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Organization of the multifunctional neural network regulating visceral organs in *Helix pomatia* L. (Mollusca, Gastropoda)

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Summary. In *Helix pomatia* L. the overlapping neuronal network was found to regulate the visceral functions (e.g. cardio-renal, respiratory and genital functions). The neural network is organized around the multifunctional interneurons, which take part in forming both afferent and efferent pathways. The interneurons are sensitive to a wide range of neurotransmitters or transmitter candidates including low molecular weight substances (ACh, 5HT, DA, octopamine, glutamate) and several oligopeptides. In this system both selection of information and modification of membrane properties (for example habituation) are carried out by a combination of simultaneously liberated active agents.
Key words. Helix pomatia L.; neural network; habituating and non-habituating neurons; interaction of neurotransmitters and peptides; FMRFamide; opiate peptides; morphine; ACh; 5HT.

Introduction

In the subesophageal ganglion complex of *Helix pomatia* a network consisting of a number of identified neurons was found to regulate the visceral organs including the cardio-renal, respiratory and genital systems[24].
The gastropod central nervous system and semi-intact preparations are commonly used for studying questions connected with the transmission, interpretation and storage of information. This involves the problems of specificity or invariance of single units in the regulatory neural networks, or on the contrary, the variability and dynamic nature of the network elements[2,15,22,26].
The data support an emphasis on the concept that recognition, analysis and regulation occur at the level of neural networks building up from overlapping neural populations. Any idea that the same unit of the network can take part in the interpretation of various pieces of information, or in the regulation of different functions, contradicts the alternative idea of networks or units specialized for one single function. The aim of our investigations was to study the interrelation of the neurons regulating various visceral organs, e.g., cardio-renal, respiratory and genital. During the investigation special attention was paid to the interaction of neurotransmitters involved in the identified neural network.

Material and methods

The experiments were performed on active snails, *Helix pomatia* L., at room temperature (20–24 °C), throughout the year. For the investigations semi-intact preparations developed earlier [21,25] were used. The preparation employed contained: 1) cardio-renal system (e.g. heart, pericardium, blood vessels, kidney and liver), 2) respiratory system (e.g. pneumostoma surrounded with a piece of mantle and body wall), 3) genital organs (e.g. hermaphroditic gland, hermaphroditic duct, female duct, spermatheca, accessory genital mass and prostate gland).
During preparation, care was taken to preserve intact the connection of anal, right parietal and intestinal nerves, innervating the cardio-renal, respiratory and genital systems, respectively, with the central nervous system (CNS).
In the majority of experiments the intracellular activity of two identified central neurons, heart contractions and the extracellular activity of the corresponding nerve were recorded simultaneously. The intracellular activity of the neurons was registered with conventional glass microelectrodes, filled with 2.5 M KCl or 0.6 M K_2SO_4, which had resistances of 5–20 MOhm. A four-channel Tektronix oscilloscope, a Gould-Brush recorder and Dagan amplifiers were employed during experiments. The inputs from various visceral organs were activated by applying tactile stimuli to the peripheral receptors.
The application of low molecular weight neurotransmitters or peptides was carried out by two methods: 1) from a micropipette 50, 100 or 200 µl of substances in the range of 10^{-4}–10^{-8} M was applied to the surface of ganglia (drop application). 2) the microelectrode with 4–6 µm tip diameter filled with the drug was positioned on the soma membrane of the

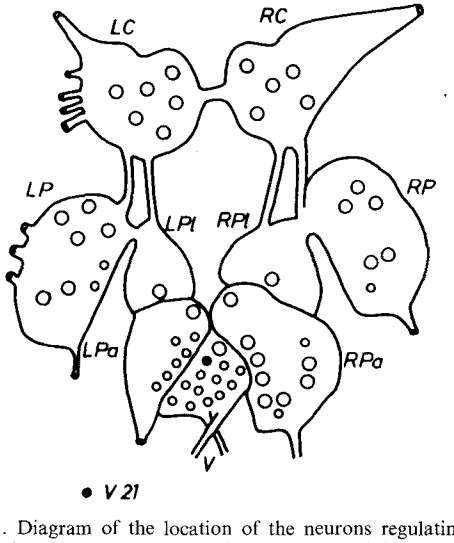

Figure 1. Diagram of the location of the neurons regulating visceral functions in *Helix pomatia* on the dorsal surfaces of the central ganglia. LC and RC, left and right parietal; LP and RP, left and right pedal; LPl and RPl, left and right pleural; LPa and RPa, left and right parietal; V, visceral ganglia.

