



## **Cardiac Nervous System in the Ostracod Crustacean *Vargula hilgendorffii***

Authors: Ando, Yumiko, Matsuzaki, Osamu, and Yamagishi, Hiroshi

Source: Zoological Science, 18(5) : 651-658

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.18.651>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Cardiac Nervous System in the Ostracod Crustacean *Vargula hilgendorffii*

Yumiko Ando, Osamu Matsuzaki and Hiroshi Yamagishi\*

*Institute of Biological Sciences, University of Tsukuba, Tsukuba,  
Ibaraki 305-8572, Japan*

---

**ABSTRACT**—We examined morphologically innervation of the heart of the ostracod crustacean *Vargula hilgendorffii*. The heart is single chambered and composed of a single layer of myocardial cells characterized by localization of myofibrils at the epicardial side. A nerve net in the heart was determined by vital staining with methylene blue. Electron microscopy revealed that a single neuron situated on the outer surface of the dorsal heart wall sends an axon into the heart wall. The axon of the dorsal neuron is branched widely and forms many neuromuscular junctions on the myocardial cells. A pair of extrinsic nerves, each of which contains several axons, enters the heart bilaterally and forms numerous nerve terminals on the dorsal neuron and myocardial cells, while no synaptic structures were found in the nerve terminals. The results suggest that the heart of *V. hilgendorffii* is neurogenic, with a single cardiac neuron having both pacemaker and motor functions.

---

## INTRODUCTION

Many investigations on the heartbeat pacemaker mechanism of crustaceans, mainly of decapods, have led to generalization that the crustacean heart is neurogenic, with the cardiac ganglion acting as the pacemaker (reviewed by Maynard, 1960; Prosser, 1973; McMahon *et al.*, 1997). Recently, however, different cardiac pacemaker mechanisms have been reported. In the branchiopod *Triops longicaudatus*, no neurons are present in the heart and the heart exhibits myogenic heartbeats arising from the endogenous rhythmic activity of the myocardium (Yamagishi *et al.*, 1997). In the heart of adults of the isopod *Ligia exotica*, both the cardiac ganglion and myocardium have endogenous rhythmic activity and the cardiac ganglion acts as the primary pacemaker entraining the myogenic rhythm to the neurogenic one *via excitatory* neuromuscular junctional potentials (Yamagishi and Hirose, 1997). Thus, the cardiac pacemaker mechanism of crustaceans appears to be more diverse than previously thought.

On the other hand, some diversity in the cardiac ganglion system has been reported among crustaceans (reviewed by Maynard, 1960). The cardiac ganglion of isopods is composed of 6 (*Ligia exotica*, Suzuki, 1934; *Ligia oceanica*, Alexandrowicz, 1952) or 12 (*Bathynomus doederleini*, Kihara and Kuwasawa, 1984) neurons, and each of the ganglion neurons has both pacemaker and motor functions (Yamagishi and Ebara, 1985; Sakurai *et al.*, 1998). In stomatopods, the cardiac ganglion is

composed of 15 motor neurons (Alexandrowicz, 1934) with several anterior neurons giving the pacemaker function (Watanabe *et al.*, 1967). In many decapods, the cardiac ganglion is composed of 9 neurons (Alexandrowicz, 1932) and these neurons are differentiated into 4 small pacemaker neurons and 5 large motor neurons (reviewed by Hagiwara, 1961; Hartline, 1979). However, the phylogenetic diversity of the cardiac ganglion system in crustaceans has not been investigated thoroughly (reviewed by Wilkens, 1999).

