the snail Helix pomatia L. Comp. Biochem. Physiol. *85C* (1986) 419–425.
30 S.-Rózsa, K., and Solntseva, E.J., Modulation of cholinergic transmission by opiate peptides and FMRFamide on identified neurons of Helix pomatia L. (Gastropoda, Mollusca). Acta physiol. hung. *67* (1986) 429–433.
31 Stefano, G.B., Hiripi, L., S.-Rózsa, K., and Salánki, J., Behavioural effects of morphine on the land snail Heleix pomatia. Demonstration of tolerance. Adv. Physiol. Sci. Vol. 23. Neurobiology of Invertebrates, pp. 285–294. Ed. J. Salánki. Akadémiai Kiadó, Budapest, Pergamon Press 1981.

0014-4754/87/090965-08$1.50 + 0.20/0

Neural control of the circulatory system of *Aplysia*

by J. Koester and U.T. Koch*

*Center for Neurobiology and Behavior, Columbia University, and New York State Psychiatric Institute, 722 West 168th Street, New York (New York, USA), and *FB Biologie, Universität Kaiserslautern, Postfach 3049, D-6750 Kaiserslautern (Federal Republic of Germany)*

Key words. Aplysia; acetylcholine; serotonin; heart; cardiovascular system; respiratory system; egg-laying; feeding; arousal; excretion.

Introduction

The marine gastropod *Aplysia* offers two distinct advantages as an experimental preparation for studying cellular neuronal control of the circulation. First, its neurons are large in size and manageable in number, making it possible to identify single neurons as unique individuals that can be recognized in all members of the species. This feature allows one to work out neuronal networks in cellular detail. Second, there exists a significant body of data on the neural control of a variety of behaviors in *Aplysia*, including defensive withdrawal, locomotion, feeding, egg-laying, control of water balance, and respiration[28]. Because circulatory function typically changes as part of an overall pattern of physiological adjustment, it is necessary to understand the control of all of the major organ systems in the body before one can fully describe the mechanisms and the nature of cardiovascular control. Although we are still a long way from such an understanding, a promising beginning has been made.

The goal of this paper is to review the present state of our knowledge of the cellular and network mechanisms of cardiovascular control in *Aplysia*. The first part describes the circulatory system and the neurons that are known to affect it directly. The second part describes what is known about how the actions of these cells are integrated by the central nervous system during excretory, egg-laying, feeding, and respiratory behaviors. With a few exceptions, the work described below has been performed on *A. californica*.

Anatomy and function of the circulatory system

Eales' description[18] of the circulatory system of *A. punctata* fits quite closely the circulatory system of *A. californica*. Blood enters the two-chambered heart from the efferent veins of the kidney and the gill, which are in parallel (fig. 1). The auricle pumps blood into the ventricle, which feeds the systemic circulation by three parallel pathways. Two of these arterial pathways go exclusively to visceral organs, while one goes primarily to somatic tissue. The visceral tissues are supplied by: 1) the abdominal aorta, which perfuses the hepatopancreas and the ovotestis; and 2), the gastroesophageal artery, which goes to the esophagus and stomach. The so-

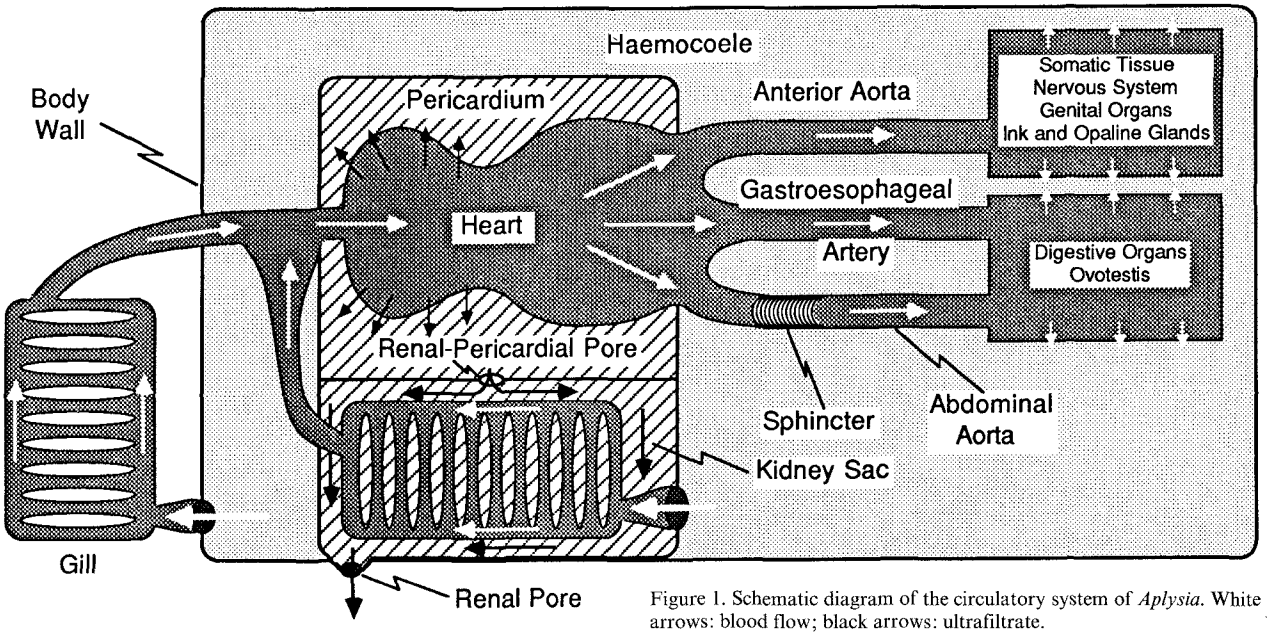

Figure 1. Schematic diagram of the circulatory system of *Aplysia*. White arrows: blood flow; black arrows: ultrafiltrate.

matic tissues are supplied by the anterior aorta, which perfuses the nervous system, the muscle of the buccal mass, and the body wall, including the foot, parapodia, mantle shelf, siphon and tentacles. This artery also provides minor branches to various internal organs, including the opaline gland, the hermaphroditic duct, the accessory genital mass, and the penis. In this open circulatory system, the fine branches of the arterial tree open directly into interstitial spaces. Blood flows from the various organs into the hemocoele, which in turn empties into muscular venous sinuses that supply the kidney and the gill.

The heart is enclosed in a pericardium, a remnant of the true coelom. It is thought that filtration across the wall of the heart forms an ultrafiltrate that becomes the pericardial fluid[18]. This fluid flows through the renal-pericardial pore into the other portion of the coelomic space, which is within the kidney. There it is elaborated by secretion and reabsorption into urine, which is excreted through the renal pore into the mantle cavity.

The arteries, which are relatively thin-walled, lack significant elasticity. Arteries that supply the viscera have primarily circular muscle, while those that perfuse somatic tissues generally have both circular and longitudinal muscle. The proximal end of the abdominal aorta has an especially thick ring of circular muscle that functions like a sphincter.

The heart beat is myogenic, though its strength and frequency are modulated by neural input from the abdominal ganglion. The circular and longitudinal muscle of the arteries also receives innervation from the abdominal ganglion as well as other parts of the nervous system.

There have been only a few attempts to study the functional role of the circulatory system in *Aplysia*. The standard function of supplying oxygen and nutrients and removing waste products from the tissues is consistent with the fact that the blood contains the respiratory pigment hemocyanin[18], and with early physiological studies[83]. In addition, because its shell is merely a rudimentary disc, *Aplysia* must rely to some extent on a hydrostatic skelton for postural support and to assist in movements. The contribution of the circulatory system to the latter function remains undocumented, as does its putative role in the formation of the ultrafiltrate that is elaborated into urine. While a number of cardiovascular adjustments have been found to be elicited by environmental stimuli (see below), we know little about the functional effects of the adjustments on the other organs of the body. There clearly exists a need for better understanding of the basic cardiovascular physiology of *Aplysia* before it will be possible to gain a comprehensive picture of its neural control.