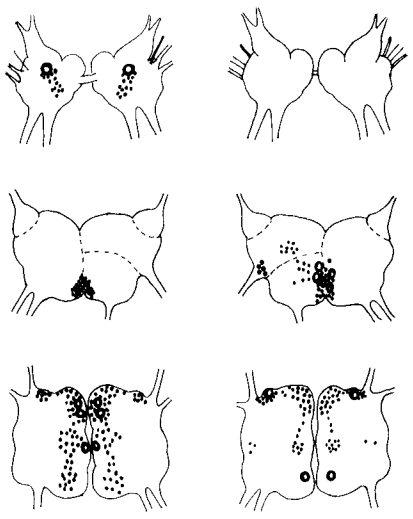

Figure 2. Distribution of serotonergic cells in the CNS of *Helix pomatia* marked by pigment accumulation in their soma following 5,6-dihydroxytryptamine treatment.

cell being investigated and a controlled amount of the drug was released under pressure. The inhibitors were added to the bath.

The following substances were used and dissolved in Meng's saline[16]: acetylcholine chloride, ACh (Sigma); 5-hydroxytryptamine creatinine sulphate, 5HT (Sigma); dopamine hydrochloride, DA (Merck); octopamine hydrochloride, OP (Merck); L-glutamate, Glu (Merck); FMRFamide, Leu-enkephalin (Peninsula Lab.); morphine (CHINOIN); naloxone (Calbiochem); 5,6-dihydroxytryptamine, 5,6-DHT (Calbiochem).

Results and discussion

1. Distribution of the central neurons innervating visceral organs of Helix pomatia L.

The cells regulating visceral organs were found to be distributed throughout the central nervous system of *Helix pomatia* L.[21-23,25]. The main population of the involved neurons was located on the dorsal surface[5] of the visceral, left and right parietal ganglia (fig. 1). For identification of the neurons both morphological and physiological criteria were applied[21]. Among the neurons regulating visceral organs there were two heart inhibitory and four heart excitatory motoneurons, and one heart relaxing one; further, six interneurons have been identified[22]. These neurons are connected to respiratory and genital organs, too[24]. The number of sensory cells was found to be variable.

Among the neurons regulating visceral organs a set of serotonergic cells can be identified using 5,6-dihydroxytryptamine (5,6-DHT) as a marker[29]. It has been demonstrated that 5,6-DHT treatment of the animal induced biphasic processes in the connections of the central neurons; at the first phase of action a chemical degeneration of the serotonergic endings can be obtained, and then by the 40–60th day of incubation these connections are restored with simultaneous accumulation of a brownish pigmentation in the soma of the serotonergic cells (fig. 2). These pigment labeled cells can be used for studying the electrophysiological and chemical properties of soma as well as formation of serotoneric pathways[29].

Labeling the identified neurons by $CoCl_2$ and horse radish peroxidase contributed also to the mapping of the regulatory network of visceral functions[9,10]. The majority of the sensory cells proved to be bipolar with rich arborization, while motoneurons were unipolar or pseudounipolar and the interneurons also had pseudounipolar or bipolar axons, one of them terminating at the periphery[21].

2. Characterization of the neuronal network innervating visceral organs

It was shown earlier that the neurons regulating visceral organs can respond to several modalities and regulate more than one visceral function[21,22]. This kind of interrelated neural network is organized around multifunctional interneurons fulfilling an integrative role[23]. The network was found to be organized from variable elements. In this system the sensory inputs originating from one organ (e.g. heart, pneumostoma or hermaphroditic ducts, respectively) can modify the work of another organ due to the information running to motor-, and interneurons from the receptor field of the innervated organs.

One of the most-studied units of the network regulating visceral function is the interneuron V21. This multifunctional interneuron contains more than one neurotransmitter and receives inputs using the same type[9] of neurotransmitters[9,10]. This interneuron has a characteristic pattern of firing (tonic or phasic) changing from one to another type according to the activation of particular sensory pathways originating from a visceral organ[21,24] (fig. 3).

Changing the firing pattern in the neuron V21 to a tonic one (fig. 3) always leads to inhibition of heart activity. The interneuron V21 receives at least seven afferent pathways and forms several efferent paths to visceral organs including the genital organs[9,10].