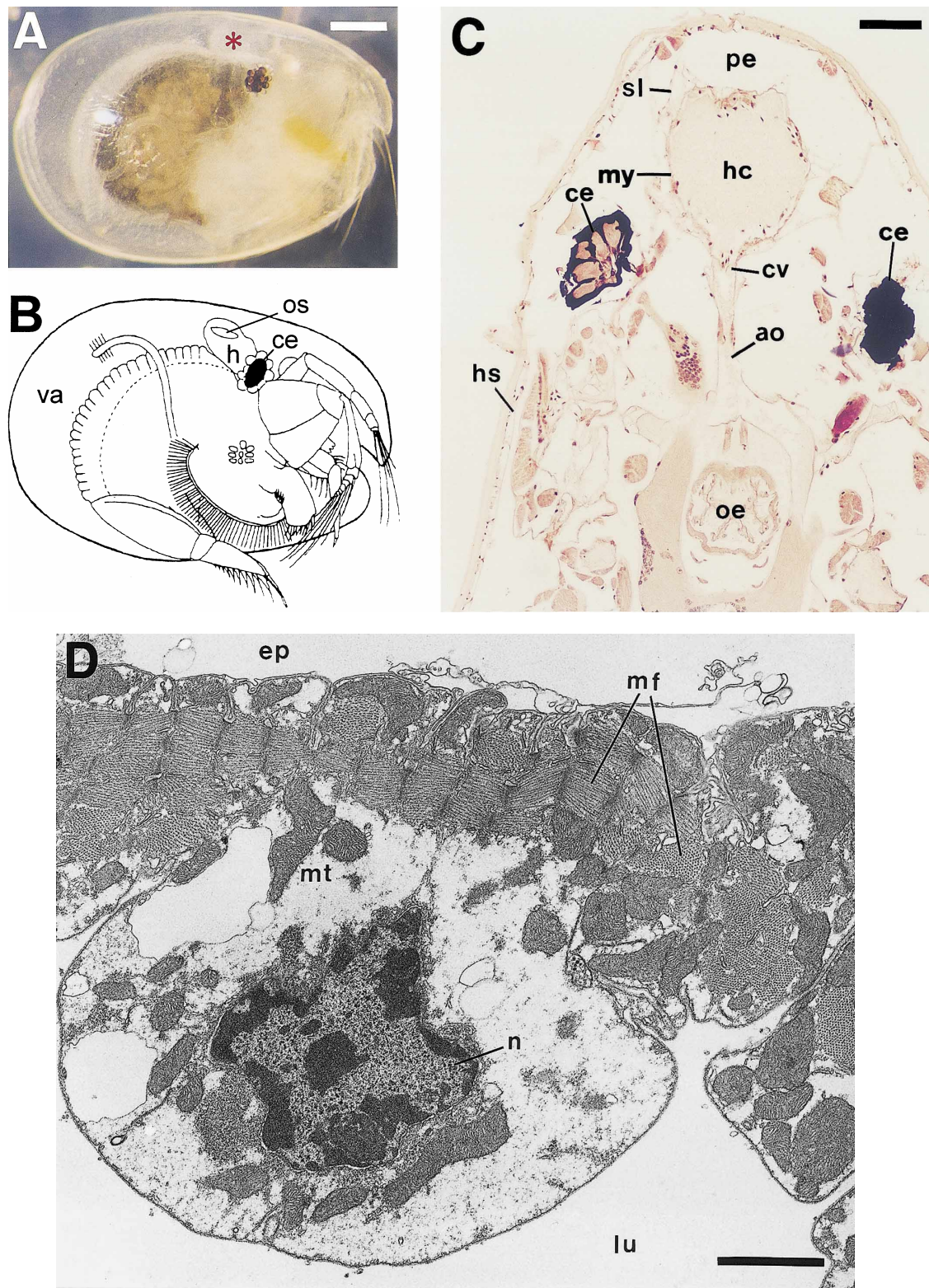
In the ostracod *Gigantocypris mülleri*, Cannon (1940) has reported anatomically the presence of only a single neuron in the heart, perhaps indicating that ostracods have the simplest cardiac ganglion system among crustaceans. The nervous components of the heart were not examined in the light and electron microscopic studies of two ostracods, *Cypridina norvegica* (Christ, 1982) and *V. hilgendorffii* (Abe and Vannier, 1995). In order to explore phylogenetic diversity of the cardiac ganglion system in crustaceans, we examined innervation of the heart of *V. hilgendorffii*. The results suggest that the heart of *V. hilgendorffii* is neurogenic, with a single cardiac neuron acting as the pacemaker. Some of the results presented here have been appeared previously in abstract form (Ando *et al.*, 1998).

## MATERIALS AND METHODS

### Animals

Adult males and females of the marine ostracod crustacean *V. hilgendorffii* (Müller) were collected using baited traps at the seashores at Tateyama and Shimoda, Japan. They were kept in an artificial seawater (ASW; SWING Hi-marine, Hi-pet Co.) aquarium in the laboratory, at room temperature, for two to three months. Over a hundred

\* Corresponding author: Tel. +81-298-53-6670;  
FAX. +81-298-53-6614.  
E-mail: yamagishi@biol.tsukuba.ac.jp



**Fig. 1.** Anatomy of *Vargula hilgendorffii*. **A**, Light micrograph of a living specimen of *V. hilgendorffii*. Lateral view of the body from the right side is shown. Internal structures of the body can be observed through translucent valves. The heart (asterisk) is located dorsally just behind the compound eye. **B**, Anatomy of *V. hilgendorffii* drawn after removing the right valve. ce, compound eye; h, heart; os, ostium; va, left valve. **C**, Light micrograph of a transverse paraffin section through the heart, aorta and compound eyes. ce, compound eye; cv, cardioarterial valve; hc, heart cavity; he, hemolymph sinus; my, myocardium; oe, oesophagus; pe, pericardium; sl, suspensory ligament. **D**, Electron micrograph of a transverse section of myocardial cells. The heart wall is composed of a single layer of myocardial cells. Each myocardial cell is globular shaped and surrounded by epicardium and basal lamina. ep, epicardium; lu, lumen of the heart; mf, myofibrils; mt, mitochondrion; n, nucleus. Scale bars represent 500  $\mu$ m in A, 200  $\mu$ m in C and 2  $\mu$ m in D.

specimens, 3.0 to 3.5 mm in body length, were used for experiments.

