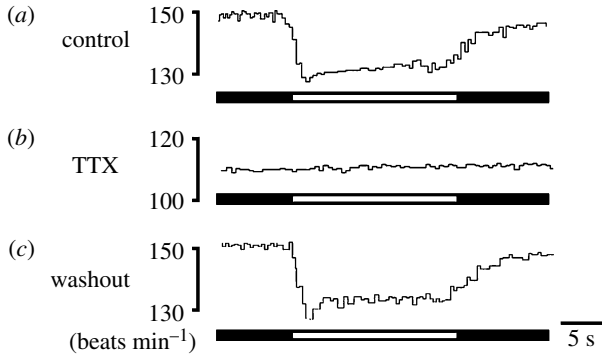


Figure 5. Effects of tetrodotoxin (TTX) on heart photoresponse. To change reversibly the heartbeat from neurogenic to myogenic, 10 μM TTX was applied. Photoresponses (*a*) before application, (*b*) during application and (*c*) after washout of TTX are shown. Black and white parts of the horizontal bar indicate the periods of constant darkness and illumination, respectively. White light of 6.0 mW $cm^{-2}$ was applied for 20 s.

## 4. DISCUSSION

### (a) *Photosensitivity of the heart*

The results of the present study show that the isolated adult heart of the isopod crustacean *L. exotica* responds to illumination by white light by decreasing its beat frequency (figure 1). Moreover, the magnitude of the heart photoresponse depended on the intensity (figure 2) and wavelength (figure 3) of the light stimulus. These results suggest that the adult heart of *L. exotica* is photosensitive.

The heart of adult *L. exotica* is basically neurogenic; the cardiac ganglion acts as the primary pacemaker with the myocardium acting as a secondary pacemaker (Yamagishi & Hirose 1997). Periodic bursts of the cardiac ganglion, each of which consists of two or three impulses, induce the action potentials of the myocardium through excitatory junctional potentials (Yamagishi & Ebara 1985; Sakurai *et al.* 1998). The frequency of bursting activity of the cardiac ganglion decreased in response to light (figure 4*a*) and a heartbeat always followed each

ganglionic burst discharge (figure 4*b*). Moreover, the heart photoresponse vanished reversibly when the heartbeat was reversibly changed from neurogenic to myogenic by application of TTX (figure 5). These results suggest that the cardiac ganglion is responsible for the heart photoresponse in adult *L. exotica*. This idea is supported by the following observations. The cardiac pacemaker of *L. exotica* is transferred from the myocardium to the cardiac ganglion during juvenile development (Yamagishi & Hirose 1997), and the heart photoresponse appears in association with the cardiac pacemaker transfer during development (Miyamoto & Yamagishi 2004). We conclude that photosensitivity in the heart of *L. exotica* is not in the myocardium but in the cardiac ganglion.

### (b) *Photoresponse characteristics of the cardiac ganglion*

The *Ligia* cardiac ganglion is composed of six neurons that lie longitudinally along the midline of the inner surface of the dorsal heart wall (Alexandrowicz 1952; Yamagishi & Ebara 1985). All the cardiac ganglion neurons are glutamatergic motoneurons with pacemaker property and discharge synchronously periodic bursts of impulses via electrical connections among the neurons (Yamagishi & Ebara 1985; Sakurai *et al.* 1998). Partial illumination by white light to the anterior or posterior half of the heart resulted in a smaller decrease in the heartbeat frequency than that by the illumination to the whole heart (H. Miyamoto 2005, unpublished observations). These results suggest that all the six ganglion neurons are photosensitive and their summed response is reflected in the frequency change of the heartbeat.

The pacemaker bursting activity of the *Ligia* cardiac ganglion decreased in frequency by injection of a hyperpolarizing current into a cardiac ganglion neuron (Yamagishi & Ebara 1985). A frequency decrease of the cardiac ganglion activity in response to light (figure 4*a*) predicts generation of a hyperpolarizing photoreceptor potential in the cardiac ganglion neurons. Extraocular photosensitive neurons have been found in the central nervous system of many invertebrates, but most of which produces a depolarizing receptor potential (reviewed by Musio 1997). Photosensitive neurons producing a hyperpolarizing receptor potential are found in the central nervous system of the molluscs, *Onchidium verruculatum* (Hisano *et al.* 1972; Gotow & Nishi 2002) and *Aplysia californica* (Brown & Brown 1973; Andersen & Brown 1979). These hyperpolarizing receptor potentials, however, are monophasic and no biphasic receptor potentials predicted from the heart response are found yet. It is interesting to examine the membrane potential responses of the cardiac ganglion neurons to light and these investigations are now in progress.

The spectral sensitivity curve of the heart photoresponse peaked at a wavelength around 520 nm. Maximum responses in green light have been reported in extraocular photoreceptors of various invertebrates (Felisberti *et al.* 1997; Nishi & Gotow 1998). Moreover, the spectral sensitivity curve of the *Ligia* heart photoresponse well resembles those reported in crayfish extraocular photosensitive neurons (Sandeman *et al.* 1990) and *Ligia* green photoreceptor cells, one of the three types photoreceptor cells found in the compound eye (Hariyama *et al.* 1993). On the other hand, the spectral sensitivity curve of the heart photoresponse seems to fit well to the vitamin A1-based visual pigment absorption curve (dashed line in figure 3*b*) obtained from the template by Govardovskii *et al.* (2000). In addition, selective light adaptation experiments resulted in no spectral shift in the heart photoresponse (see §3). These results suggest that the spectral response of the cardiac ganglion is caused by one type of visual pigment. The cardiac ganglion neurons of *L. exotica* may have a visual pigment similar to that of the green photoreceptor neurons in the compound eye.

When the monochromatic light of the most effective wavelength of 530 nm was applied, the threshold light intensity to induce the heart photoresponse was $7.26\times10^{12}$ quanta $cm^{-2}\,s^{-1}$ (figure 3*a*). This threshold value is comparable with those obtained in extraocular photosensitive neurons of other invertebrates (Larimer 1967; Andersen & Brown 1979; Nishi & Gotow 1998).

### (c) *Physiological role of the heart photosensitivity*

Most of the extraocular photosensitive neurons found in the invertebrate central nervous systems are inter-neurons making synaptic connections with many other neurons; so, their functions are still uncertain. In contrast, the photosensitive cardiac ganglion neurons of *L. exotica* are motoneurons of the myocardium with a pacemaker function. Therefore, changes in the pacemaker activity of the cardiac ganglion neurons by light stimulus affect directly the cardiac outflow in the hemolymph circulation.

*Ligia exotica* is a largely diurnally active seashore animal, whose terrestrial habitat is well lit by sunlight (Hariyama *et al.* 1986). In addition, white light whose intensity is much weaker than sunlight (approx. 100 mW $cm^{-2}$) could induce the heart photoresponse, even when it was applied passing through the dorsal carapace (see §3). These facts suggest that, in living animals, sunlight passing through the dorsal carapace directly affects metabolic activities of all tissues and organs by changing the cardiac output, aside from any possible regulation through the central nervous system. The physiological role of the heart photosensitivity is uncertain in the present study. Investigations on its function in the diurnal activity and circadian rhythm of living animals are required.

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