Our results showed that regulation of visceral organs at the cellular level is carried out at the network level, rather than at that of the single neurons. If one compares network function to human cognition, it may seem that the information coming from various peripheries is understood, treated, interpreted and stored within a broad network and the output is then formed on the basis of all the information occurring in the system in close correlation with the inputs. Under these circumstances the role of the units can vary depending on the quality and quantity of the information and, further on, the prohibition of or preference for certain connections can decide whether one or another part of the network is involved in regulation[7,21,22].

In Gastropoda the regulation of visceral organs is carried out with redundantly informed networks. The elements, as a rule, do not form fixed pathways but a dynamic nature of the

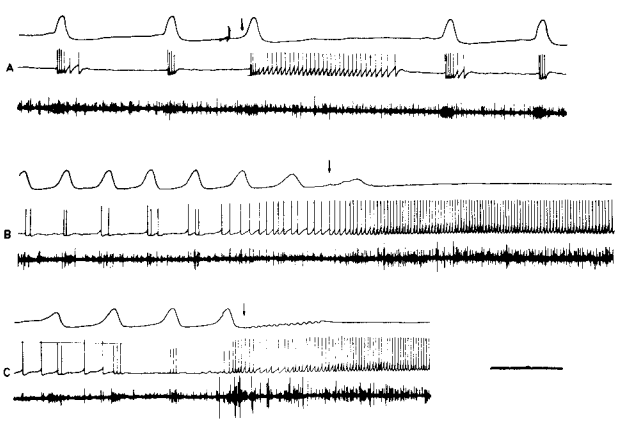

Figure 3. Firing pattern of the multifunctional interneuron, V21. Change of phasic pattern of firing to a tonic one as a result of stimulation of various visceral organs. *A* Heart stimulation; *B* stimulation of pericardium; *C* kidney stimulation. In each record, heartbeats appear above, intracellular activity of the interneuron V21 appears in the center, and extracellular recording from the intestinal nerve appears below.