#### Vital staining with methylene blue

The body of *V. hilgendorffii* is covered with translucent left and right valves (Fig. 1A). One of the valves of the specimen was fixed to a small sheet of Parafilm with an adhesive. Then, the specimen was placed in the Silgard seated chamber filled with ASW and was fixed to the bottom of the chamber by pins through the Parafilm sheet. The heart was exposed by breaking the upper side valve and isolated by removing tissues around the heart. Several drops of 0.5% methylene blue (Wako) solution were added into the chamber. Nerves stained on the heart were observed under a stereomicroscope and drawn.

#### Light and electron microscopy

Fresh specimens were fixed with Bouin's solution at room temperature for 3 to 7 days and dehydrated in graded ethanol-n-butanol series. Then the specimens were embedded in paraffin (melting point 56 to 57°C) and prepared for microtome serial sections (5 to 10 µm in thickness). The sections were stained with Mayer's hematoxyline and eosin, and then observed with a light microscope.

For electron microscopy, the heart with some surrounding tissues was isolated and prefixed for 1 hr at room temperature and 2 hr at 4°C with 2.5% glutaraldehyde in ASW. After washing, the preparation was postfixed for 1.5 hr at 4°C with 2% osmium tetroxide in distilled water and dehydrated through a graded ethanol series. Then the preparation was cleaned in n-butyle glycidyl ether (QY-1) and infiltrated in an n-butyl glycidyl ether/epoxy resin (Epon 812 or Ager 100) mixture. Embedding was achieved in flat molds filled with epoxy resin at 60°C. Ultrathin sections were cut with a diamond knife (Diatome). The sections on Formvar-covered single hole grids were stained in saturated uranyl acetate solution and examined with a JEOL JEM-1010 transmission electron microscope at 80 kV.

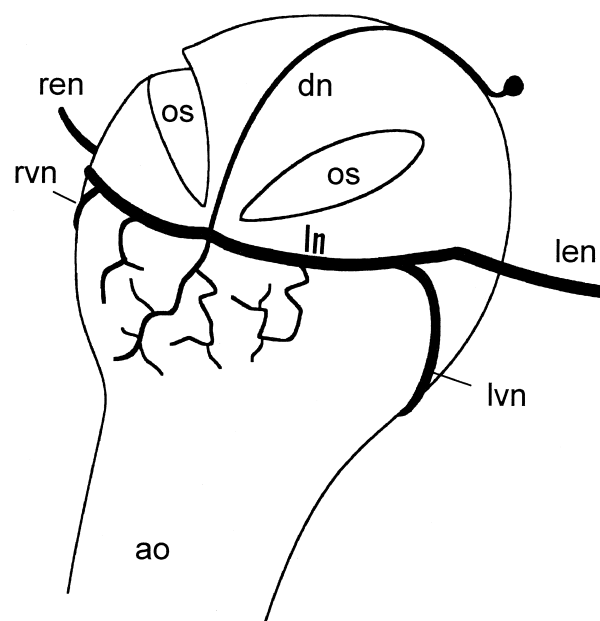
## RESULTS

#### Structure of the heart

The single chambered heart of *V. hilgendorffii* is globular (approximately 250 µm in diameter) and is located dorsally behind the lateral eyes (Fig. 1 A and B). The heart is suspended in the pericardial cavity by suspensory ligaments and has two ostia and one ventral aorta (Fig. 1C). The heart wall consists of a single layer of globular myocardial cells surrounded by epicardium and basal lamina. Electron microscopy revealed that bundles of myofibrils are localized at the pericardial side and cross each other in each myocardial cell (Fig. 1D).

#### Cardiac nervous system

Fig. 2 is a schematic drawing of the cardiac nervous system of *V. hilgendorffii* as observed by vital staining with methylene blue. The cardiac nervous system consists of three main nerves which we refer as the dorsal, lateral, and ventral nerves. The dorsal nerve runs transversely along the midline of the dorsal heart wall and the posterior end of the dorsal nerve projects out of the heart forming a neuron-like globular structure. The dorsal nerve connects at the anterior portion of the heart with the lateral nerve. The lateral nerve runs horizontally at the central portion of the anterior heart wall and a pair of extrinsic nerves joins the nerve bilaterally. The ventral nerves are derived from the lateral nerve at the left and right sides of the heart and extends to the ventrolateral regions of the heart.