Motoneurons, neurotransmitters and hormones that act on the circulatory system

Control of the heart

The heart is innervated by four, and possibly more classes of neurons. All of the identified cardiac control neurons are located in the abdominal ganglion.

Heart excitor RB_{HE}. Hill showed in 1964 that the hearts of *A. fasciata* and *A. depilans* are excited by serotonin. Histological and neurochemical studies then demonstrated that *Aplysia* heart contains serotonin, localized primarily to nerve fibers[8, 11, 78]. A neuron in the RB cluster, cell RB_{HE}, was found to mimic the actions of serotonin on the heart of *A. californica*[41, 48]. This neuron is thought to be serotonergic because its motor effects are blocked by a serotonergic antagonist, it contains the metabolic machinery for synthesizing serotonin[41], and it exhibits serotonin immunoreactivity[31].

The primary action of serotonin on *Aplysia* heart in situ is to cause an increase in rate that outlasts the presence of the transmitter by several tens of seconds[48]. While a positive inotropic effect is sometimes also observed, it is more commonly seen with isolated cardiac tissue. At least some of the effect of serotonin on *Aplysia* heart is mediated by an accumulation of myocardial cyclic AMP[17, 30, 43, 45], which in turn may act on intracellular calcium stores[68].

Heart excitor LD_{HE}. Unlike RB_{HE}, cell LD_{HE} produces an increase in heart rate that typically lasts for only one beat. Its transmitter type is unknown[48].

Multi-modal motoneuron L_7. Cell L_7 has well-described actions as a motoneuron to the gill and siphon[7, 37, 53, 54]. The excitation of the gill is effected by direct connections to muscle, as well as by indirect connections via gill motoneurons located in the branchial ganglion[40]. In addition, L_7 produces conventional fast excitatory junctional potentials (ejps) in the auricle of *Aplysia* muscle. These ejps can initiate a beat in the quiescent heart[1]. The transmitter used by cell L_7 has not been identified, although it is thought not to be acetylcholine or serotonin[22, 31].

Neurosecretory cells R_7, R_8, and R_{15}. The heart has also been shown to contain the processes of two types of neurosecretory neurons.

Cells R_7 and R_8 are members of a cluster of 12 neurosecretory neurons, R_3–R_{14}. The cells in this cluster all express a common gene that is thought to encode three neuropeptides with molecular weights of 1.3, 3.3, and 5.0 kd[52]. One of the peptides encoded in the DNA, peptide II, has been isolated from extracts of the abdominal ganglion. There is strong pharmacological, neurochemical and morphological evidence that the cells of the R_3–R_{14} cluster also utilize glycine as a transmitter[25, 51, 57, 58, 69]. Two of these 12 neurons, cells R_7 and R_8, were found to send processes to the heart[61]. Activity in R_7 and R_8 has not yet been demonstrated to affect the heart, and peptide II appears not to affect the circulatory system[64]. Glycine, on the other hand, has been shown (at mM concentrations) to increase heart rate and frequency, and to cause release of preloaded Ca^{++} from ventricular muscle[69].

Cell R_{15} also has been shown to send processes to the heart, as well as to a number of other vascular spaces[60]. This neuron elaborates a neuropeptide that has been implicated in the control of water balance and hemolymph composition[3, 39, 75]. Neither the R_{15} peptide nor R_{15} activity has yet been shown to modify cardiac function.

Heart inhibitors LD_{HI}. Hill first showed that hearts of *A. fasciata* and *A. depilans* are inhibited by acetylcholine[24]. Ten years later the twin LD_{HI} cells were found to mimic the effects of acetylcholine on the heart[41, 48]. These cells are thought to be cholinergic because they have the metabolic machinery for synthesizing acetylcholine, and their motor effects are blocked by a selective cholinergic antagonist[41]. Acetylcholine had no effect on cyclic AMP concentration in ventricles from *A. kurodai*[68].

Other cardioactive substances

Aplysia heart is excited by perfusion with two neurotransmitters which, though endogenous to the animal, are not detectable in cardiac tissues[8, 43].

Dopamine mimics the positive chronotropic effects of serotonin on the heart at from 10- to 40-fold higher concentrations[30, 41, 82]. It has been suggested that dopamine and serotonin activate different types of myocardial receptors[30], because dopamine elicits a much smaller increase in adenylate cyclase activity than that produced by serotonin[17, 30]. The functional significance of these dopamine receptors is not clear.

Small cardioactive peptides (SCPs) A and B, isolated from the nervous system of *Aplysia*, both increase the strength and frequency of heart beat[9, 43]. This effect is thought to be mediated by an increase in cyclic AMP for two reasons[43]: 1) it is mimicked by the adenylate cyclase activator forskolin; and 2) the time course of the physiological effects parallels the

time course of the changes in cyclic AMP levels. The SCPs could not be detected in the hemolymph with an assay sensitive to levels as low as 10^{-11} M.

Control of the arterial system

There are at least eight classes of neurons that modulate vascular muscle in *Aplysia*. All of the known vaso-modulatory neurons are located in the abdominal ganglion.

Visceral arteries

LB_{VC} vasoconstrictor cells. The circular muscle of the abdominal aorta and gastroesophageal artery is innervated by a cluster of three neurons called the LB_{VC} cells[48]. These cells all appear to be functionally equivalent except for the fact that one of them innervates only the abdominal aorta. Pharmacological and neurochemical evidence supports the conclusion that the LB_{VC} cells are cholinergic[41]. Members of this group also innervate the circular muscle of the genital artery (unpublished observations).

Multimodal motoneuron L_7. Cell L_7 activates the circular muscle of the abdominal aorta by eliciting fast, conventional ejps. There is a powerful synergistic interaction between the contractile -- activating effects of L_7 and the LB_{VC} neurons[1].

Bag cells. The two-bag cell clusters contain approximately 800 neurosecretory cells, all of which express a small family of common genes encoding several neuropeptides[70]. Two of the peptides encoded by this gene family, egg-laying hormone (ELH) and alpha bag cell peptide (α BCP), have been demonstrated to be synthesized and released by the bag cells and to act as neurotransmitters and as hormones[50, 73]. Activating the bag cells by electrical stimulation causes a long lasting bout of phasic contractions of the longitudinal muscle of the gastroesophageal artery. Application of crude bag cell extract to the isolated artery mimics the effects of bag cell stimulation[42].

Serotonin. Glyoxylic acid treatment of the abdominal aorta reveals extensive branching of varicosity-laden fibers that fluoresce with the same characteristics as serotonin. The origin of these fibers is not yet known, but it has been found that arterial perfusion with serotonin reduces the vasoconstriction elicited by L7 activity[1].

Somatic arteries

Excitatory inputs to the anterior aorta. At least three types of fast, conventional ejps have been recorded from muscle of the proximal end of the anterior aorta[65, 66, 68]. Anatomical evidence suggests that the ejps were recorded from longitudinal muscle[59]. A cell has been identified in the ganglion that mediates one of these types of ejp, cell RD_{AAE}[65]. Based on pharmacological and ultrastructural evidence, acetylcholine, serotonin and glutamate are all plausible excitatory transmitter candidates for this muscle[59, 66–68]. Serotonin increases cyclic AMP and decreases cyclic GMP levels in the anterior aorta, and acetylcholine decreases cyclic AMP levels in the same tissue[68].

RD_{AAI}. Anterior aorta inhibitor cells. Sawada et al.[65] have identified a pair of motoneurons that inhibit the anterior aorta. The transmitter type of these cells is not yet known, but acetylcholine is a plausible candidate on the basis of pharmacological evidence[67].

Neurosecretory cell R_{14}. Like cells R_7 and R_8, R_{14} is a neurosecretory cell that is thought to release glycine and three low molecular weight peptides[52, 57] (see above). R_{14} has a widely divergent range of ramifications in the periphery, including a set of terminals that make direct contact with longitudinal muscle of the proximal anterior aorta[59, 62]. Firing R_{14} enhances the strength of phasic contractions elicited by nerve stimulation, and converts the contractions from monophasic to rhythmic. Application of 1 mM glycine to the muscle mimics the effects of R_{14} activity[69].

