Modulation of an Integrated Central Pattern Generator–Effector System: Dopaminergic Regulation of Cardiac Activity in the Blue Crab

Callinectes sapidus

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Submitted 27 May 2004; accepted in final form 29 June 2004

INTRODUCTION

The pivotal role of neuromodulation in the regulation and optimization of motor behavior is now firmly established (Katz 1999; Kupfermann 1979; Pearson 1993). Compelling evidence has come from studies of many model systems (e.g., Hultborn and Kiehn 1992; Sillar et al. 2002; Weiss et al. 1992) including, notably, the decapod crustaceans. In crustacean motor systems, modulation occurs at every level from sensory-motor integration (Edwards et al. 2002; Glanzman and Krasne 1983) through central pattern generation (Harris-Warrick and Marder 1991; Harris-Warrick et al. 1988; Miller and Sullivan 1981) to neuromuscular transmission (Breen and Atwood 1983; Florey and Rathmayer 1978; Kravitz et al. 1980).

In neuromodulatory architectures, two broad themes have been distinguished: in the terminology of Cropper (1987) and Katz and Frost (1996), “intrinsic” and “extrinsic” modulation. Intrinsic modulation is locally integrated into the structure of the “mediating” network. It is automatically set in motion when the network is active to provide local adjustments, although these adjustments may have global consequences in a well-connected, dynamically sensitive network such as a central pattern generator (CPG). The functional roles of intrinsic modulation have been relatively well studied (see, e.g., Brezina and Weiss 1997; Katz 1999; Katz and Frost 1996; Marder and Thirumalai 2002).

Extrinsic modulation, by contrast, arrives from an external source such as a modulatory neuron that is not itself part of the mediating circuitry. Such extrinsic modulatory systems of crustaceans have been described as overall “gain setters” (Kravitz 1988; Ma et al. 1992). Because extrinsic modulation is not obligatorily coupled to the activity of any particular part of the mediating system, it is free to exert widespread actions. In crustaceans, dopamine is of particular interest in this regard because it appears to regulate multiple motor systems (Barthe et al. 1989; Berlind 1977; Harris-Warrick et al. 1998; Meyrand and Moulin 1985; Miller et al. 1984, 1985; Rajashekhar and Wilkens 1992; Wood 1995). In those species in which it has been localized, dopamine has been found to be present in a relatively small number of neurons with extensive projections (Cournil et al. 1994, 1995; Tierney et al. 2003; Wood and Derby 1996). Based on observations of this kind, it has been conjectured that the role of extrinsic modulation in motor systems is to modulate multiple parts of motor circuits, and multiple motor circuits, so as to integrate their activities into a global, coordinated whole. Whether external modulation really plays this role, and what functional consequences might flow from such actions, has, however, not been rigorously established. In part this has been because of the lack of a suitable simple experimental preparation. Our first aim in this paper is to identify and characterize such a preparation.

The cardiac system of decapod crustaceans is an exceptionally simple system that is known to receive substantial modulatory regulation. The heartbeat in marine species is driven by a simple (usually 9 neurons) central pattern generating circuit, the cardiac ganglion (CG), positioned within the dorsal wall of the heart (Cooke 1988, 2002). The CG is directly controlled by
a small number of modulatory fibers that originate in the CNS (Delgado et al. 2000; Field and Larimer 1975; Maynard 1966; Yazawa and Kuwasawa 1994). The heartbeat is also regulated by modulators that originate from the pericardial organs (POs), neurohaemal structures that flank the heart and release bioactive products into the general circulation (Alexandrowicz and Carlisle 1953; Cooke and Sullivan 1982). Recognition of the crustacean CG as “an autonomously active, rhythmic, pattern-forming, neural system integrating its own spontaneity with sensory and neurohumoral influences” (Cooke 1988) led early investigators to emphasize its utility as a very simple model for more complex nervous systems (Alexandrowicz 1932; Hagiwara 1961; Welsh and Maynard 1951).

Perhaps the most important aspect of global coordination is that between center and periphery. Experimental studies suggest that motor systems are, as theoretical studies suggest they must be, coordinately modulated both in the center and in the periphery to ensure efficient, adaptive behavior (Brezina et al. 2000b; Calabrese 1989; Chiel and Beer 1997; Meyrand and Marder 1991). The present study was intended to establish the crustacean cardiac system, seen as a simple model of a CPG complete with its effector system, as a suitable preparation in which to study the coordination of central and peripheral modulation. Anatomical and physiological methods were used to show that a single central dopaminergic neuron is likely to exert actions both on the periphery (neurohemoronal modulation of contractions of the heart muscle) and on the central pattern generator (direct innervation of the CG) of the cardiac system of the blue crab Callinectes sapidus. These inferences were examined with experiments in which dopamine was applied to intact hearts, isolated cardiac ganglia, and a novel semi-intact working heart preparation in which central and peripheral effects could be compared directly.

**Methods**

Specimens of Callinectes sapidus (male and female) were captured in the San José Lagoon in the Hato Rey district of San Juan, Puerto Rico. They were housed under ambient light and temperature conditions in water that was obtained from collection sites. To reduce fat deposits within the heart, crabs were not fed. They were typically used within 3 wk of capture.

**Histology**

TYROSINE HYDROXYLASE (TH) IMMUNOHISTOCHEMISTRY. Specimens were covered in ice (30 min) to achieve immobilization. Tissues were dissected, secured to Sylgard-lined petri dishes with minute pins, and fixed for 1 h in freshly prepared 4% paraformaldehyde. Standard whole mount immunohistochemical protocols were followed (see Miller et al. 1991 for detailed buffer composition, incubation, and wash procedures). Ganglia were washed (5×, room temperature with agitation) in PTA (0.1 M phosphate buffer containing 2% Triton X-100 and 0.1% sodium azide). After preincubation with normal goat serum (0.8%), tissues were immersed (48 h, room temperature) in a 1:200 dilution of the primary TH antibody (mouse monoclonal; Immunostar, Stillwater, MN). After repeated PTA washes (5×, ≥30 min each, room temperature), ganglia were incubated in secondary antibodies conjugated to a fluorescent marker [Alexa 488 goat anti-mouse IgG (H+L) conjugate; Molecular Probes, Eugene, OR: A-11029]. The secondary antibody dilutions ranged from 1:1,000 to 1:3,000. The Alexa 488 was viewed with the G-2A filter block of the Nikon Optiphot or using the preconfigured FITC channel of a Zeiss Pascal LSM5 laser-scanning confocal microscope. Standard images were captured using the ACT1 (Nikon) software package. Confocal images were reconstructed (AIM Software) from sequential images captured in the z-axis plane of the tissue. Images were transported as TIFF files to Adobe Photoshop (Version 6) for adjusting overall contrast and brightness. Finally, they were imported to Corel Draw 9 for addition of labels, cropping, and organization of panels.