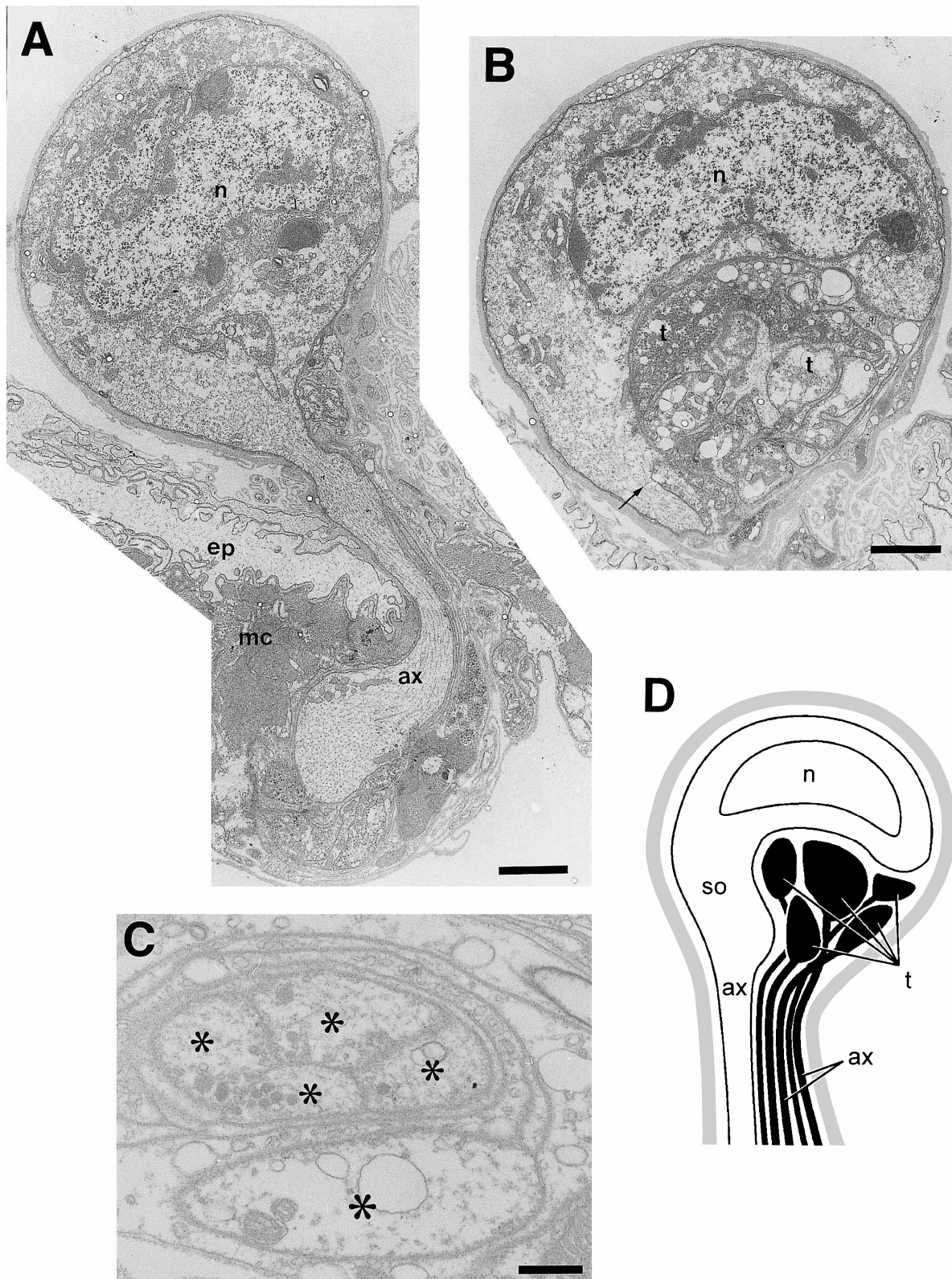
**Fig. 2.** Cardiac nervous system of *V. hilgendorffii*. Schematic drawing of the cardiac nervous system after staining with methylene blue; laterofrontal view of the heart from the right side. ao, aorta; dn, dorsal nerve; len, left extrinsic nerve; ln, lateral nerve; lvn, left ventral nerve; os, ostium; ren, right extrinsic nerve; rvn, right ventral nerve.

In the anterior region of the heart some thin nerves are derived ventrally from the lateral nerve and branch finely.