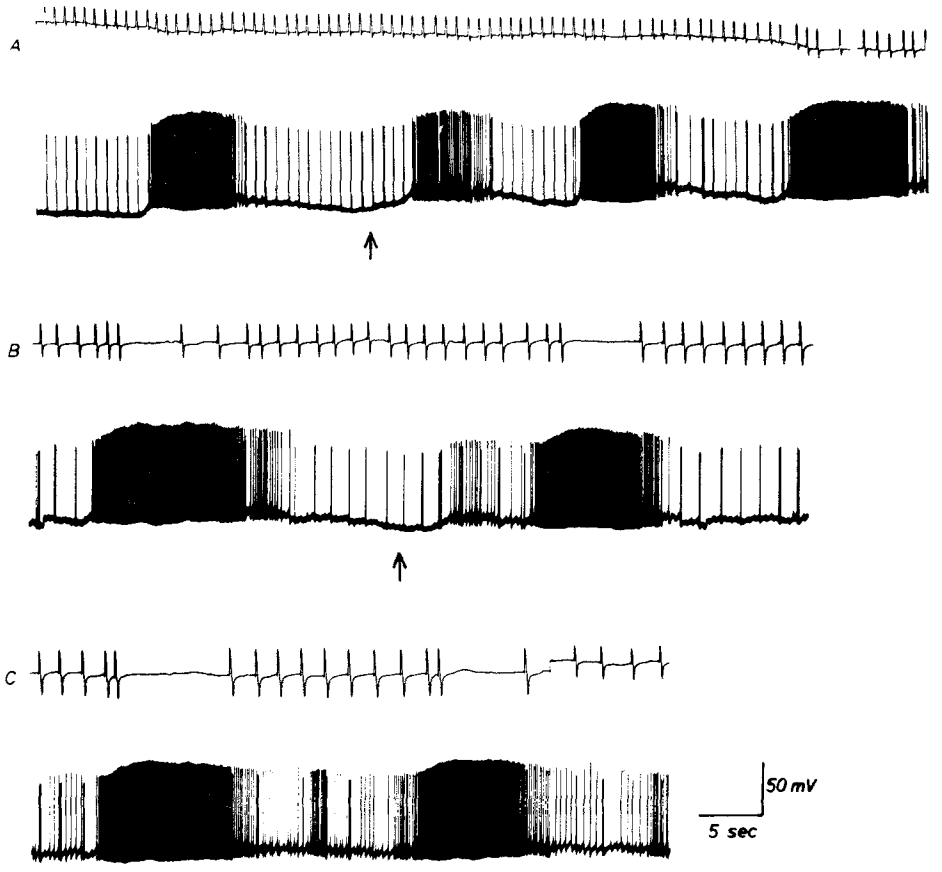

Figure 4. Spontaneous high frequency firing of the interneuron V21. Note that at the presence of high frequency firing the interneuron V21 became insensitive to the low molecular weight neurotransmitters. Above: heart activity. At the bottom: intracellular activity of interneuron V21 showing spontaneous high frequency burst firing (*A, B, C*). The arrows show the application of low molecular weight neurotransmitter.

network prevails, based on the actual combination of the units involved, activated by various inputs. The same unit can be involved in the dynamic organization of the network, with various combinations and qualities (sensory unit or interneuron), increasing in this way the functional plasticity of the nervous system.

3. Neurotransmitters used by the central neurons innervating visceral organs

The moto- and interneurons were found to be sensitive to the majority of low molecular weight neurotransmitters[23]. These neurons more often than not showed biphasic responses to the neurotransmitters (table 1). The appearance of excitatory or inhibitory responses was found to be extremely variable. The variability occurred irrespective of whether the substances were applied to the surface of the neurons by pressure injection or by drop application[23] to the bath.

This variation in the response to neurotransmitters can partially be explained by the fact that in the nervous system of an intact animal, or in semi-intact preparations, each neuron is exposed to a large number of synaptic and non-synaptic influences, and this results in a permanent modification of their condition[21]. As a consequence the cells in these situations behave differently from voltage clamped membranes, showing great variability in their responses to neurotransmitters. This variability can be attributed to dynamic alterations in response to neurotransmitters at the synapses. As was stated, the sensitivity of the same neurons to neurotransmitters also depends on the sequence or combination of substances applied to the cell membrane. This variability could be partly modelled by choosing a correct sequence for the application of substances[23].

Table 1. Reactions to various neurotransmitters of the neurons regulating visceral functions in the CNS of *Helix pomatia* L.

Cell	Function	Neurotransmitter				
		ACh	5HT	DA	OP	Glutamate
V12	Heart inhibitory motoneuron	H,D	D,H	H,D	H,D	O,H
V13	Heart inhibitory motoneuron	D	D	H,D	H,D	O
V38	Heart excitatory motoneuron	H,D	D	H,D	O,H	O
V41	Heart excitatory motoneuron	H,D	D	D,H	O,H	O
V42	Heart excitatory motoneuron	H,D	H	D,H	O,H	O
RPa39	Heart excitatory motoneuron	O,H,D	D	D,H	H,D	D,H
V43	Motoneuron relaxing the heart	O,H,D	D	O,D,H	O	O,H,D
V20	Interneuron	H	H	D,H	D	H
V21	Multifunctional interneuron	D,H	D,H	D	H	H,D
RPa2	Inhibited by heart afferents	D,H	D	H	H,D	D,H

H, hyperpolarization; D, depolarization; O, no response.

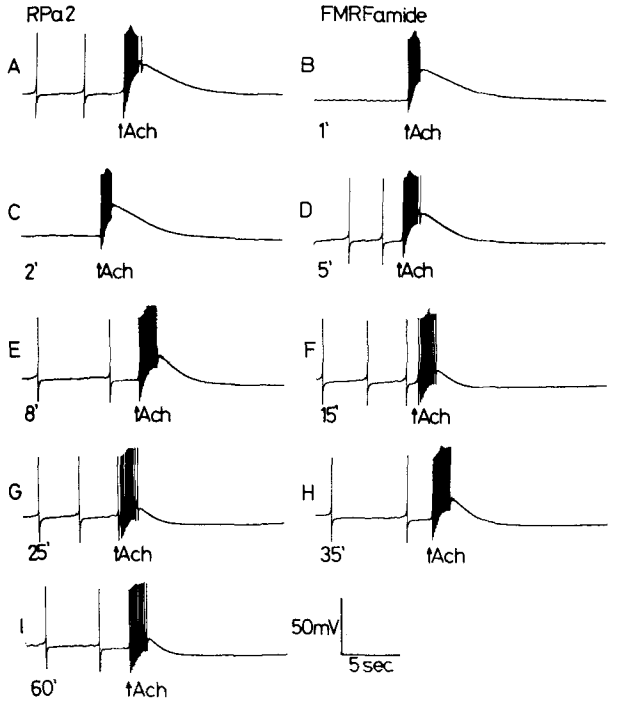

Figure 5. Interaction between FMRFamide (10^{-4} M) and ACh (10^{-4} M) on the giant neuron RPa2 of *Helix pomatia*. *A* Control effect of ACh; *B–I* Effect of ACh following FMRFamide treatment from 1st to the 60th min. Here and in the following figures, an arrow indicates the local application of neurotransmitters to the surface of the soma.