**Nerve Backfills.** The biotin–avidin protocol followed the methods of Xion et al. (1999) with modifications based on Diaz-Ríos et al. (1999). The tissue of interest was pinned out near a small Vaseline well that was formed on the Sylgard surface. The nerve being examined was cut and drawn into the well. Care was taken to avoid contact between the end of the nerve and the Vaseline. The tip of the nerve was cut one more time and then the crab saline inside the well was withdrawn and replaced with a saturated aqueous solution (1.6 mg/30 μl) of biocytin (Sigma Chemical, St. Louis, MO). The walls of the well were then built up with successive layers of Vaseline, forming an “igloo” that effectively isolated the biocytin pool from the saline surrounding the ganglion. The preparation was covered and incubated overnight at 14°C. The well was then removed, and ganglia were washed 3–5 times, repinned, and fixed in paraformaldehyde as described above. The fixed ganglia were transferred to microcentrifuge tubes, washed 5 times (30 min each) with PTA solution and incubated overnight (room temperature, with shaking) in Rhodamine-ε60 Avidin D (Vector Laboratories, Burlingame, CA) diluted 1:3,000 in PTA (24–48 h, room temperature). Tissues were then washed 5 times with PTA and the quality of the backfill was assessed before further immunohistochemical processing. In the double-labeling experiments, THli was visualized using the Alexa 488 goat anti-mouse secondary antibody (see above). A barrier filter (546 nm green interference) was used to eliminate “bleed through” of rhodamine when examining and photographing THli fluorescence.

**Neurobiotin Injection.** Methods for intracellular staining were modified from the methods of Delgado et al. (2000). Microelectrode tips were filled with 4% Neurobiotin (Vector Laboratories) dissolved in 0.5 M KCl and 50 mM Tris (pH 7.6). The electrode shafts were filled with 2 M KCl, resulting in resistances ranging from 10 to 30 MΩ. Depolarizing current pulses (1–2 nA; 0.5 s; 1 Hz; 10–60 min) were used to ejection the Neurobiotin. This procedure did not appear to affect the resting potential or spontaneous electrical activity of the injected neuron. The preparations were usually left at room temperature for 2–3 h to allow material to diffuse from the injection site (cell body) to distant processes and dye-coupled cells. They were then repinned if necessary, and fixed in paraformaldehyde as described above. The fixed ganglia were transferred to microcentrifuge tubes, washed 5 times (30 min each) with PTA solution, and incubated in Rhodamine-ε60 Avidin D diluted (1:3,000 to 1:5,000) in PTA (24–48 h, room temperature). Tissues were then washed 5 times with PTA, examined, and processed for THli as described above.

**Physiology**

**Working Heart (Fig. 1A).** Hearts were removed intact and the sternal artery was cannulated with a modified syringe needle and mounted in a 20-mL organ bath. The heart was suspended using a fine monofilament nylon thread attached to the force plates of a Grass FT03 isometric force transducer and placed under a resting load (~0.5 g). Perfusion with saline was maintained at a constant rate (2 ml/min) and pressure. The crab saline composition was based on Pantin’s saline for Cancer pagurus: 487 mM NaCl, 13.6 mM KCl, 13.4 mM CaCl2, 13.6 mM MgCl2, 1.4 mM sodium sulfate, 3 mM HEPES (N-[2-hydroxyethyl] piperazine-N’-[2-ethanesulfonic acid]), adjusted...
to pH 7.4 with sodium hydroxide. Perfusion rate and pressure were maintained when dopamine trials were performed.

SEMI-INTACT WORKING HEART (FIG. 1B). Hearts were dissected and pinned in Sylgard-lined petri dishes, in an arrangement as similar as possible to that in the intact crab. A small incision was made in the ventral wall of the heart to expose part of the nerve ring containing the motor neuron axons. The ring was cut and the severed end proximal to the ganglion was drawn into an extracellular suction electrode. The heart was then connected to the force plates of a Grass FT03 isometric force transducer with a hook and nylon thread and placed under a resting load (∼0.5 g). The preparation was continually internally perfused with saline at a constant rate (2 ml/min) and pressure. Perfusion rate and pressure were maintained when dopamine trials were performed.

ISOLATED CARDIAC GANGLION (FIG. 1C). Hearts were pinned ventral side up in Sylgard-lined petri dishes. A cut was made in the ventral musculature exposing the cardiac ganglion. Dissection was achieved principally by teasing away the adhering muscles. Previous investigators (Tazaki and Cooke 1979a) noted that the region within the confluence of the motor roots at each end of the ganglion contains the dendritic endings of the ganglionic neurons (see also Fig. 2B). A small noncontracting remnant was therefore retained at either end of the ganglion. Extracellular suction electrode recordings were obtained from at least one of the 4 cut ganglionic roots. Membrane potentials were recorded from anterior and/or posterior motor neurons using 2 M KCl-filled or Neurobiotin-tipped microelectrodes (10–30 MΩ). Preparations were continuously superfused with saline (2 ml/min).

In all physiological preparations, effects of dopamine were evaluated by comparing parameter values averaged over a 1-min period during the strongest response to the applied dopamine (typically about 5 min after the perfusion switch) to control values averaged over a 1-min period before the dopamine application. The preparation was washed (≥20 min) between the application of different dopamine concentrations.
RESULTS

Functional topography of the Callinectes cardiac system

We first sought to characterize features of the Callinectes cardiac system relevant to its suitability for examining the coordination of central and peripheral modulation. Injection of Neurobiotin into any of the large neurons within the cardiac ganglion revealed, through dye coupling, its full complement of 5 motor neurons. The cell bodies of 3 of the motor neurons were located at the anterior end of the ganglion (Fig. 2A, asterisks). These cells projected large-caliber axons into the ganglionic trunk in the posterior direction. Two large posterior motor neurons (Fig. 2B, asterisks) projected axons in the anterior direction. The 5 axons came into close apposition within the anterior half of the trunk. The axons originating from the anterior neurons bifurcated on reaching the posterior end of the ganglion and projected branches into each of the posterior roots of the lateral connectives. The axons of the posterior motor neurons project in the anterior direction. Finer dendritic processes extend from their posterior pole. These processes branch near the cell body (one dendritic branch point is indicated by an arrowhead) and project into the muscle fibers adjacent to the ganglion within the confluence of the 2 posterolateral connectives. In the most posterior region of the ganglion (dashed box) the cell bodies of the small interneurons exhibited lower levels of dye coupling. Calibration bar in both A and B = 100 μm. C: intracellular recording from an anterior motor neuron (Ant. MN) and a posterior motor neuron (Post. MN) together with simultaneous extracellular recording of the motor neuron impulse pattern from an anterior connective (Ant. con.) and a posterior connective (Post. con.). Rhythmic (≈0.5 Hz) synchronous bursting is observed in the 2 motor neurons. In both cases, nonovershooting impulses are superimposed on a slow depolarization. Impulses and the slow depolarization are both larger in the anterior neuron than in the posterior neuron. D: a single burst (boxed in C) shown on an expanded time base. Inflections on the rising phase of the depolarization in the 2 motor neurons and the extended period of synaptic input that follows the motor neuron firing (arrows) reflect excitatory postsynaptic potentials (EPSPs) originating from the small interneurons (see Tazaki and Cooke 1979a). These EPSPs also are substantially larger in the anterior motor neuron. Only the motor neuron impulses are recorded by suction electrodes on the connectives (2 bottom recordings). Identical impulse trains, precisely synchronized with the intracellularly recorded impulses, are recorded from both connectives.