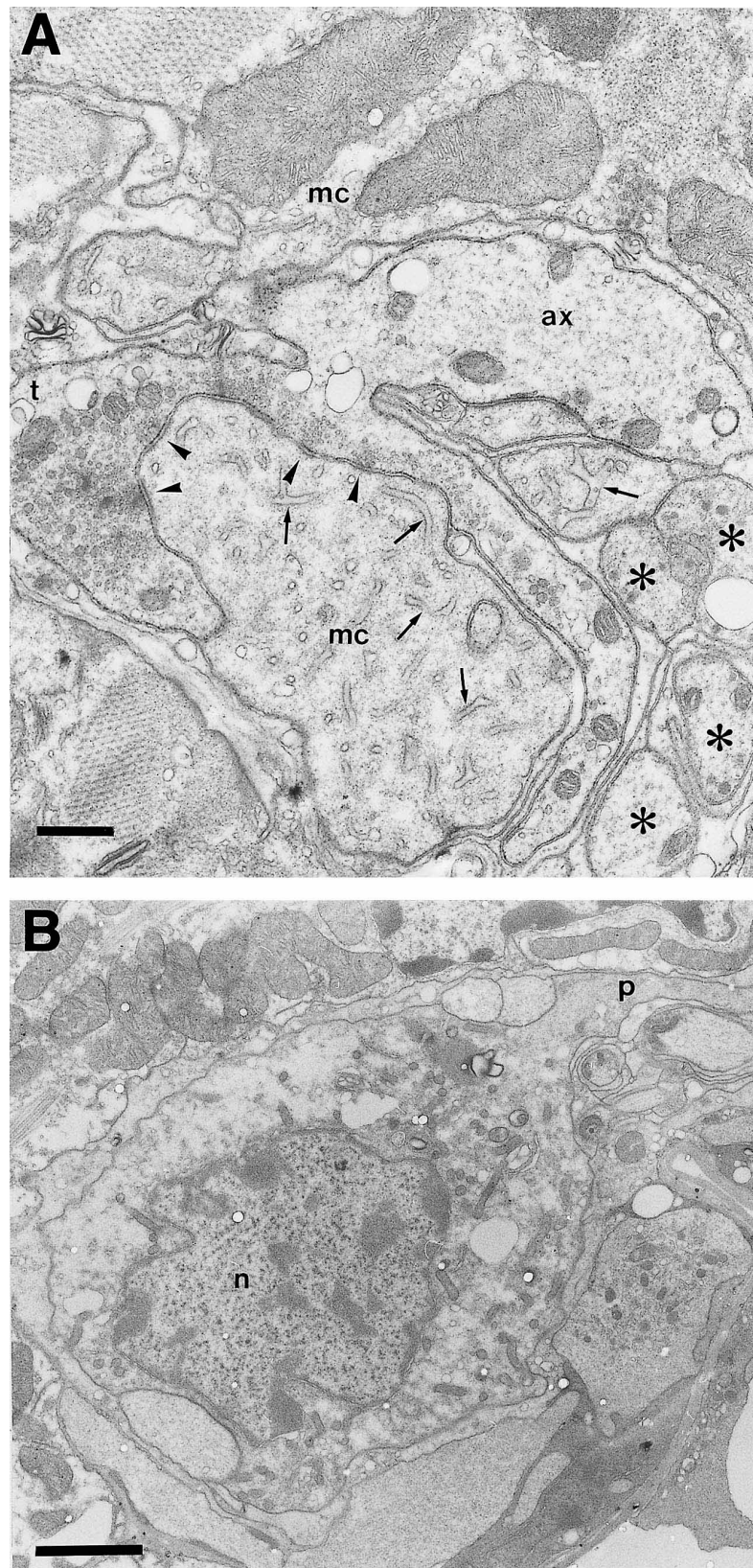
#### Fine structure of the cardiac nervous system

Electron microscopy revealed that the globular structure on the outer surface of the dorsal heart wall was the soma of a neuron that we refer to as the dorsal neuron. The soma of the dorsal neuron was 12 to 16 µm in diameter, contained a nucleus and sent an axon into the heart wall (Fig. 3A). Several nerve terminals containing many vesicles surrounded the soma of the dorsal neuron (Fig. 3B). However, no definite synaptic structures were found between the nerve terminals and the soma of the dorsal neuron. The dorsal nerve was composed of five axons (Fig. 3C). The axon of the dorsal neuron could be distinguished from the other four axons in the dorsal nerve because it was always larger in diameter than the other four axons and contained no dense vesicles. The four axons were extrinsic axons; two of them are in the left extrinsic nerve and the other two are in the right extrinsic nerve; they enter the dorsal nerve bilaterally through the lateral nerve. The nerve terminals surrounding the soma of the dorsal neuron arise from the four extrinsic axons which run in the dorsal nerve with the axon of the dorsal neuron (Fig. 3D).

The axon of the dorsal neuron runs anteriorly in the dorsal nerve, bifurcates at the connection with the lateral nerve, and extends into the left and right ventral nerves. Over its course, the axon of the dorsal neuron gives rise to many fine branches and forms nerve terminals on the myocardial cells. In the nerve terminals, typical synaptic structures, aggregation of vesicles at the presynaptic site and thickening of the postsynaptic membrane, were clearly found. At the postsyn-



**Fig. 3.** Electron micrographs of the cardiac nervous system. **A**, Sagittal section of the soma of the dorsal neuron. The soma of the dorsal neuron is located on the outer surface of the dorsal heart wall and sends an axon into the heart wall. ax, axon; ep, epicardium; mc, myocardial cell; n, nucleus. **B**, Sagittal section of the soma of dorsal neuron. Nerve terminals surrounding the soma of the dorsal neuron are shown. Many neuro-filaments (arrow) are found in the neuron. n, nucleus; t, nerve terminal. **C**, Transverse section of the dorsal nerve. The dorsal nerve is composed of five axons (asterisks), including an axon of the dorsal neuron and four extrinsic axons of smaller diameter. **D**, Schematic drawing of the soma of the dorsal neuron. The soma of the dorsal neuron is surrounded by nerve terminals derived from the four extrinsic axons in the dorsal nerve. ax, axon; n, nucleus; t, terminal; so, soma. Scale bars represent 2  $\mu\text{m}$  in A and B, and 0.5  $\mu\text{m}$  in C.