Dopamine. Glyoxylic acid treatment of the proximal end of the anterior aorta reveals moderate branching of varicosity-laden fibers with catecholamine-like fluorescence properties (unpublished observations). The origin of these fibers, which presumably contain dopamine, is not yet known.

Neuronal and hormonal integration of cardiovascular function

Heart rate

Heart rate recorded in the intact animal has been shown to change in response to a number of environmental stimuli. Heart rate increases are elicited by noxious or food stimuli (fig. 2), by increased temperature, and by moderate hypoxia. Heart rate also increases during and following a meal, and during spontaneous increases in overall activity of the animal. In addition to these studies on the intact animal, phasic spontaneous increases in heart rate have been recorded in a semi-intact preparation[36]. Heart rate is decreased by exposure to air[20], by extreme hypoxia, during eating in animals that are nearly satiated[16], and during respiratory pumping[6].

Three of these heart rate responses have been found to be under direct neural control. Experiments utilizing nerve lesion or intracellular recording suggest that the spontaneous increase in heart rate, the increase triggered by food stimuli, and the decrease during respriratory pumping are all neurally mediated[16, 20, 36]. The details of this neural control are presented here and in the following section, which also describes changes in blood flow and blood pressure.

Excretion. The spontaneous heart rate increases recorded in the semi-intact preparation were shown to be mediated by bursts of action potentials generated endogenously by cell L_{10}. This abdominal ganglion neuron acts as a central interneuron and a peripheral motoneuron. In the ganglion, it excites RB_{HE} and inhibits the LD_{HI} cells, thereby causing an

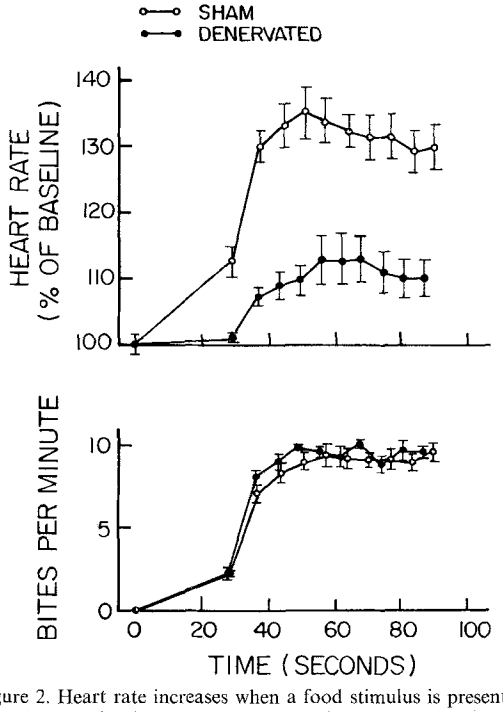

Figure 2. Heart rate increases when a food stimulus is presented to the animal. The stimulus was presented at time zero and was maintained throughout the recording period. Cutting the pericardial nerve eliminated most of the heart rate response, with no effect on the biting response[16].

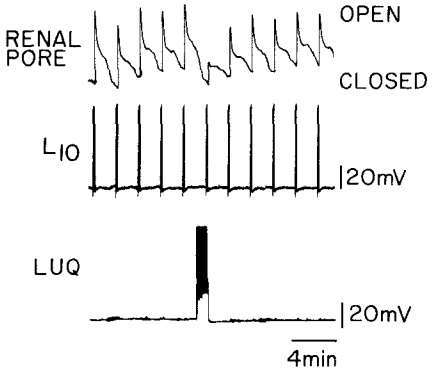

Figure 3. Firing L_{10} (7 Hz) by current injection causes transient opening of the renal pore, and firing an LUQ cell (1 Hz) closes the pore and antagonizes the effect of L_{10}. Pore opening was monitored with a photocell.

increase in heart rate[36]. The former effect is most important, because the LD_{HI} cells are normally silent. Cell L_{10} inhibits all cells in the cluster of identified cells L_2, L_3, L_4, L_5, and L_6, which also have endogenous bursting properties[27]. Neurons L_2–L_6, which are members of the left upper quadrant (LUQ) group, exhibit FMRFamide immunoreactivity[4], apparently due to the presence of a neuropeptide that contains a portion of the FMRFamide sequence[72]. A subset of these five cells send their axons to the renal pore, which closes when they are active. Cell L_{10} also sends an axon to the pore, and when it fires the pore opens (unpublished observations and fig. 3). Thus the overall effect of L_{10} is to promote renal excretion. The increase in heart rate caused by L_{10} may facilitate this process by enhancing the rate of filtration across the wall of the heart.

The spontaneous heart rate increases recorded in the semi-intact preparation have not been detected in recordings from the intact animal. Perhaps the appropriate stimulus for triggering L_{10} activity has not been tested. Alternatively, the motor effect of RB_{HE} may be more inotropic than chronotropic in the intact animal.

Blood pressure and blood flow

Heart rate as such is important to the animal only in so far as it affects blood flow and pressure. Recent studies have focussed on the changes in blood pressure and regional blood flow, which are the physiologically relevant outputs of the cardiovascular system. These studies have used both intact animals and semi-intact preparations:

Egg-laying. Egg-laying is a complex behavioral pattern that involves finding an appropriate location for egg deposition, packaging and releasing eggs, and attaching the eggs to the substrate with a stereotyped pattern of head movements[2]. The entire behavior takes about 30–60 min. Early in the behavioral sequence, all of the cells in the bag cell cluster are activated in a burst of activity that lasts for several minutes[12]. ELH released by this burst triggers ovulation by acting on the ovotestis[63]. The peptides released by the bag cells have a wide variety of effects throughout the nervous system, but none of these central actions have been related directly to features of the egg-laying behavior observed in the intact animal.

There are two cardiovascular adaptations that seem likely to occur during egg-laying, based on the known effects of bag cell activity. First, because L10 is excited and the LUQ cells L2–L6 are inhibited by bag cell activity[46,47], one might expect an increase in renal excretion accompanied by an increase in heart rate. Second, recording in the semi-intact preparation, Ligman and Brownell have shown that bag cell activity initiates phasic contractions of longitudinal muscle in the anterior aorta and gastroesophageal artery[42]. They have suggested that the effect of these contractions may be to divert a greater fraction of cardiac output to the ovotestis and hermaphroditic duct.

Food elicited arousal. Susswein, Weiss and Kupfermann have shown that exposure of *Aplysia* to food (seaweed) elicits an arousal state that builds up gradually over the course of a few minutes[77]. The arousal is manifest as a progressive increase in the strength and frequency of biting in response to a stimulus of fixed intensity. Part of the mechanism for this enhanced efficiency of eating is mediated centrally, and part of it is due to peripheral changes at the buccal muscle[80].

Cardiovascular changes have also been recorded during food arousal with a time course that parallels that of the changes in the biting movements. As mentioned above, heart rate increases in synchrony with the increase in biting rate (fig. 2)[16]. In addition, there is an increase in mean blood pressure and flow recorded from the anterior aorta during arousal (fig. 4)[32]. Short-term occlusion of the anterior aorta caused a 40–50% decrease in the efficacy of biting, suggest-

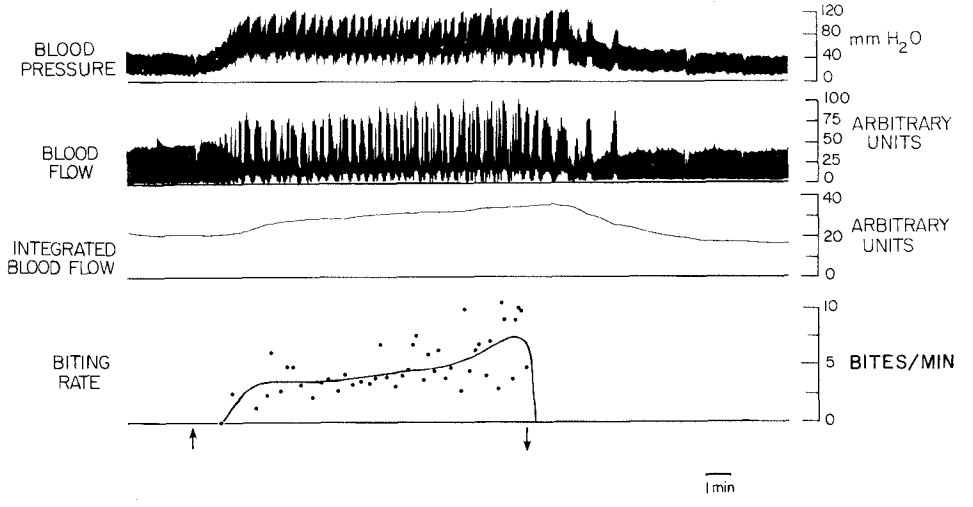

Figure 4. Blood pressure and blood flow recorded from the proximal anterior aorta increases during food arousal, along with biting rate. The choppiness of the instantaneous flow and pressure records is the result of time-sharing of cardiac output between two vascular beds (c.f. fig. 6). Food was presented between the two arrows[32].