FIG. 2. Functional topography of the Callinectes cardiac ganglion. A: neurobiotin fill of the 3 large anterior motor neurons (asterisks). Each motor neuron gives rise to a large-caliber axon that projects in the posterior direction into the ganglionic trunk (arrows). B: cell bodies of 2 large motor neurons located at the posterior end of the ganglion (asterisks). Same preparation as in A; the trunk connecting the 2 ends (~5 mm in length) is not shown. Axons originating from the 3 anterior motor neurons bifurcate (one bifurcation is indicated by arrow) near the posterior end of the ganglion and project a branch into each of the posterior roots of the lateral connectives. Axons of the 2 posterior motor neurons project in the anterior direction. Finer dendritic processes extend from their posterior pole. These processes branch near the cell body (one dendritic branch point is indicated by arrowhead) and project into the muscle fibers adjacent to the ganglion within the confluence of the 2 posterolateral connectives. In the most posterior region of the ganglion (dashed box) the cell bodies of the small interneurons exhibited lower levels of dye coupling. Calibration bar in both A and B = 100 μm. C: intracellular recording from an anterior motor neuron (Ant. MN) and a posterior motor neuron (Post. MN) together with simultaneous extracellular recording of the motor neuron impulse pattern from an anterior connective (Ant. con.) and a posterior connective (Post. con.). Rhythmic (~0.5 Hz) synchronous bursting is observed in the 2 motor neurons. In both cases, nonovershooting impulses are superimposed on a slow depolarization. Impulses and the slow depolarization are both larger in the anterior neuron than in the posterior neuron. D: a single burst (boxed in C) shown on an expanded time base. Inflections on the rising phase of the depolarization in the 2 motor neurons and the extended period of synaptic input that follows the motor neuron firing (arrows) reflect excitatory postsynaptic potentials (EPSPs) originating from the small interneurons (see Tazaki and Cooke 1979a). These EPSPs also are substantially larger in the anterior motor neuron. Only the motor neuron impulses are recorded by suction electrodes on the connectives (2 bottom recordings). Identical impulse trains, precisely synchronized with the intracellularly recorded impulses, are recorded from both connectives.

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Tazaki and Cooke 1979a, 1983a), each of the large motor neurons in *Callinectes* received synchronous excitatory postsynaptic potentials (EPSPs) in the initial and late phases of each burst (Fig. 2D, arrows). Impulses corresponding to these EPSPs were not detected in the motor trunks, indicating that they originate from the small interneurons, the projections of which are confined to the CG (Miorilli et al. 1987; Tazaki and Cooke 1979a, 1983a,b).

In the crab cardiac ganglia that have been investigated to date, the spatiotemporal properties of the electrical coupling and synaptic signaling result in precise synchrony of the motor neuron firing (Berlind 1982; Miorilli et al. 1987; Tazaki 1972; Tazaki and Cooke 1979a,b, 1983a,b). Simultaneous recording from an anterior motor neuron, a posterior motor neuron, a branch of the anterior connective, and a branch of the posterior motor neuron revealed that such synchrony of firing is also a property of the *Callinectes* CG (Fig. 2, C and D). Intracellular recordings from any of the anterior motor neurons (Fig. 2, C and D, Ant. MN) revealed, in each burst, 5 to 10 nonoverlapping impulses (10–15 mV) superimposed on a slow potential (500–900 ms in duration). The motor neuron slow potential has been shown to reflect the combined actions of an endogenous regenerative driver potential and the chemically mediated EPSPs originating from the small interneurons (Tazaki and Cooke 1979a, 1983a). In somatic recordings of posterior motor neurons (Fig. 2, C and D, Post. MN), the amplitudes of the impulses (3–5 mV) and the EPSPs (0.5–2 mV) were considerably smaller than those in the anterior motor neurons (10–15 and 2–4 mV), probably reflecting their origin in the anterior half of the ganglion (see Tazaki and Cooke 1983a). Examination of a single burst with an expanded time axis revealed that the impulses of the posterior neurons occurred in precise synchrony with those of the anterior motor neurons (Fig. 2D, 2 top records). Moreover, recordings from each of the major connectives (Fig. 2D, 2 bottom records) exhibited identical impulse trains that corresponded to the synchronous firing of the 2 sets of motor neurons.

**The semi-intact working heart preparation**

The observed synchrony of motor neuron firing suggested that recording from any of the 4 connectives would reflect the motor output of the ganglion and thus the patterned input to the entire cardiac musculature. This reasoning prompted us to develop a preparation, termed the semi-intact working heart (S-IWH; see METHODS), in which we could record the motor output of the ganglion in a minimally dissected contracting heart (Fig. 1B). Because this preparation required some removal of cardiac muscle and severing one connective, we performed an initial comparison of the parameters of heartbeat activity in the S-IWH with those in the fully intact working heart (WH) preparation (Fig. 1A). Contraction amplitudes were substantially reduced in the S-IWH (compare Fig. 1, A2 and B2), attributed presumably to the reduced mass of heart muscle and the reduced motor drive resulting from cutting the connective. However, the heartbeat frequency of the S-IWH (15.8 ± 1.8 beats/min, mean ± SE, n = 12) did not differ significantly from that of the intact WH (16.5 ± 1.2 beats/min, n = 20; 2-tailed Student’s *t*-test, *P* = 0.74; Fig. 3A1). Moreover, the time to peak contraction (WH: 336.6 ± 29.7 ms, S-IWH: 364.9 ± 30.5 ms; *P* = 0.53) and the contraction duration (WH: 594.0 ± 104.7 ms, S-IWH: 612.0 ± 112.6 ms; *P* = 0.91) did not differ between the 2 preparations (Fig. 3, A2 and A3).