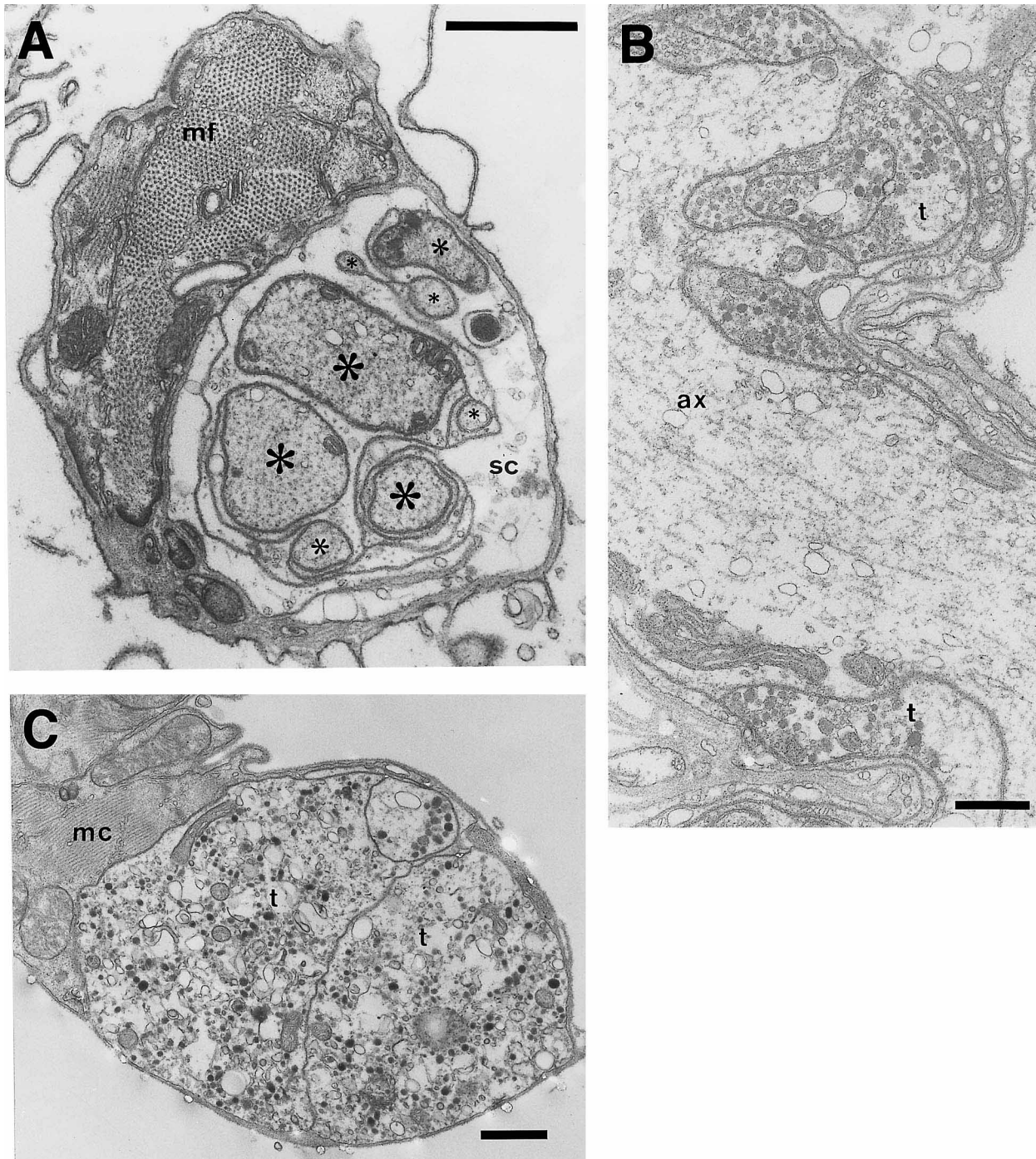
**Fig. 4.** Electron micrographs of the cardiac nervous system. **A**, Transverse section of the dorsal nerve. The axon of the dorsal neuron (ax) and four extrinsic axons (asterisks) are shown. The nerve terminal (t) of the dorsal neuron axon forms clear synaptic structures (arrowheads) with aggregation of vesicles at presynaptic sites and thickening of the postsynaptic membrane with the myocardial cell (mc). Invagination of sarcolemma is observed at many sites in the myocardial cell (arrows). **B**, Transverse section at the central portion of the lateral nerve. Neuron-like cell structures having a nucleus (n) and cell processes (p) are observed. Scale bars represent 0.5  $\mu\text{m}$  in A and 1  $\mu\text{m}$  in B.

aptic region of the myocardial cell, no myofibrils but many sites of invagination of the sarcolemma were observed (Fig. 4A.).