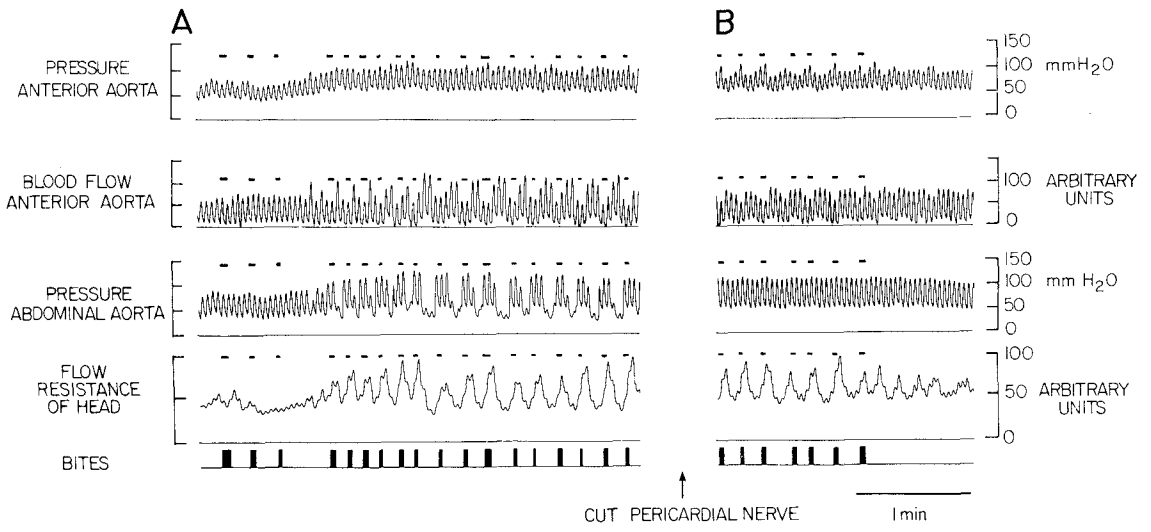

Figure 5. *A* During food arousal, resistance of the distal anterior aorta and of the proximal abdominal aorta change in alternation. Pressure was recorded in the anterior aorta proximal to the main site of resistance increase, while in the abdominal aorta the recording was made distal to the major site of resistance change (fig. 6). With this configuration, increased pressure indicates increased resistance in the former case, and decreased resistance in the latter. *B* Cutting the pericardial nerve eliminates the resistance changes in the abdominal aorta, which are caused by phasic firing of the LB_{VC} motoneurons.
Food presentation began at the start of the left hand set of traces, and was begun some time before the beginning of the right hand set[32]. Horizontal bars above traces indicate protraction of the buccal mass.

ing that the increases in flow and pressure measured in this vessel during arousal are important for the normal functioning of the feeding apparatus.

In addition to these tonic changes in pressure and flow during arousal, there are also phasic changes[32]. The first step in initiating biting is for the buccal mass to move forward into a cocked position. From there it alternates between a protracted and a retracted position[81]. During the retraction phase of biting, the flow resistance of the distal anterior aorta rises considerably above baseline, and most of the cardiac output is diverted to the gastroesophageal artery and the abdominal aorta. During the protraction phase, flow resistance of the anterior aorta returns to normal, and the resistance of the abdominal aorta increases dramatically (figs 5 and 6). It is not known whether the resistance changes in the anterior aorta are the passive results of the biting movements, or whether they are caused by vasomotor activity. Whatever the cause, they are balanced by the alternating changes in resistance of a parallel vessel, the abdominal aorta. The result of this alternation is to dampen partially the surges in systemic pressure that result from the phasic increases in flow resistance of the anterior aorta. Thus by 'time-sharing' the cardiac output, each of the two major vascular beds can maintain an adequate time-averaged blood flow, even when experiencing transient peaks in resistance.

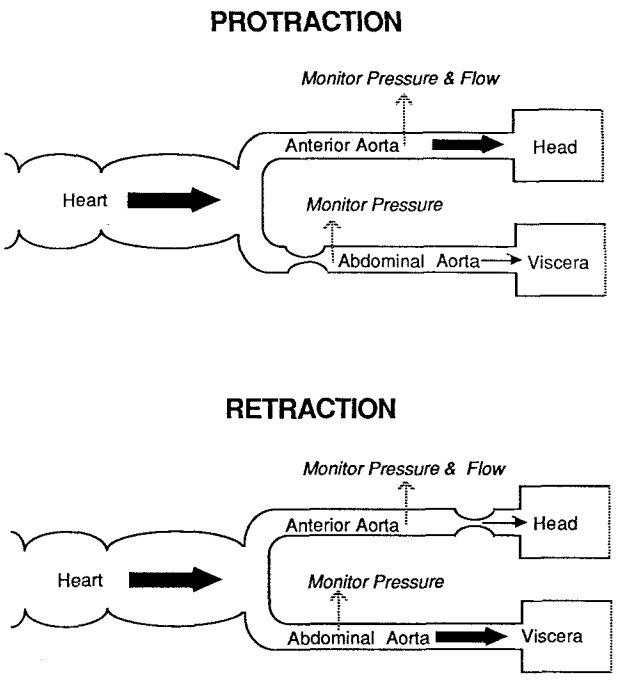

Figure 6. Summary of the vascular resistance changes that occur during biting (c.f. figs 4 and 5).

The neural mechanisms that mediate the cardiovascular components of arousal were investigated by recording from cardiovascular motoneurons in the semi-intact preparation while the animal was aroused with food[33]. Both RB_{HE} and the LB_{VC} cells were found to increase their firing rates during arousal, with the LB_{VC} cells firing in phase with the protraction phase of biting (fig. 7). These results, together with the results of lesion experiments (figs 2 and 5), lead to three conclusions: 1) the increase in heart rate is mediated in part by RB_{HE}; 2) increased firing in RB_{HE} and LB_{VC} both contribute to the increased flow and pressure recorded in the anterior aorta; 3) the phasic decreases in flow and pressure recorded in the distal abdominal aorta are mediated by the activation of the LB_{VC} cells during the protraction phase of biting. The results of the lesion experiments suggest that other, unspecified cells or hormones must also play a significant role in producing the increases in heart rate and particularly the increase in systemic pressure (fig. 3)[16, 32].

The fact that the tonic heart rate effects are mediated by the serotonergic cell RB_{HE}, while the phasic vasomotor effects are generated by the cholinergic LB_{VC} cells, fits with a general pattern of transmitter function found in *Aplysia*. Serotonergic cells are employed most typically where slow, modulatory actions are required, often mediated by changes in cyclic AMP. Cholinergic neurons, on the other hand, are more frequently employed to produce rapid, mediatory effects[7, 21, 29, 41, 80].