We further compared the motor patterns recorded in the S-IWH with those recorded in the isolated cardiac ganglion (ICG) preparation (Fig. 1C). The burst frequency of the ICG (24.0 ± 3.2 bursts/min, n = 9) was significantly greater than that of the S-IWH (16.5 ± 1.2 bursts/min, n = 12; *P* < 0.05; Fig. 3B1), as was the number of impulses per burst (ICG: 15.4 ± 2.6, S-IWH: 7.1 ± 0.8; *P* < 0.05) and the burst duration (ICG: 310.4 ± 74.2 ms, S-IWH: 138.9 ± 12.1 ms; *P* < 0.05; Fig. 3, B2 and B3).

Together, these observations demonstrated that the semi-intact working heart preparation retained the essential properties of the intact heart. However, there were significant differences between the motor patterns produced by the S-IWH and the isolated CG, underscoring the importance of examining the isolated CG (P < 0.05; see main text). Means ± SE are plotted throughout, with the number of preparations *n* indicated.**

![Figure 3](http://jn.physiology.org/)
cardiac modulation in the context of the entire, integrated CPG–effector system.

**Dopaminergic regulation of the cardiac system: anatomical substrates**

In a series of immunohistochemical experiments, we used a monoclonal antibody generated against tyrosine hydroxylase (TH) to identify possible sources and modes of dopaminergic modulation of the *Callinectes* cardiac system. In a previous mapping of the catecholaminergic system of *Callinectes* by Wood and Derby (1996), the staining pattern of TH-like immunoreactivity (THli) was found to be virtually identical to that observed with an antibody against dopamine (see also Cournil et al. 1994). In view of this, and the reported inability to detect norepinephrine and epinephrine in crustacean nervous tissue using chromatographic techniques (Barker et al. 1979; Sullivan et al. 1977), THli is commonly equated with the localization of dopamine in these species.

We observed THli in a limited number of neurons in the brain and ventral nerve cord (Figs. 4 and 5). In agreement with the previous description (Wood and Derby 1996) the largest central THli neuron was observed in the commissural ganglion (Fig. 4A, arrow). This cell corresponds to a dopaminergic neuron, termed the “L-cell” (Selverston et al. 1976), that has been described in a number of crustacean species, including the crab *Carcinus maenas* (Cooke and Goldstone 1970), the lobsters *Panulirus interruptus* (Kushner and Maynard 1977), *Homarus gammarus* (Cournil et al. 1984, 1994), *Homarus americanus* (Pulver et al. 2003; Siwicki et al. 1987), and the crayfish

![Diagram](http://jn.physiology.org/)

**FIG. 4.** Tyrosine hydroxylase-like immunoreactivity (THli) in the L-cells and their projections. A: THli in the commissural ganglion (CoG). A diffuse system of fine THli fibers coursed throughout the central neuropil region of the CoG. THli was located in 5 neurons, all in the anterior portion of the ganglion. Cell body of the L-cell (arrow) was substantially larger than the others (arrowheads) and typically had an irregular shape (see also Cooke and Goldstone 1970). Its stout axon coursed through the central neuropil region of the ganglion and then turned abruptly to enter the circumesophageal connective (C conn.). A second fine fiber present in the superior esophageal nerve (son) could not be associated definitively with any of the neurons in the CoG. B: each L-cell soma (arrows) in the paired commissural ganglia gave rise to a single large-caliber axon that exited the ganglion and ascended toward the brain along the lateral edge of the C conn. After its reversal of direction (C and D) the L-cell axon (arrowheads) coursed past the CoG along the medial edge of the C conn. C: within the brain, groups of THli somata were present within the anterior medial ventral protocerebrum (filled arrowhead; cluster 6 according to the nomenclature of Sandeman et al. 1992) and laterally in cluster 12 of the deutocerebrum (unfilled arrowhead). Prominent bundles of THli fibers, probably originating from neurons in the eyestalk ganglia (Wood and Derby 1996), projected from the optic nerve (opt. n.) to ventral regions of the anterior medial protocerebrum, where their staining became impossible to follow. A more diffuse, but widespread THli innervation of the deutocerebrum and tritocerebrum originated, in part, from a limited number (4 to 6) of fibers that ascended in each C conn. Largest such fiber, originating from the L-cell (A and B), approached the brain in the most lateral edge of each connective, but reversed its direction before reaching the posterior tritocerebrum (arrow). D: higher magnification of the junctional area between the C conn. and tritocerebrum from the same preparation as in C. Near the point at which the L-cell fiber reversed direction (arrow), it gave rise to a collateral that in turn branched (arrowhead) to innervate medial and lateral regions of the tritocerebrum. Calibration bars = 100 μm in A and D, 200 μm in B and C.
Oronectes rusticus (Tierney et al. 2003). The L-cell axon gave rise to multiple neurites within the commissural ganglion before its entry into the circumesophageal connective (C conn.). Four additional smaller THii neurons were observed in the commissural ganglion (Fig. 4A, arrowheads). A slender fiber projected into the superior esophageal nerve (son; Fig. 4A). This fiber did not appear to be a collateral of the L-cell fiber projected into the superior esophageal nerve (son; Fig. 4B). Each L-cell projected anteriorly toward the brain (Fig. 4A) appeared to contribute to the central neuropil network, but their projections to the pericardial organ in the lateral region of the thorax. On reaching the subesophageal ganglion, the L-cell axon descended in the lateral intersegmental (LIS) tract (Maynard 1961). Toward the posterior edge of the nerve, a single large-diameter fiber (arrow) could be followed into the ganglion, where it turned sharply to enter the C conn. B2; TH-like immunoreactivity; same preparation and field of view as in B1 shows labeling of the L-cell axon. Calibration bar = 200 μm.

FIG. 5. Projection of the L-cell axon into the first segmental nerve (SN1). A: whole mount preparation of the ventral nerve cord. Several fine THii fibers descending in the C conn. contributed to longitudinal intersegmental tracts (arrowheads) projecting to the most posterior portions of the ganglionic mass. Within the abdominal ganglia, 2 large THii cell bodies were observed near the midline of the cord. These cells are likely to correspond to the anterior unpaired medial (aum) cells that were shown to provide a catecholaminergic innervation of the hindgut in crayfish (Mercier et al. 1991). On reaching the subesophageal ganglion, the L-cell axon descended in the lateral intersegmental (LIS) tract (Maynard 1961), coursed slightly past the origin of SN1, and then turned back and laterally to enter the nerve (arrows). sa, sternal artery. Calibration bar = 500 μm. B: double-labeling experiments to confirm that the L-cell axon projects into SN1. B1: biocytin backfill of SN1 revealed a bundle of fibers (arrowhead) that coursed in the anterior direction on entering the subesophageal ganglion, likely corresponding to projections of the C-cell cluster in the anterior thoracic ganglia (Matsumoto 1954; Maynard 1961). Toward the posterior edge of the nerve, a single large-diameter fiber (arrow) could be followed into the ganglion, where it turned sharply to enter the C conn. Only this fiber was observed to label for THii (Fig. 5B2, arrow), indicating that the sole dopaminergic fiber in SN1 originates from the L-cell. Occasionally, a smaller THii fiber also entered SN1, but it always terminated close to the ganglion and was never back-filled.