At the region of the connection between the dorsal and lateral nerves, a neuron-like cell structures (10 to 15  $\mu\text{m}$  in diameter) containing a nucleus was found on the luminal side of the heart wall (Fig. 4B). This cell sent processes bilaterally

into the lateral nerve. The cell appears to be a neuron but no nerve terminals were found in the processes.

Each of the paired extrinsic nerves contained two thick and several thin axons (Fig. 5A). The thick axons were found in the dorsal, lateral and ventral nerves and the thin axons were found also in the lateral and ventral nerves. Over their



**Fig. 5.** Electron micrographs of the cardiac nervous system. **A**, Transverse section of an extrinsic nerve at the portion just before entering the heart. In addition to two thick axons (asterisks), several thin axons are observed (asterisks). Some myofibrils (mf) attach to the extrinsic nerve at this portion. sc, supporting cell. **B**, Longitudinal section of the dorsal nerve. Nerve terminals (t) of the extrinsic axons on the dorsal neuron axon (ax) are shown. **C**, Transverse section of a branch derived anteriorly from the lateral nerve. Nerve terminals (t) of extrinsic axons on the luminal surface of the myocardial cell (mc) are shown. Scale bars represent 1  $\mu\text{m}$  in A and C, and 0.5  $\mu\text{m}$  in B.

courses, the axons form nerve terminals on the axon of the dorsal neuron (Fig. 5B) and on the luminal surface of the myocardial cells (Fig. 5C). However, no definite synaptic structures were found in the nerve terminals.

## DISCUSSION

The results of the present study show that the heart of ostracods has a cardiac nervous system as suggested by Canon (1940). Moreover, the finding of an intrinsic motor neuron is the first evidence for the presence of the cardiac ganglion system in Ostracoda, the most primitive order in Crustacea investigated so far.

As reported by Abe and Vannier (1995), the heart of *V. hilgendorffii* has two ostia and one ventral aorta, and the heart wall is composed of a single layer of globular myocardial cells. In each myocardial cell, bundles of myofibrils are localized at the epicardial site and cross each other (Fig. 1). These characteristics of the myocardial cells of *V. hilgendorffii* are similar to those of the ostracod *Cypridina norvegica* (Christ, 1982). Ultrastructure of the myocardial cells has been investigated in various crustaceans (reviewed by Nylund *et al.*, 1987). The characteristics of the myocardial cells of *V. hilgendorffii* were similar to that of branchiopods, one of the most primitive orders in Crustacea (Tjønneland *et al.*, 1980; Økland *et al.*, 1982; Yamagishi *et al.*, 2000).

Electron microscopy revealed that a neuron situated on the outer surface of the dorsal heart wall (dorsal neuron) sends an axon into the heart wall where it branches widely (Fig. 3). Moreover, the dorsal neuron connects by many neuromuscular junctions with the myocardial cells along the course of its path (Fig. 4A). No other neuromuscular junctions except those of the dorsal neuron were found in the heart. These results suggest that the dorsal neuron is an intrinsic motor neuron innervating the myocardium.

In the ostracod *Gigantocypris mülleri*, light microscopic observations of serial sections by Cannon (1940) revealed the presence inside the heart of a neuron that sends axons bilaterally out of the heart. In *V. hilgendorffii*, a neuron-like cell was found on the inner surface of the anterior heart wall (Fig. 4B), however, no nerve terminals were found in the processes of this cell. The function of this cell is uncertain in the present study, but it is unlikely that the cell is a motoneuron in the heart.

Two extrinsic axons enter the *V. hilgendorffii* heart bilaterally and branch widely in the heart forming many nerve terminals on the dorsal neuron and myocardial cells. In addition to the two axons, some thin axons which form similar nerve terminals were found in the extrinsic and other nerves (Fig. 5). As it was uncertain whether the thin axons are branches of the two thick axons, we could not determine the precise number of axons in the extrinsic nerve. In many crustaceans, a pair of extrinsic cardioregulatory nerves, each of which contains two or three axons, enter the heart and make synaptic connections with the cardiac ganglion neurons or with both the cardiac ganglion neurons and myocardial cells (revived

by McMahon *et al.*, 1997). In *V. hilgendorffii*, however, no synaptic structures were found in the nerve terminals of the extrinsic axons (Figs. 3 and 4). The extrinsic axons appear not to be presynaptic axons against the cardiac neuron and myocardium but may function as neurosecretory axons.

The results of the present study suggest that the heart-beat of the ostracod *V. hilgendorffii* is neurogenic, with the simplest cardiac ganglion composed of a single neuron acting as a pacemaker. Application of tetrodotoxin, which blocks impulse generation in the crustacean cardiac ganglion, caused a diastolic arrest of the heart (Ishii and Yamagishi, 2000). This supports the above idea but electrophysiological evidence is required to unequivocally demonstrate a neurogenic heart-beat.