At the same time the liberation of more than one neurotransmitter from the nerve endings raises the question of whether the combination of the simultaneously liberated neurotransmitters has a role in filtering information. It is commonly accepted that the selection of information takes place above all at the synapses. Without selection of information the analytical-synthetic function of the brain could not be carried out.

In the central nervous system all the neurons are permanently affected by a number of synaptic and non-synaptic factors so that they are functioning all the time under modulated conditions. In this situation the effects of the neurotransmitters cannot be constant, but depend on the momentary characteristics of the cell receptors.

In the spring, the peptidergic cells are activated to release large amounts of their secretory products, causing a high frequency burst firing of the multifunctional neuron V21. At that time, all the other pathways running to this neuron and using low molecular weight neurotransmitters (ACh or monoamines but not amino acids), may be inactivated or 'closed' (fig. 4). This kind of interaction of peptides and classical neurotransmitters can be taken as an example of selection of information. The same interaction can be demonstrated between FMRFamide and ACh or 5HT (see below).

4. Interaction of FMRFamide and low molecular weight neurotransmitters

Among molluscan species the peptide FMRFamide was first demonstrated in the ganglia of a marine snail, *Macrocallista nimbosa*, and consequently in the CNS of numerous molluscan species[12, 17]. FMRFamide has been tested on a wide variety of molluscan tissues. It has proved to have either stimulatory or inhibitory effects on the hearts of various species of molluscs[11]. In *Helix* cerebral ganglia the giant serotonergic cells were hyperpolarized or depolarized by FMRFamide, depending on the level of membrane potential[4–6].

In vertebrate and invertebrate neurons the co-existence of peptides and low molecular weight neurotransmitters has been found to be rather common. In such cases the peptides are thought to play the role of modulators[3, 13]. In the molluscan nervous system the interaction of dopamine and serotonin with opiate peptides has been described[14, 26, 31].

In our experiments FMRFamide caused variable effects on the neurons regulating visceral functions, although an excitatory response was more common.

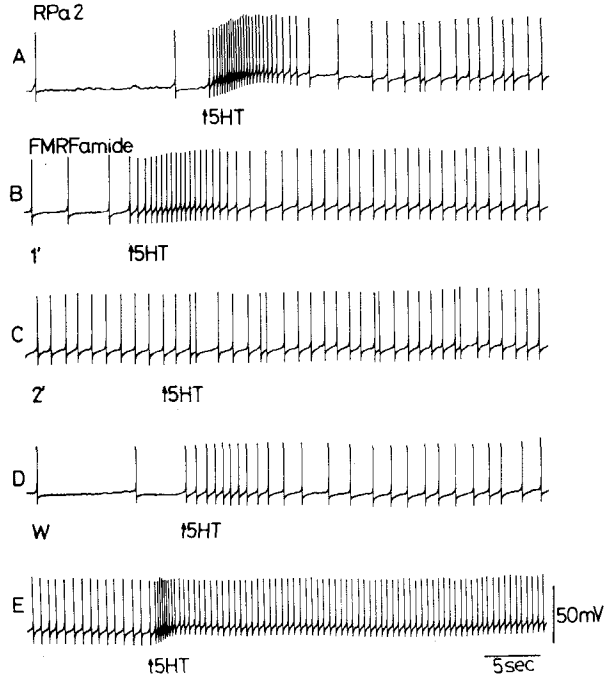

Figure 6. Interaction between FMRFamide (10^{-4} M) and 5 HT (10^{-4} M) on the giant neuron RPa2 of *Helix pomatia* L. *A* Control effect of 5HT. Effect of 5HT at 1st (*B*) and 2nd (*C*) min of FMRFamide application. Washout of the FMRFamide effect, 10 min (*D*) and 60 min (*E*).