Respiratory pumping. Aplysia exhibit, with varying degrees of frequency, a stereotyped behavioral pattern called respira-

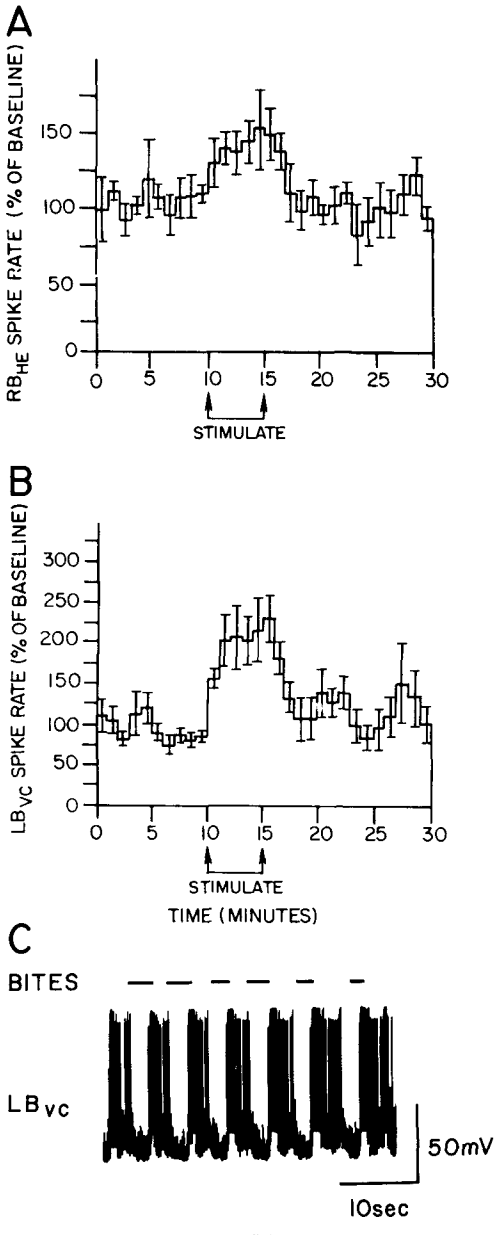

Figure 7. *A* and *B* RB_{HE} and LB_{VC} firing rates increase during arousal elicited by food stimuli (between arrows); *C* During rhythmic biting, the LB_{VC} cells are activated during the protractions phase of biting, and inhibited during retraction. (Retraction marked by horizontal bars)[33].

tory pumping. It consists of a brief, phasic contraction of the parapodia and the mantle organs. As the gill and siphon contract, seawater is flushed out of the mantle cavity, and within a few seconds is replaced by freshwater as the organs relax. Internally, these events are accompanied by cardiac inhibition. As the gill contracts, it forces blood through the relaxed heart, into the systemic circulation.

It is has been suggested that the effect of this behavior is to enhance the gradient for the exchange of blood gases across the gill[6, 36]. The hypothesis that this behavior enhances respiration is supported by the fact that its frequency is increased in situations that lead to respiratory stress. For example, respiratory pumping occurs more frequently following a meal, when the animal is kept in poorly oxygenated seawater for prolonged periods[35], when ambient CO_2 levels are increased[15], during locomotion[23], and following prolonged periods of exposure to air at low tide[38].

Besides occurring spontaneously, respiratory pumping can also be triggered. Weak tactile stimuli to the gill or siphon activate tactile afferents that monosynaptically excite motoneurons that mediate a graded withdrawal reflex involving both organs[10]. Stronger stimuli activate, with a slight delay, an additional all-or-none component of withdrawal that represents the triggering of the respiratory pumping mechanism[55]. Both the direct reflex and the respiratory pumping component exhibit simple forms of non-associative learning. While learning modifies the direct reflex by a graded effect on amplitude[29], the feature of the respiratory pumping component that is modified is its probability of being triggered[56]. There are dozens of identified motoneurons in the branchial, abdominal, and pedal ganglia that mediate respiratory pumping [6, 23, 34]. More recently, two symmetrical clusters of interneurons located in the abdominal ganglion have been shown to act as trigger cells for the behavior: there are 10–15 R_{25} cells in the right hemiganglion, and a symmetrically located cluster of 10–15 L_{25} cells in the left hemiganglion (fig. 8)[5, 6, 34]. There is extensive electrical and chemical synaptic coupling between the cells in the two clusters.

Activity in the R_{25} and L_{25} network is *necessary* for initiating and coordinating the various components of respiratory pumping. When the behavior occurs spontaneously, or when it is triggered by a tactile stimulus to the gill or siphon, the entire population of R_{25} and L_{25} cells begins to burst in synchrony slightly before the onset of the behavior[5, 6, 34]. Hyperpolarizing one or two members of this network can prematurely terminate an individual bout of respiratory pumping, and can also slow the frequency of the spontaneously occurring behavior. Moreover, most, and probably all of the cells in the two clusters project to different subsets of the motoneurons that mediate the behavior. One can not conclude that the population of R_{25} and L_{25} neurons is *sufficient* to account for the initiation of respiratory pumping, however, because there are hundreds of unidentified cells in the ganglion. Any one of these unidentified neurons could contribute to the population burst in the R_{25}–L_{25} cells that triggers the behavior.

The burst generation in the R_{25} and L_{25} cells that drives respiratory pumping can be initiated by either of two related mechanisms. When the behavior is *triggered* by tactile stimuli, RE and LE tactile sensory neurons excite polysynaptically the cells in the R_{25} and L_{25} clusters[6, 34]. As the interneurons begin the fire, they excite each other via their electrical and chemical excitatory synapses. Over the course of 5–10 s this reverberatory activity builds up to a crescendo, with all of the interneurons simultaneously reaching their maximal firing rates. The same effect can be achieved artificially by firing a single R_{25} or L_{25} neuron by current injection (fig. 9). After a brief delay, the positive feedback connections between the cells leads to a network-wide burst that is relayed to the motoneurons that drive the behavior. When the behavior occurs *spontaneously*, the same mechanism is operative, except that the trigger for the burst is a slow pacemaker potential that occurs synchronously in all cells of the network. Tetrodotoxin applied to the ganglion blocks the sodium channels that mediate the action potentials, unmasking slow plateau potentials that are generated by the pacemaker potentials (fig. 10). The slope of the pacemaker potential can be increased by activity in either of two identified neurons called the R_{20} cells.

Burst termination in the pool of interneurons is mediated by two processes. Each cell in the R_{25}–L_{25} network generates an endogenous, long lasting after-hyperpolarization following a high frequency train of action potentials[34]. In addition, a subset of the interneurons elicits slow, long-lasting inhibitory post-synaptic potentials (IPSPs) in other cells in the network (fig. 11).

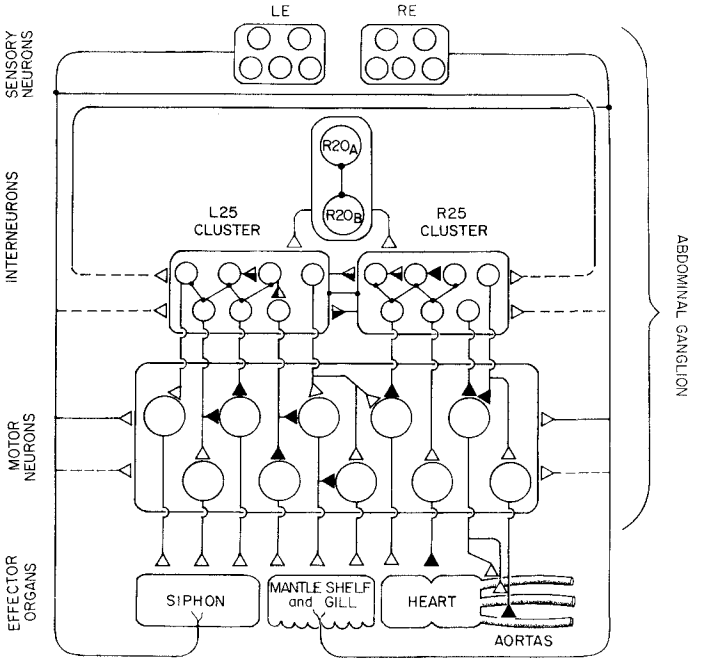

Figure 8. Schematic diagram of the classes of cells that mediate respiratory pumping. Clear terminals are excitatory and black terminals are inhibitory. Black circles indicate electrical synopses. Dotted lines indicate polysynaptic pathways[5,6,34].