## ACKNOWLEDGEMENTS

We thank Dr. J. L. Wilkens for valuable suggestions. We also thank Dr. R. S. J. Weisburd for critical reading of the manuscript. This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and by the University of Tsukuba Research Project to H.Y. Contribution no. 657 from Shimoda Marine Research Center, University of Tsukuba.

## REFERENCES

- Abe K, Vannier J (1995) Functional morphology and significance of the circulatory system of Ostracoda, exemplified by *Vargula hilgendorffii* (Myodocopida). *Mar Biol* 124: 51–58
- Alexandrowicz JS (1932) The innervation of the heart of crustacea. I. Decapoda. *Q J Microscop Sci* 41: 291–310
- Alexandrowicz JS (1934) The innervation of the heart of crustacea. II. Stomatopoda. *Q J Microscop Sci* 76: 511–548
- Alexandrowicz JS (1952) Innervation of the heart of *Ligia oceanica*. *J Mar Biol Ass UK* 33: 709–719
- Ando Y, Matsuzaki O, Yamagishi H (1998) Ultrastructure of the heart and cardiac nervous system of *Vargula hilgendorffii* (Ostracoda, Crustacea). *Jap Soci Comp Biochem Physiol* 15(Suppl): 257
- Cannon HG (1940) On the anatomy of *Gigantocypris mülleri*. *Discov Rep* 19: 185–224
- Christ I (1982) The cardiac ultrastructure of *Cypridina norvegica* Barid (crustacea, ostracoda). *Zool Anz Jena* 208: 323–329
- Hagiwara S (1961) Nervous activities of the heart in Crustacea. *Erg Biol* 24: 287–311
- Hartline DK (1979) Integrative neurophysiology of the lobster cardiac ganglion. *Am Zool* 19: 53–65
- Ishii Y, Yamagishi H (2000) Pacemaker mechanism in the heart of the ostracod crustacean *Vargula hilgendorffii*. *Zool Sci* 17 (Suppl): 89
- Kihara A, Kuwasawa K (1984) A neuroanatomical and electrophysiological analysis of nervous regulation in the heart of an isopod crustacean, *Bathynomus doederleini*. *J Comp Physiol* 154: 883–894
- McMahon BR, Wilkens JL and Smith PJS (1997) Invertebrate circulatory systems. In "Handbook of Physiology" Sec 13 Comparative Physiology vol II Ed by Dantzler WH, Oxford Univ Press New York Oxford, pp 931–1008
- Maynard DM (1960) Circulation and heart function. In "The Physiology of Crustacea 1" Ed by TH Watermann, Academic Press New York, pp 161–226
- Nylund A, Økland S, Tjønneland A (1987) The crustacean heart ultrastructure and its bearing upon the position of the isopods in



- eumalacostracan phylogeny. *Zool Scr* 16: 235–24
- Økland S, Tjønneland A, Larsen A, Nylund A (1982) Heart ultrastructure in *Branchinecta pludosa*, *Artemia salina*, *Branchipus shaefferi* and *Streptocephalus sp.* (crustacea, anostraca). *Zoomorphology* 101: 71–81
- Prosser CL (1973) Circulation of body fluid. In "Comparative Animal Physiology" Ed by C CL Prosser, Saunders WB Co Washington DC, pp. 822–856
- Sakurai A, Mori A, Yamagishi H (1999) Glutamatergic neuromuscular transmission in the heart of the isopod crustacean *Ligia exotica*. *J Exp Biol* 201: 2833–2842
- Suzuki S (1934) Ganglion cells in the heart of *Ligia exotica* (Roux). *Sci Rep Tohoku Imp Univ* 9: 213–217
- Tjønneland A, Midtumm B, Økland S, Liebich HO (1980) Heart ultrastructure in *Lepidurus arcticus* Pallas (crustacea, branchiopoda, notostraca). *Cell Tiss Res* 211: 20–212
- Watanabe A, Obara S, Akiyama T, Yumoto K (1967) Electrical properties of the pacemaker neurons in the heart ganglion of a stomatopod, *Squilla oratoria*. *J Gen Physiol* 50: 813–834
- Wilkens JL (1999) Evolution of the cardiovascular system in Crustacea. *Am Zool* 39: 199–214
- Yamagishi H, Ando H and Makioka T. (1997) Myogenic heartbeat in the primitive crustacean *Triops longicaudatus*. *Biol. Bull.*, 193: 350–358
- Yamagishi H, Ando Y, Matsuzaki O (2000) Myocardial depolarizing response to glutamate in the myogenic heart of the branchiopod crustacean *Triops longicaudatus*. *Zool Sci* 17: 27–32
- Yamagishi H, Ebara A (1985) Spontaneous activity and pacemaker property of neurons in the cardiac ganglion of an isopod, *Ligia exotica*. *Comp Biochem Physiol* 81A: 55–62
- Yamagishi H, Hirose E (1997) Transfer of the heart pacemaker during juvenile development in the isopod crustacean *Ligia exotica*. *J Exp. Biol* 200: 2393–2404

(Received September 11, 2000 / Accepted April 19, 2001)