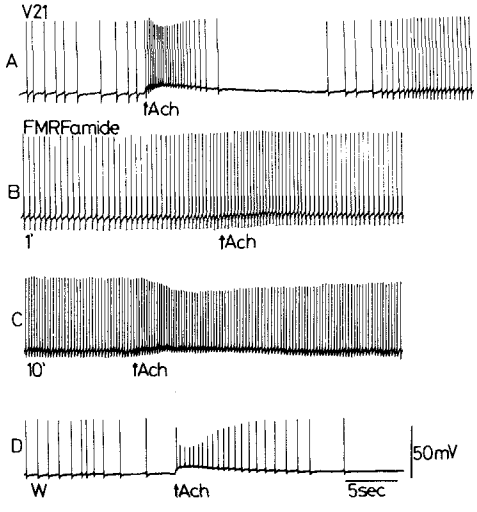

Figure 7. Interaction of FMRFamide (10^{-4} M) and ACh (10^{-4} M) on the multifunctional interneuron V21 of *Helix pomatia* L. *A* Control effect of ACh. Effect of ACh at the 1st (*B*) and 10th (*C*) min of FMRFamide application. *D* Effect of ACh after washout of the FMRFamide.

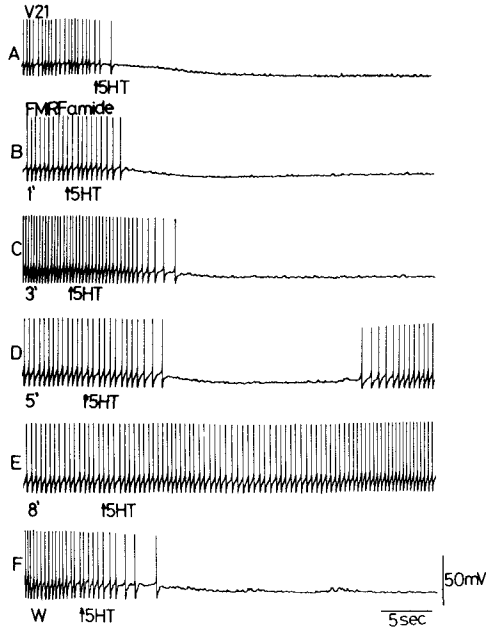

Figure 8. Interaction between FMRFamide (10^{-4} M) and 5 HT (10^{-4} M) on the interneuron V21 of *Helix pomatia* L. *A* Control effect of 5HT. Effect of 5HT at the 1st (*B*), 3rd (*C*), 5th (*D*) and 8th (*E*) minute of FMRFamide treatment. *F* Effect of 5HT after washout of the FMRFamide.

Two well-known *Helix* neurons, V21 and RPa2, were used for testing the modulatory influence of FMRFamide on the acetylcholine (ACh) and 5-hydroxytryptamine (5HT) effects. The RPa2 neuron is the largest one in the right parietal ganglion, while the visceral cell V21 is the multiaction interneuron inhibiting heart activity[21]. Both cells are members of the neural network regulating visceral functions; their axons run into the intestinal nerve[21], and have a variable firing pattern modified by sensory inputs from the regulated organs[28].

The giant neuron RPa2 reacted to the local application of ACh[18, 30] with a wave of depolarization on which a high-frequency burst of firing appeared (fig. 5). Pretreatment of the ganglia with FMRFamide decreased the amplitude of the ACh-elicited depolarization to 13% of its control value within 35 min, and it remained at this level for more than an hour. The ACh-elicited increase in firing frequency of the neuron RPa2 was less influenced by FMRFamide (fig. 5). When applied to the neuron RPa2, 5HT also caused a wave of depolarization which, however, was lower in amplitude than in the case of Ach. Following 5HT application, during the depolarizing wave, an increased frequency of firing also appeared (fig. 6). Treatment of the ganglia with FMRFamide led to the elimination of both the membrane depolarization and the increase in firing rate of the RPa2 neuron in about 2–5 min (fig. 6). The effect of 5HT was restored by long-lasting washing out of the FMRFamide, but the degree of depolarization of the membrane as well as the increase in frequency of firing elicited by 5HT remained lower than that of the control (fig. 6).