Conclusions

The neural circuitry that coordinates the cardiovascular system with the other organ systems of *Aplysia* is quite complex. At least 12 types of motor- and modulatory neurons and nearly an equal number of neurochemical substances have been implicated in this process. This knowledge has accumulated even though our search for control neurons has been limited to the abdominal ganglion, and only a small fraction of the vascular system has been examined in detail for neuronal and hormonal influences. Judging from the surprising complexity of the regulatory mechanisms described thus far, it is clear that efficient control of the circulation has considerable adaptive significance for this organism.

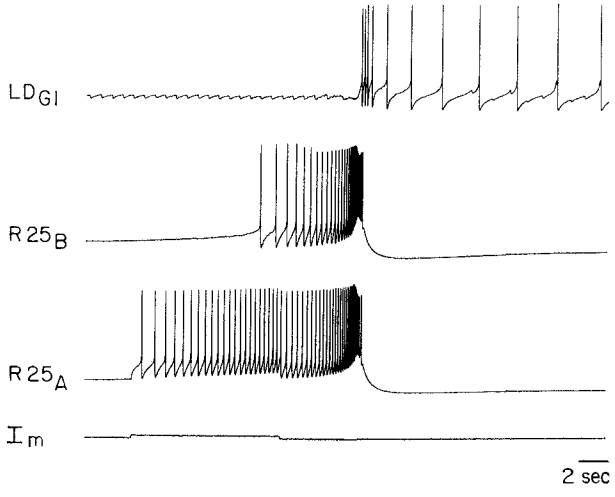

Figure 9. Firing a single R_{25} cell by current injection triggers by reverberation the entire L_{25}-R_{25} network. Current was injected into R_{25A}, and the burst generation in the rest of the network was monitored by recording in a second interneuron (R_{25B}) and in a gill motoneuron (LD_{G1}).

Cardiovascular changes during food arousal

The high degree of temporal resolution found in the neural control of the abdominal aorta (figs 5–7) contrasts with the tonic modulation generally associated with vasomotor control in other organisms. It seems likely that an adaptive advantage might be gained by a precise matching of the timing of vasomotor activity to that of somatic muscle. Such coordination would be particularly important in this instance if it were to increase the efficiency of feeding, for *Aplysia* in the wild spend from 1–3 h per day grazing on seaweed[38,76].

Interneurons that trigger respiratory pumping

It is interesting to speculate as to why so many (20–30) interneurons have evolved to generate respiratory pumping. Given the fact that a single neuron can make widespread synaptic connections, and the fact that many individual neurons in *Aplysia* have been shown to possess endogenous pacemaker activity, why is there not simply a single command neuron for respiratory pumping, rather than the multineuronal network that has been described?

There are at least three plausible explanations for the existence of such a large number of R_{25} and L_{25} neurons: 1) When excitatory input to the network is subliminal for triggering an all-or-none burst, individual neurons in the network may play a role in generating graded behaviors that differ from

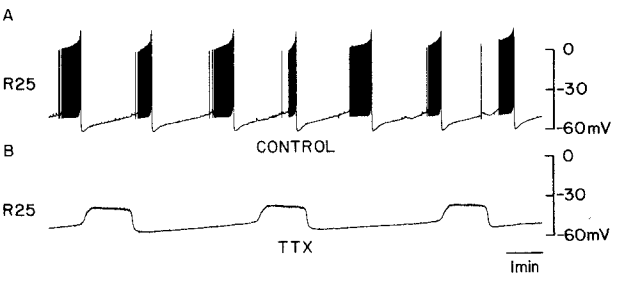

Figure 10. Tetrodotoxin (30 μM) blocks spike initiation in the R_{25} and L_{25} neurons, revealing an underlying pacemaker potential.

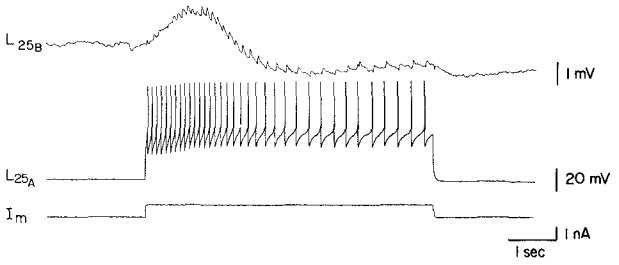

Figure 11. Firing L25A by injecting a step of current into the cell elicits in a second L25 cell rapid EPSPs riding on the envelope of a slow IPSP. The fast EPSPs contribute to burst initiation, and the slow IPSP contributes to burst termination.

respiratory pumping[6]. 2) A large interneuron pool may be required to implement a behavior that can occur both spontaneously and in a triggered mode. This hypothesis follows from the fact that other known individual neurons in *Aplysia* with endogenous bursting capabilities do not respond to an excitatory input with an all-or-none burst. The endogenous pacemaker potential mechanism found in the R_{25}–L_{25} neurons, combined with the positive feedback reverberatory nature of their synaptic potentials, enables respiratory pumping to occur both spontaneously and in a triggered mode. 3) There may be a need for multiple trigger cells that derives from the apparent inability of neurons in *Aplysia* to segregate different receptor types for the same transmitter[71]. For example, assume that there is a single trigger cell for respiratory pumping, and that cell is cholinergic. Such a cell could not make the excitatory connections to the LD_{HI} cells that drive them during respiratory pumping because the LD_{HI} cells also receive inhibitory connections from other cholinergic cells (e.g. L_{10}), and an individual neuron in *Aplysia* cannot segregate excitatory cholinergic receptors to one synapse and inhibitory cholinergic receptors to another. This constraint could be overcome if the trigger cell were to use a transmitter different from actylcholine, e.g. dopamine. But the same limitation would arise again if LD_{HI} (or other excitatory follower cells of the trigger cell) received dopaminergic inhibitory input from another source. One way around this constraint would be for the trigger source to be composed of a network of coupled cells, each of which projects to only a subset of the motoneurons, and at least some of which use different transmitters. The first two conditions are known to apply to the R_{25}–L_{25} network. The last prediction, that cells in the network are non-homogeneous with respect to transmitter type, has not yet been tested.

Acknowledgments. This work was supported in part by NIH grant NS 14385 and the Science Commission of NATO through DAAD. We thank I. Kupfermann and K.R. Weiss for critically reading an earlier draft of this manuscript.