The large L-cell axon in SN1 could be followed to the pericardial organ in the lateral region of the thorax. On reaching the PO, it ramified repeatedly, projecting branches into each of the major bars and longitudinal trunks of the PO (Fig. 6; terminology of Alexandrowicz 1953). Within the central core of each bar and trunk, multiple smooth THii fibers ran in parallel fashion (Fig. 6, C and D). These fibers gave rise to finer, more irregular branches that reached the superficial cortex or secretory layer of the PO (Fig. 6E; see Maynard and Maynard 1962). No immunoreactive cell bodies were observed in the PO. These observations are consistent with previous descriptions of the anatomical features of catecholaminergic projections to the pericardial organs of 6 other brachyuran species (Cooke and Goldstone 1970) and the embryonic lobster Homarus americanus (Pulver and Marder 2002).

Unexpectedly, however, a single branch of the L-cell axon was observed to depart from the anterior bar region of the PO in a side twig that gave rise to the dorsal nerve projecting to the heart (Fig. 6B, arrow). Within the heart, the THii fiber extended, without branching, to the cardiac ganglion. No THii innervation of the myocardium was observed. On reaching the CG, each L-cell fiber produced collaterals that formed a distributed varicos innervation surrounding the 3 large anterior motor neurons (Fig. 7A). The fibers then coursed to the posterior region of the CG where they branched locally and terminated abruptly in the vicinity of the small interneurons (Fig. 7B, arrowhead). No THii innervation was observed around the cell bodies of the 2 posterior motor neurons. Moreover, no
THli fibers were observed to leave the CG in any of its motor roots or dendritic processes.

Together, the distribution of THli material in *Callinectes* suggested that a single central dopaminergic neuron, the L-cell, might regulate cardiac activity in complementary ways. Dopamine released from its terminals in the pericardial organs is likely to act in a neurohormonal fashion on the entire cardiac system, whereas dopamine released from its terminals in the cardiac ganglion might act as a local modulator within specific regions of the ganglion.

Cardioactive actions of dopamine

We tested the effects of exogenous dopamine on each of the 3 preparations: the fully intact working heart, the semi-intact working heart, and the isolated cardiac ganglion. In the intact WH, DA produced increases in contraction frequency and amplitude (Fig. 8). For both parameters, the DA dose–response relation revealed threshold responses in the nanomolar range, with gradual increases in the magnitude of the response up to the micromolar range (Fig. 8, A, C, and D). Despite increases in contraction amplitude of 80–100% above control values in the presence of high DA concentrations, no obvious changes in the shape of the contractions (i.e., in their temporal characteristics such as rise time and decay time) were noted in the presence of DA (Fig. 8B, inset). Phase plots in which the rate of change of the force was plotted against the force of contraction (Fig. 8B) had similar shapes in the presence of DA as under control conditions, indicating that the rates of rise and decay both changed in direct proportion to the increase in the force of contraction.

Of the 3 preparations, we focused particularly on the S-IWH because it allowed simultaneous recording of both the contractions and the underlying motor activity of the CG. The effects of DA on the contractions could then be compared with those in the WH, whereas the effects on the motor activity could be compared with those in the ICG. Furthermore, by showing that the contractions were always coupled one-for-one with the bursts of the underlying motor pattern (Figs. 1B2 and 9A), the S-IWH provided one parameter—the frequency of the contractions or bursts—that could be compared across all 3 preparations.

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**Cardioactive actions of dopamine**

**We tested the effects of exogenous dopamine on each of the 3 preparations: the fully intact working heart, the semi-intact working heart, and the isolated cardiac ganglion. In the intact WH, DA produced increases in contraction frequency and amplitude (Fig. 8). For both parameters, the DA dose–response relation revealed threshold responses in the nanomolar range, with gradual increases in the magnitude of the response up to the micromolar range (Fig. 8, A, C, and D). Despite increases in contraction amplitude of 80–100% above control values in the presence of high DA concentrations, no obvious changes in the shape of the contractions (i.e., in their temporal characteristics such as rise time and decay time) were noted in the presence of DA (Fig. 8B, inset). Phase plots in which the rate of change of the force was plotted against the force of contraction (Fig. 8B) had similar shapes in the presence of DA as under control conditions, indicating that the rates of rise and decay both changed in direct proportion to the increase in the force of contraction.**

Of the 3 preparations, we focused particularly on the S-IWH because it allowed simultaneous recording of both the contractions and the underlying motor activity of the CG. The effects of DA on the contractions could then be compared with those in the WH, whereas the effects on the motor activity could be compared with those in the ICG. Furthermore, by showing that the contractions were always coupled one-for-one with the bursts of the underlying motor pattern (Figs. 1B2 and 9A), the S-IWH provided one parameter—the frequency of the contractions or bursts—that could be compared across all 3 preparations.
As already described, with the exception of absolute contraction amplitudes, the basal properties of the contractions in the S-IWH were similar to those in the WH (Fig. 3A). The effects of DA on the contractions were also similar: both contraction frequency and amplitude were increased (Fig. 9, A–C). Apparent thresholds were in the nanomolar range, and

FIG. 8. Actions of dopamine on the WH preparation. A: contractions recorded from the WH (see Fig. 1A) shown with a compressed time base. Responses to 3 concentrations of dopamine (DA). Onset of DA perfusion is indicated by upward arrows. Calibration bars: vertical = 1 g, horizontal = 30 s. B: preservation of shape of the modulated contraction. Inset: superimposed contractions from the WH under control conditions (Con) and in the presence of 1 × 10^{-6} M dopamine (DA). Although the contraction amplitude was increased by about 90%, the time to peak contraction and the decay time remained unchanged. Main plot: phase plot of the rate of change of the force (y-axis) as a function of the force of contraction (x-axis). Ten contractions are plotted under control conditions and in the presence of 1 × 10^{-6} M DA. Similar shapes of the phase plots indicate that the rates of rise and decay of the contractions are both directly proportional to the amplitude of the contractions under the 2 conditions. C and D: DA concentration dependency of contraction frequency (C) and amplitude (D). Means ± SE from 10 preparations. Smooth curves are best fits of the DA concentration dependency values with the equation: \% Change = a[1 + (EC_{50}/\log_{10}[DA])^b], where [DA] is the DA concentration and a, b, and EC_{50} are parameters of the fit.