1 Alevizos, A., and Koester, J., Identified gill motoneuron L7 of *Aplysia* is a multimodal motoneuron that also innervates the cardiovascular system. Soc. Neurosci. Abstr. *17* (1986) 499.
2 Arch, S., and Smock, T., Egg-laying behavior in *Aplysia californica*. Behav. Biol. *19* (1977) 45–54.
3 Bablanian, G., and Treistman, S., The effect of hyperpolarization of cell R15 on the hemolymph composition of intact *Aplysia*. J. comp. Physiol. *155* (1985) 297–303.
4 Brown, R.O., Gusman, D., Basbaum, A.I., and Mayeri, E., Identification of *Aplysia* neurons containing immunoreactive FMRFamide. Neuropeptides *6* (1985) 517–526.
5 Byrne, J., Identification and initial characterization of a cluster of command and pattern-generating neurons underlying respiratory pumping in *Aplysia californica*. J. Neurophysiol. *49* (1983) 491–508.
6 Byrne, J., and Koester, J., Respiratory pumping: neuronal control of a centrally commanded behavior in *Aplysia*. Brain Res. *143* (1978) 87–105.
7 Carew, T.J., Pinsker, H., Rubinson, K., and Kandel, E.R., Physiological and biochemical properties of neuromuscular transmission between identified motoneurons and gill muscle in *Aplysia*. J. Neurophysiol. *37* (1974) 1020–1040.
8 Carpenter, D., Breese, G., Schanberg, S., and Kopin, I., Serotonin and dopamine: distribution and accumulation in *Aplysia* nervous and non-nervous tissues. Int. J. Neurosci. *2* (1971) 49–56.
9 Cawthorpe, D.R.L., Rosenberg, J., Colmers, W.F., Lukowiak, K., and Drummond, G.I., The effects of small cardioactive peptide-B on the isolated heart and gill of *Aplysia californica*. Can. J. Physiol. Pharmac. *63* (1985) 918–924.
10 Castellucci, V., Pinsker, H., Kupfermann, I., and Kandel, E.R., Neuronal mechanisms of habituation and dishabituation of the gill-withdrawal reflex in *Aplysia*. Science *167* (1970) 1745–48.
11 Chase, T.N., Breese, G.R., Carpenter, D.O., Schanberg, S.M., and Kopin, I.J., Stimulation induced release of serotonin. Adv. Pharmac. *6* (1968) 351–364.
12 Cobbs, J.S., and Pinsker, H.M., Role of bag cells in egg deposition of *Aplysia brasiliana*. J. comp. Physiol. *147* (1982) 523–535.
13 Coyer, P.E., Responses of regularly-firing *Aplysia* R3–R13 neurons to normoxia and hypoxia. J. exp. Biol. *107* (1983) 1–8.
14 Critz, S.D., Harper, J.F., and Byrne, J.H., Evidence for the inhibitory subunit of adenylate-cyclase (NI) in nervous and heart-tissue of *Aplysia*. Neurosci. Lett. *64* (1986) 145–150.
15 Croll, R.P., Sensory control of respiratory pumping in *Aplysia californica*. J. exp. Biol. *117* (1985) 15–27.
16 Dieringer, N., Koester, J., and Weiss, K., Adaptive changes in heart rate of *Aplysia californica*. J. comp. Physiol. *123* (1978) 11–21.
17 Drummond, G.I., Wernham, S., and Lukowiak, K., Stimulation of adenylate-cyclase in the heart of *Aplysia californica* by biogenic-amines. Comp. Biochem. Physiol. *80* (1980) 129–133.
18 Eales, N.B., *Aplysia*. Liverpool mar. Biol. Committee, Proc. Trans. Liverpool biol. Soc. L.M.B.C. Mem. vol. 35, *24* (1921) 183–266.
19 Eberly, L.B., and Pinsker, H.M., Neuroethological studies of reflex plasticity in intact *Aplysia*. Behav. Neurosci. *98* (1984) 609–630.
20 Feinstein, R., Pinsker, H., Schmale, M., and Gooden, B.A., Bradycardial response in *Aplysia* exposed to air. J. comp. Physiol. *122* (1977) 311–324.
21 Glanzman, D.L., Mackey, S., and Kandel, E.R., Depletion of serotonin in *Aplysia* interferes with facilitation produced by sensitizing stimuli. Soc. Neurosci. Abstr. *12* (1986) 1339.
22 Giller, E., and Schwartz, J.H., Choline acetyltransferase in identified neurons of abdominal ganglion of *Aplysia californica*. J. Neurophysiol. *34* (1971) 93–107.
23 Hening, W.A., Central Generation and Coordination of a Complex Behavioral Sequence in *Aplysia californica*: Locomotion and Respiratory Pumping. PhD. thesis, Dept. of Physiology, New York University, 1982.
24 Hill, R.B., Some effects of acetylcholine and of active amines on the isolated ventricles of *Aplysia dactylomela* and *Aplysia fasciata*. Pubbl. Staz. zool. Napoli *34* (1964) 75–85.
25 Iliffe, E.M., McAdoo, D.J., Beyer, C.B., and Haber, B., Amino acid concentrations in the *Aplysia* nervous system: neurons with high glycine concentrations. J. Neurochem. *28* (1977) 1037–1042.
26 Kaldany, R.R.J., Campanelli, J.T., Makk, G., Evans, C.J., and Scheller, R.H., Proteolytic processing of a peptide precursor in *Aplysia* neuron R14. J. biol. Chem. *261* (1986) 5751–5757.
27 Kandel, E.R., Frazier, W.T., Waziri, R., and Coggeshall, R., Direct and common connections among identified neurons in *Aplysia*. J. Neurophysiol. *30* (1967) 1352–1376.
28 Kandel, E.R., Behavioral Biology of *Aplysia*. W.H. Freeman and Company, San Francisco 1979.
29 Kandel, E.R., and Schwartz, J.H., Molecular biology of learning: modulation of transmitter release. Science *218* (1982) 433–443.
30 Kebabian, P.R., Kebabian, J.W., and Carpenter, D.O., Regulation of cyclic AMP in heart and gill of *Aplysia* by the putative neurotransmitters dopamine and serotonin. Life Sci. *24* (1979) 1757–1764.
31 Kistler, H.B., Hawkins, R.D., Koester, J., Steinbusch, H.W.M., Kandel, E.R., and Schwartz, J.H., Distribution of serotonin-immunoreactive cell-bodies and processes in the abdominal ganglion of mature *Aplysia*. J. Neurosci. (1985) 72–80.
32 Koch, U.T., and Koester, J., Time sharing of heart power: cardiovascular adaptations to food arousal in *Aplysia*. J. comp. Physiol. *149* (1982) 31–42.
33 Koch, U.T., Koester, J., and Weiss, K.R., Neuronal mediation of cardiovascular effects of food arousal in *Aplysia*. J. Neurophysiol. *51* (1984) 126–135.
34 Koester, J., Respiratory pumping in *Aplysia* is controlled by two coupled clusters of interneurons. Soc. Neurosci. Abstr. *9* (1983) 542.

35 Koester, J., Dieringer, N., and Mandelbaum, D.E., Cellular neuronal control of molluscan heart. Am. Zool. *19* (1979) 103–116.

36 Koester, J., Mayeri, E., Liebeswar, G., and Kandel, E.R., Neural control of circulation in *Aplysia*. II. Interneurons. J. Neurophysiol. *37* (1974) 476–496.

37 Kupfermann, I., and Kandel, E.R., Neuronal controls of a behavioral response mediated by the abdominal ganglion of *Aplysia*. Science *164* (1969) 847–850.

38 Kupfermann, I., and Carew, T.J., Behavior patterns of *Aplysia* in its natural environment. Behav. Biol. *12* (1974) 317–337.

39 Kupfermann, I., and Weiss, K.R., Water regulation by a presumptive hormone contained in identified neurosecretory cell R15 of *Aplysia*. J. gen. Physiol. *67* (1976) 113–123.

40 Kurokawa, M., and Kuwasawa, K., Electrophysiological studies on the branchial ganglion in the opisthobranch mollusks *(Aplysia* and *Dolabella)*. J. comp. Physiol. *156* (1985) 35–44.

41 Liebeswar, G., Goldman, J.E., Koester, J., and Mayeri, E., Neural control of circulation in *Aplysia*. III. Neurotransmitters. J. Neurophysiol. *38* (1975) 767–779.

42 Ligman, S.H., and Brownell, P.H., Differential hormonal action of the bag cell neurons on the arterial system of *Aplysia*. J. comp. Physiol. *157* (1985) 31–37.

43 Lloyd, P.E., Kupfermann, I., and Weiss, K.R., Two endogenous neuropeptides (SCP_A and SCP_B) produce a cAMP-mediated stimulation of cardiac activity of *Aplysia*. J. comp. Physiol. *156* (1985) 659–667.

44 Mahon, A.C., Lloyd, P.E., Weiss, K.R., Kupfermann, I., and Scheller, R.H., The small cardioactive peptide-A and peptide-B of *Aplysia* are derived from a common precursor molecule. Proc. natl Acad. Sci. USA *82* (1985) 3925–3929.

45 Mandelbaum, D.E., Koester, J., Schonberg, M., and Weiss, K.R., Cyclic AMP mediation of the excitatory effect of serotonin in the heart of *Aplysia*. Brain Res. *177* (1979) 388–394.

46 Mayeri, E., Brownell, P., and Branton, W.D., Multiple, prolonged actions of neuroendocrine bag cells on neurons in *Aplysia*. II. Effects on beating pacemaker and silent neurons. J. Neurophysiol. *42* (1979) 1185–97.

47 Mayeri, E., Brownell, P., Branton, W.D., and Simon, S.B., Multiple, prolonged actions of neuroendocrine bag cells on neurons in *Aplysia*. I. Effects on bursting pacemaker neurons. J. Neurophysiol. *42* (1979) 1165–1184.

48 Mayeri, E., Koester, J., Kupfermann, I., Liebeswar, G., and Kandel, E.R., Neural control of circulation of *Aplysia*. I. Motoneurons. J. Neurophysiol. *37* (1974) 458–475.

49 Mayeri, E., and Rothman, B.S., Neuropeptides and the control of egg-laying behavior in *Aplysia*, in: Model Neural Networks and Behavior, pp. 285–301. Ed. A.I. Selverston. Plenum Press, New York 1985.

50 Mayeri, E., Rothman, B.S., Brownell, P.H., Branton, W.D., and Padgett, L., (1985). Nonsynaptic characteristics of neurotransmission mediated by egg-laying hormone in the abdominal ganglion of *Aplysia*. J. Neurosci. *5* (1985) 2060–2077.

51 McAdoo, D.J., Iliffe, T.M., Price, C.H., and Novak, R.A., Specific glycine uptake by identified neurons of *Aplysia californica*. II. Biochemistry. Brain Res. *154* (1978) 41–51.

52 Nambu, R.R., Taussig, R., Mahon, A.C., and Scheller, R.H., Isolation of genes using probes from individual *Aplysia* neurons: neuropeptide modulators of cardiovascular physiology. Cell *35* (1983) 47–56.

53 Perlman, A.J., Central and peripheral control of siphon-withdrawal reflex in *Aplysia californica*. J. Neurophysiol. *42* (1979) 510–529.

54 Peretz, B., Central neuron initiation of periodic gill movements. Science *166* (1969) 1167–1172.

55 Pinsker, H., Kupfermann, I., Castellucci, V., and Kandel, E., Habituation and dishabituation of the gill-withdrawal reflex in *Aplysia*. Science *167* (1970) 1740–1742.

56 Pinsker, H.M., Integration of reflex activity and central pattern generation in intact *Aplysia*. J. Physiol. Paris *78* (1982–83) 775-785.

57 Price, C.H., and McAdoo, D.J., Localization of axonally transported ^{3}H-glycine in vesicles of identified neurons. Brain Res. *219* (1981) 307–315.

58 Price, C.H., McAdoo, D.J., Farr, W.F., and Okuda, R., Bidirectional axonal transport of free glycine in identified neurons R3–R14 of *Aplysia*. J. Neurobiol. *10* (1979) 551–571.

59 Price, C.H., Fowle, W., and Rittenhouse, A.R., Anatomy and innervation of the anterior aorta of *Aplysia* and the ultrastructure of specialized neuromuscular junctions on vascular smooth muscle. J. comp. Neurol. *222* (1984) 366–382.

60 Rittenhouse, A.R., and Price, C.H., Peripheral axons of the parabolic burster neuron R15. Brain Res. *333* (1985) 330–335.

61 Rittenhouse, A.R., and Price, C.H., Anatomical and electrophysiological study of multitransmitter neuron-R14. J. comp. Neurol. *247* (1986) 447–456.

62 Rittenhouse, A.R., and Price, C.H., Axons and target tissues of *Aplysia* neurons R3–14 and their status as multifunctional, multimessenger neurons. J. Neurosci. *6* (1986) 2071–2084.

63 Rothman, B.S., Weit, G., and Dudek, F.E., Direct action of egg-laying hormone on ovotestis of *Aplysia*. Science *197* (1982) 490–493.

64 Rothman, B.S., Sigvardt, K.A., Hawke, D.H., Brown, R.D., Shively, J.E., and Mayeri, E., Identification and primary structural analysis of peptide-II, an end-product of precursor processing in cells R3–R14 of *Aplysia*. Peptides *6* (1985) 1113–1118.

65 Sawada, M., Blankenship, J.E., and McAdoo, D.J., Neural control of a molluscan blood vessel, anterior aorta of *Aplysia*. J. Neurophysiol. *46* (1981) 967–986.

66 Sawada, M., Gibson, D., and McAdoo, D.J., L-glutamic acid, a possible neurotransmitter to anterior aorta of *Aplysia*. J. Neurophysiol. *51* (1984) 375–386.

67 Sawada, M., Hara, N., Ichinose, M., and Maeno, T., Excitatory and inhibitory effects of acetylcholine on the anterior aorta muscle of *Aplysia*. J. Neurosci. Res. *7* (1982) 179–192.

68 Sawada, M., Ichinose, M., Ito, I., Maeno, T., and McAdoo, D.J., Effects of 5-hydroxytryptamine on membrane potential, contractility, accumulation of cyclic-AMP, and Ca^{++} movements in anterior aorta and ventricle of *Aplysia*. J. Neurophysiol. *51* (1984) 361–374.

69 Sawada, M., McAdoo, D.J., Ichinose, M., and Price, C.H., Influences of glycine and neuron R14 on contraction of the anterior aorta of *Aplysia*. Jap. J. Physiol. *34* (1984) 747–767.

70 Scheller, R.H., Jackson, J.F., McAllister, L.B., Rothman, B.S., Mayeri, E., and Axel, R., A single gene encodes multiple neuropeptides mediating a stereotyped behavior. Cell *32* (1983) 7–22.

71 Segal, M.M., and Koester, J., Convergent cholinergic neurons produce similar postsynaptic actions in *Aplysia:* Implications for neural organization. J. Neurophysiol. *47* (1982) 742–759.

72 Shyamala, M., Fisher, J.M., and Scheller, R.H., A neuropeptide precursor expressed in *Aplysia* neuron L5. DNA *5* (1986) 203–208.

73 Sigvardt, K.A., Rothman, B.S., Brown, R.D., and Mayeri, E., The bag cells of *Aplysia* has a multitransmitter system. Identification of alpha-bag cell peptide as a second neurotransmitter. J. Neurosci. *6* (1986) 803–813.

74 Simmons, L.K., and Koester, J., Serotonin enhances the excitatory acetylcholine response in the RB-cell cluster of *Aplysia californica*. J. Neurosci. *6* (1986) 774–781.

75 Stinnakre, J., and Tauc, L., Central neuronal response to the activation of osmoreceptors in the osphradium of *Aplysia*. J. exp. Biol. *51* (1969) 347–361.

76 Susswein, A.J., Gev. S., Achituv, Y., and Marcovich, S., Behavioral patterns of *Aplysia fasciata* along the Mediterranean coast of Israel. Behav. Neural. Biol. *41* (1984) 7–22.

77 Sussweln, A.J., Weiss, K.R., and Kupfermann, I., The effects of food arousal on the latency of biting in *Aplysia*. J. comp. Physiol. *123* (1978) 31–41.

78 Taxi, J., and Gautron, J., Données cytochimiques en faveur de l'existence de fibres nerveuses serotoninergiques dans le coeur de l'Aplysie, *Aplysia californica*. J. Microsc. *8* (1969) 627–636.

79 Treistman, S.N., and Bablanian, G.M., Effects of sea water temperature on bursting pacemaker activity in cell R15 in the intact *Aplysia*. Brain Res. *346* (1985) 155–159.

80 Weiss, K.R., Cohen, J.C., and Kupfermann, I., Modulatory control of buccal musculature by a serotonergic neuron (metacerebral cell) in *Aplysia*. J. Neurophysiol. *41* (1978) 181–203.

81 Weiss, K.R., Chiel, H.J., Koch, U., and Kupfermann, I., Activity of an identified histaminergic neuron, and its possible role in arousal of feeding behavior in semi-intact *Aplysia*. J. Neurosci. *6* (1986) 2393–2402.

82 Wernham, S., and Lukowiak, K., The effects of serotonin, dopamine, and arginine vasotocin on the isolated *Aplysia* heart-I. Heart rate and amplitude of contraction. J. comp. Physiol. *75C* (1983) 295–303.

83 Wright, D.L., Cardiac Studies on *Aplysia vacaria*. Ph. D. dissertation, Department of Zoology, University of California at Los Angeles 1960.

0014-4754/87/090972-09$1.50 + 0.20/0