FIG. 9. Actions of dopamine on the S-IWH preparation. A1: top record: contraction force. Bottom record: simultaneous extracellular recording from the posterolateral connective (see Fig. 1B). A2: peak response to perfusion of 1 × 10^{-6} M DA in the same preparation as in A1. B–E: DA concentration dependency of contraction or burst frequency (B), contraction amplitude (C), burst duration (D), and the number of impulses per burst (E). Means ± SE from 6–17 preparations. Vertical dashed lines in B–D mark the half-maximally effective concentrations, EC_{50}, obtained by fitting the equation: \% Change = a[1 + (EC_{50}/\log_{10}[DA])^b] (smooth curves).
by fitting the DA dose–response values with standard sigmoidal functions (smooth curves in Fig. 9, B–E; see figure legend), we obtained estimates of the half-maximally effective DA concentration, EC$_{50}$, of 2.4 $\times$ 10$^{-7}$ M for frequency and 1.7 $\times$ 10$^{-7}$ M for amplitude (vertical dashed lines in Fig. 9, B and C, respectively). Apart from frequency, DA had much less potent effects on the parameters of the neural motor patterns. Burst duration was increased, but only by 20–40%, and only with a much higher apparent threshold ($\sim$3 $\times$ 10$^{-7}$ M) and EC$_{50}$ of 6.8 $\times$ 10$^{-7}$ M (Fig. 9D). The number of impulses per burst was not significantly changed by DA at any of the concentrations tested (Fig. 9E).

In the ICG, application of DA increased burst frequency, burst duration, and the number of impulses per burst. It had a relatively small effect on the burst frequency (maximal increase about 20%), but with a low EC$_{50}$, 1.4 $\times$ 10$^{-8}$ M (Fig. 10, A and B). On the other hand, the effects of DA on the burst duration and the number of impulses were much larger (increases of 200 and 60%, respectively), but had much higher EC$_{50}$ values, 1.2 $\times$ 10$^{-6}$ M and 2.0 $\times$ 10$^{-6}$ M, respectively (Fig. 10, C and D).

In Fig. 11 we have superimposed all of the DA dose–response curves from Figs. 8–10 for comparison. Detailed consideration of Fig. 11 is deferred until the DISCUSSION, but clearly the data could not be explained by a single unified effect of DA. Rather, there appeared to be several effects, with different magnitudes and different values of EC$_{50}$. Furthermore, remarkably, the same parameters were modulated by DA differently in the S-IWH and ICG preparations (e.g., in Fig. 11, C and D; see statistical analysis in Fig. 11 legend). How this might happen is considered in the DISCUSSION.

**DISCUSSION**

*Functional topography of the Callinectes cardiac system*

An important aim of the present work was to establish the *Callinectes* cardiac system as a suitable experimental preparation for the study of extrinsic modulation and its functional consequences. For this, it was necessary at the outset to confirm the basic functional topography of the *Callinectes* cardiac system, even though this could be expected to be in many ways similar to that studied in other crab species. Indeed, the general morphological and physiological features of the *Callinectes* CG that we have described here (see also Hawkins and House 1978) are in agreement with descriptions in other crabs, including *Eriocheir japonicus* (Tazaki 1972), *Podopthalmus vigil* (Berlind 1982), and *Carcinus maenas* (Saver et al. 1999). In particular, our characterization enables us to conclude that the number (5), position (3 anterior and 2 posterior), and physiological properties of the motor neurons in the *Callinectes* system correspond to the previous detailed description of crab cardiac functional anatomy in *Portunus sanguinolentus* (Tazaki and Cooke 1979a,b, 1983a).

Of particular importance for our subsequent investigation, the 5 motor neurons were found to fire in precise synchrony. In other crab species, the synchrony is thought to reflect highly effective electrical coupling close to the region of synaptic integration and impulse initiation of all 5 motor neurons. This critical integrative area is located within the anterior portion of the CG (Miorilli et al. 1987; Tazaki and Cooke 1979a, 1983a). The synchrony appears to be unique to crab cardiac systems. In lobsters, the motor neurons of the CG also fire in coordinated bursts but—because the individual neurons have unique, often multiple sites of impulse initiation—not in a precisely synchronized fashion (Friesen 1975a; Hartline 1967). The synchrony of motor neuron firing in the crab CG is experimentally advantageous because it permits monitoring of the motor output from any of the connectives projecting from the CG to the heart musculature. Recordings from muscle fibers demonstrate that excitatory junctional potentials corresponding to this motor pattern occur throughout the myocardium (Benson 1981; our observations). No evidence for synaptic drive to the muscle from any other source is observed.

**FIG. 10.** Actions of dopamine on the isolated cardiac ganglion. *A1:* top record: intracellular recording from a CG motor neuron. Bottom record: simultaneous extracellular recording from the posterolateral connective (see Fig. 1C). *A2:* peak response to perfusion of 1 $\times$ 10$^{-6}$ M DA in the same preparation as in A1. *B–D:* DA concentration dependency of burst frequency (B), burst duration (C), and the number of impulses per burst (D). Means ± SE from 6–10 preparations. Vertical dashed lines mark the half-maximally effective concentrations, EC$_{50}$, obtained by fitting the equation: % Change = $a/(1 + (EC_{50}/log_{10}[DA])^b)$ (smooth curves).
**Catecholaminergic innervation of the cardiac system**

In this work we have used THli as a marker of catecholaminergic localization. For the reasons already presented in RESULTS, the catecholamine that the THli reflects in *Callinectes*, and probably other crustaceans, is very likely to be dopamine.

The distribution of THli in *Callinectes* (see also Wood and Derby 1996) indicates that the catecholaminergic innervation of the cardiac system originates from a single CNS neuron, the L-cell. The convoluted course of the L-cell axon that we have observed in the CNS is very characteristic. Maynard (1961b) reported that the largest fiber in the crab segmental nerve 1, which he designated the “a” fiber, followed a unique course on entering the subesophageal ganglion, turning “sharply” toward the circumesophageal connective. Using histofluorescent methods in *Carcinus maenas*, Cooke and Goldstone (1970) then determined that the “a” fiber originated from a large catecholaminergic cell in the commissural ganglion. They were able to trace the large axon of this cell to the brain, back to the subesophageal ganglion, and into a segmental nerve projecting to the pericardial organ. In *Callinectes* itself, Wood and Derby previously identified the L-cell and followed its axon to the brain and back past the commisseral ganglion. These investigators likewise postulated that the *Callinectes* L-cell projected to the PO. Here we have confirmed this projection. In addition, we have made the novel finding, critical for understanding the regulation of the cardiac system, that a branch of the L-cell axon then continues beyond the PO, to the heart. Within the heart, the innervation of the cardiac ganglion by this fiber is consistent with previous descriptions of cardioaccelerator fibers in several crustacean species (Field and Larimer 1975; Maynard 1960; Sakurai and Yamagishi 1998; Yazawa and Kuwasawa 1994).

In studies of the stomatogastric system of lobsters, the L-cell was found to receive depolarizing input corresponding to the esophageal motor rhythm of the foregut (Selverston et al. 1976). Subsequently, the firing pattern of the L-cell of *Homarus* was shown to be influenced by 4 distinct foregut rhythms (Robertson and Moulins 1981). Given the previously demonstrated excitatory effects of dopamine on the pyloric central pattern generator in the stomatogastric ganglion (Anderson and Barker 1981), the L-cell was postulated to regulate the activity of the gut through a positive feedback loop by release of dopamine from its terminals in the PO (Robertson and Moulins 1981). The projections of the L-cell that we have documented here suggest that the L-cell may play an even broader integrative role that includes cardiac responses to increased metabolic or behavioral demands (see Guirguis and Wilkens 1995). The demonstration of long-lasting (16–18 h) increases in heart rate associated with food detection and consumption in *Callinectes* (McGaw and Reiber 2000) is consistent with such a broader role.

Neurons that correspond to the L-cell in a range of decapods all appear to exhibit a catecholaminergic phenotype: *Carcinus maenas* (Cooke and Goldstone 1970); *Panulirus interruptus* (Barker et al. 1979; Kushner and Barker 1983); *Homarus gammarus* (Cournil et al. 1984, 1994); *Homarus americanus* (Siwicki et al. 1987); *Cancer irroratus* and *boealis* (Marder 1987); *Callinectes sapidus* (Wood and Derby 1996; this study); and *Macrobrachium rosenbergii* (Sosa et al. 2002). However, there appears to be substantial variability in the cotransmitter content of L-cells. In *Homarus gammarus* (Cournil et al. 1984) and *Macrobrachium rosenbergii* (Sosa et al. 2002), the L-cell contains serotonin immunoreactivity. Proctolin-like immunoreactivity is present in the L-cell of *Homarus americanus* (Siwicki et al. 1987), *Cancer irroratus* and *C. boealis* (Marder 1987), and *Callinectes sapidus* (Marder 1987).
et al. 1986), but not in *Procambarus clarkii* (Siwicki and Bishop 1986), *Panulirus interruptus* (Siwicki and Bishop 1986), or *Callinectes*, the subject of the present study (Wood and Derby 1998; Wood et al. 1996). In view of such cotransmitter diversity, it will be interesting to examine the generality of the L-cell projection to the CG that we have identified. It is possible that the cotransmitter diversity reflects somewhat different modes of use of the L-cell in different species. Some species may use the L-cell mostly for hormonal release through the PO, others for direct modulation of the CG, and still others in the dual mode of modulation that, we propose, occurs in *Callinectes*.

The limited distribution of THli within the CG appears to arise exclusively from the single pair of L-cell fibers that enter the heart by the dorsal nerves (Fig. 7; see also Yazawa and Kuwasawa 1994). The presence of dopamine within these fibers may account for earlier biochemical measurements of catecholamines in the lobster cardiac ganglion (Ocorr and Berlind 1983). The localization of THli does not support a role for dopamine as the neurotransmitter of the motor neurons, a function for which L-glutamate is currently a leading candidate (Benson 1981; Cooke 1966; Delgado et al. 2000; Yazawa et al. 1998). The absence of THli innervation in the region of the cell bodies of the posterior motor neurons (Fig. 7B) suggests that the innervation of the 5 motor neurons may not be uniform. Direct modulation of the posterior motor neurons cannot be excluded, however, because their synaptic input and impulse initiation occur in the anterior portion of the ganglion, in a region that receives substantial THli innervation (Fig. 7A; see Cooke 2002; Mirolli et al. 1987; Tazaki and Cooke 1983a). It is also notable that the THli projections are confined to the ganglion itself. In this regard, they differ from the “System II” cardio regulatory fibers described by Alexandrowicz (1932) and the catecholaminergic innervation of the hermit crab, where the regulatory axons innervate myocardial cells (Yazawa and Kuwasawa 1994). Finally, the localization of THli terminals to the neuropil and somatic regions of the ganglion indicates that the distal dendritic processes projecting into adjacent muscle fibers (Fig. 2B) are also not targets of this regulation. This contrasts to GABAergic inhibitory regulation for which extensive dendritic innervation has been observed (“System I” fiber of Alexandrowicz 1932; Delgado et al. 2000).

In sum, we propose that the pattern of L-cell innervation that we have found indicates a dual mode of action of the L-cell neurotransmitter, dopamine, on the cardiac system of *Callinectes*. Dopamine released from the L-cell terminals in the pericardial organs is likely to act in a neurohormonal fashion on the entire cardiac system, including its periphery, the cardiac musculature. At the same time, dopamine released from the terminals in the cardiac ganglion acts as a local modulator of the central motor pattern generated by the ganglion. We propose that this dual innervation provides an anatomical substrate for the physiological actions of dopamine that we have found, as discussed next.

**Cardioactive actions of dopamine**

The cardioactive effects observed here are in many ways similar to those reported in other arthropods where dopamine typically produces increases in contraction frequency and amplitude (Augustine et al. 1982; Berlind et al. 1970; Cooke and Sullivan 1982; Florey and Rathmayer 1978). Because dopamine also produces increases in burst frequency, burst duration, and the number of impulses per burst in isolated cardiac ganglia (Berlind 1998; Miller et al. 1984), it is easy to assume that all of the effects observed in the cardiac system are a simple consequence of its central actions. However, when the actions of dopamine are quantitatively compared on the CG with and without the peripheral cardiac musculature, as we have done here, it becomes clear that matters are likely to be considerably more complex.

The similarity between the basal contraction parameters of the S-IWH and the fully intact WH (Fig. 3, A1–A3), coupled with the comparable dose dependency of the effect of dopamine on contraction amplitude (Fig. 11A), support the conclusion that (except for the absolute magnitude of the contraction) the properties of the S-IWH faithfully reflect those of the intact system. In the S-IWH, and so presumably in the WH, there is essentially no effect of dopamine on the burst parameters of the underlying motor pattern, such as burst duration (Fig. 11C) or the number of impulses per burst (Fig. 11D). Yet there is a large increase in contraction amplitude (Fig. 11A). This increase could be a secondary consequence of the dopamine-induced increase in burst and contraction frequency (Fig. 11B; see Mahadevan et al. 2004). Alternatively or in addition, however, it could also be produced by a direct action of dopamine on the peripheral cardiac musculature. Peripheral effects of modulators, including dopamine, acting both presynaptically at neuromuscular junctions and postsynaptically on the myocytes themselves, are common in other muscles of decapods (e.g., Breen and Atwood 1983; Djokaj et al. 2001; Fischer and Florey 1983; Florey and Rathmayer 1978; Jorge-Rivera et al. 1998; Kravitz et al. 1980; Lingle 1981), other arthropods, and invertebrates generally (Calabrese 1989; Evans and Myers 1986; Worden 1998). A peripheral dopaminergic action would presumably be attributable to dopamine released neurohormonally from the pericardial organs (dashed arrows in the summary diagram presented in Fig. 12A and in more detail in Fig. 12B), given that the cardiac musculature is not directly innervated by the dopaminergic L-cell.

All of the other effects of dopamine are likely to be initiated centrally, through the direct innervation by the L-cell of the CG. The data are consistent with a single central effect of dopamine on the burst frequency of the motor pattern and, consequently, because contractions follow the bursts one-for-one (Fig. 1B2), on contraction frequency (Fig. 11B). The effects of dopamine on burst or contraction frequency are statistically indistinguishable in the WH and S-IWH (see Fig. 11 legend). In the ICG, the effect is significantly smaller, but in a characteristic way. Because the absolute burst frequency is higher in the ICG under control conditions (Fig. 3B1, and “Control” inset of Fig. 11B), the smaller increase brings the frequency at high dopamine concentrations to almost exactly the same absolute value in the ICG as in the S-IWH (“10−6 M DA” inset of Fig. 11B) and WH. This maximal frequency, in the range of 20–25 bursts or beats/min, may thus constitute a ceiling above which the cardiac ganglion cannot accelerate. A single central effect of dopamine on frequency thus appears to be the most parsimonious explanation. In previous studies using ligatures and Vaseline wells, dopamine was found to affect ganglionic burst frequency most strongly when applied to the posterior region of the CG (Miller et al. 1984; see also
Berlind, 1998, 2001a). We propose therefore that the effect on frequency is achieved primarily by dopamine release from the projections of the L-cell that terminate in the area of the small interneurons (thin solid arrows in Fig. 12, A and B), which act as pacemakers of the crab cardiac system (Tazaki and Cooke, 1979a, 1983a,b). There is yet a third effect of dopamine, which is seen in the ICG and is thus presumably a direct central effect: the increase in burst duration and the number of impulses per burst, which extends into the muscle surrounding the CG (Fig. 2, A and B). The “high” DA concentrations required to produce these effects are still only of the order of 10⁻⁶ M, and thus quite possibly reached with endogenous release of dopamine. However, the physiological significance of these observations remains to be established.

The most intriguing feature of this third effect of dopamine is that it is seen in the isolated CG but not when the CG remains embedded within the cardiac musculature. Furthermore, as with frequency, even the basal values of these parameters, the burst duration and the number of impulses per burst, are different in the ICG and S-IWH (Fig. 3, B2 and B3). We conclude that, in the intact system, feedback from the cardiac musculature regulates the parameters of the motor pattern produced by the CG as well as the modulation of these parameters. Several feedback mechanisms that could help explain our data are already known to exist in crustacean cardiac systems. First, the CG burst duration is typically found to be reciprocally related to the burst frequency. This relation is attributable, at least in part, to the properties of the motor neuron driver potentials, which are smaller at higher frequencies (Tazaki and Cooke, 1990). Because dopamine increases burst frequency by acting on the small pacemaker interneurons, this relation, opposing the simultaneous increases in burst duration and the number of impulses per burst produced by the action of dopamine directly on the motor neurons, could serve to maintain these parameters approximately constant (Fig. 12B), as we have observed is the case in the intact system. However, at least in its simplest form, this mechanism would provide purely central feedback, operating within the CG itself. More interestingly, Sakurai and Wilkens (2003) recently reported that increasing tension of the cardiac musculature [i.e., contraction amplitude (Fig. 12B)] exerts negative feedback effects on parameters of the motor pattern produced by the CG. Mechanistically, this kind of negative feedback from the periphery to the center could be implemented by direct mechanosensitive hyperpolarization of the motor neuron dendrites that extend into the muscle surrounding the CG (Fig. 2B; Sakurai and Wilkens, 2003; see Cooke, 2002) or by a retrograde diffusible messenger (e.g., NO; see Mahadevan et al., 2004).

Central-peripheral integration through extrinsic modulation

The relation between the CG motor pattern and contractions of the myocardium is not well understood and is likely to be complex. Development of tension in crustacean muscle fibers is thought to be directly related to the degree to which the muscle membrane is depolarized (Orkand, 1962). For example, Anderson and Cooke (1971) showed that each burst of impulses from a lobster CG produced a complex synaptic depolarization that preceded and accompanied the early phase of cardiac contraction (see also Benson, 1981). Contraction was graded and could be elicited with depolarizations as small as 5 mV. Regenerative membrane responses were not detected in the lobster myocardium, but they have been observed in other crustacean hearts (compiled in Anderson and Cooke, 1971). Precisely to bridge such complexities of contraction–excitation coupling, a general analytical approach, termed the neuromuscular transform, was recently developed (Brezina et al., 2000a,b, 2003a,b). This approach simply considers the input–output relation between the motor neuron firing pattern and the muscle contraction. In the S-IWH preparation that we have developed here, we can record and, preliminary work (Fort et al., 2004) shows, also experimentally manipulate simultaneously, both the motor neuron firing pattern and the contractions. This ability promises to make the Callinectes cardiac system highly suitable to the neuromuscular-transform approach. Indeed, the work presented here has already produced a body of data that can begin to be analyzed in this way.

Work with the neuromuscular transform in Aplysia (Brezina et al., 2000b), as well as more general computational and neuroethological considerations (Chiel and Beer, 1997), suggest that central motor commands and the properties of the peripheral musculature must be matched and coordinated if...
functional behavior is to be produced. This coordination must be maintained throughout all plastic changes in the behavior. Such coordination can ideally be implemented by extrinsic modulation, originating from a single source but ramifying to both the center and the periphery. In this work, we have documented the likely existence of such a mechanism in the Callinectes cardiac system. The anatomy of the L-cell suggests that it releases dopamine both as a neurohormone onto the entire heart and as a local neurotransmitter within the CG, and we have found multiple physiological effects of dopamine that probably correspond. Furthermore, by comparing the 3 preparations in which the cardiac musculature was removed from the CG to different degrees, we have found evidence for feedback coupling from the periphery to the center. Such feedback—in more complex systems, implemented by dedicated sensory neurons—is common in neuromuscular systems, and its presence immediately raises important dynamical issues, of stability for example, that the mechanisms of central-peripheral coordination and modulation must deal with. These issues were not addressed in the original work with the neuromuscular transform in Aplysia, largely because of a lack of suitable experimental preparation. In its exceptional simplicity and experimental tractability, yet already with multiple sites of modulation and feedback, we believe that the Callinectes cardiac system will prove to be a suitable preparation for the study of these questions.

GRANTS

This work was supported, in part, by a National Science Foundation CAREER Award IBN-9722349, DBB0115825, and National Institutes of Health Grants NS-07464, NS-039405, NS-045546-02, NS-41497, MH-048190, RR-03051, and GM-08224.

REFERENCES