The multifunctional interneuron V21 reacted to the application of ACh with a depolarizing wave and an increased firing frequency, which was followed by an inhibitory phase (fig. 7). FMRFamide modulated the ACh effect on the interneuron V21, too. In one case, FMRFamide eliminated the depolarization as well as excitatory and inhibitory effects of ACh, while in another case only an inhibitory phase was induced in the same neuron. The complex effect of ACh was modified with FMRFamide in a long-lasting manner, but it could be restored by washing out (fig. 7). However, sometimes the interaction between ACh and FMRFamide was less pronounced, in that only a temporary elimination of the ACh-elicited depolarizing wave and ACh-induced inhibitory

Table 2. The effect of FMRFamide and opiates on the membranes of *Helix* central neurons regulating visceral functions

Neurone	Effect of FMRFamide	Morphine	leu-enkephalin
LPa2	H	D	O
LPa5	H	O	O
RPa1	–	H	H
RPa2	D	D	D
RPa3	H	O	D
RPa8	H	O	O
V4	D	O	O
V9	D	D	O
V11	D	O	D
V12	D	O	O
V21	D	D	D
NS	D	D	D

H, hyperpolarization; D, depolarization; O, ineffective; NS, neurosecretory cell at visceral ganglion; LPa, left parietal; RPA, right parietal; V, visceral ganglia.

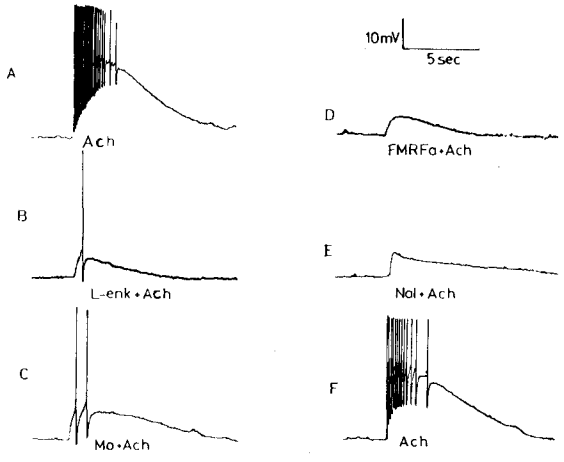

Figure 9. Interaction of leu-enkephalin (L-enk), morphine (Mo), FMRFamide (FMRF) and naloxone with ACh on the neuron LPa5 of *Helix pomatia* L.

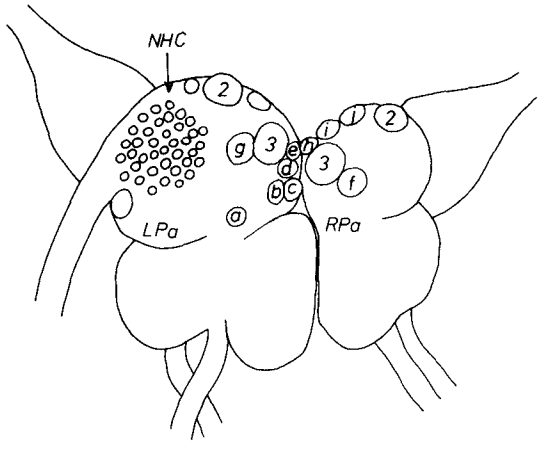

Figure 10. Distribution of the habituating and non-habituating neurons on the dorsal surface of the left (LPa) and right (RPa) parietal ganglia of *Helix pomatia* L. NHC: non-habituating cells. *A–J* habituating (white) cells.

phase was observed. In such cases, the effect of ACh was partially restored in the presence of FMRFamide, although a complete restoration of the ACh effect was not achieved even by washing out.

The action of 5 HT on the interneuron V21 was to cause a long-lasting hyperpolarization and inhibition of firing (fig. 8). This inhibition could only be eliminated with repeated washing out of 5HT. The treatment of the ganglia with FMRFamide first diminished the hyperpolarization caused by 5HT, then its inhibitory phase became shorter, and by the 8th or 10th minute of FMRFamide application the 5HT effect was completely eliminated. With repeated washing out of FMRFamide the effect of 5HT could be restored (fig. 8).

Leu-enkephalin, morphine and naloxone all modified the ACh-elicited response in a similar manner to FMRFamide (fig. 9). The interaction between FMRFamide or opiate peptides and low-molecular weight neurotransmitters could not be demonstrated in all the neurons involved in the regulation of visceral organs. For example, on one of the motoneurons of the respiratory system, the neuron RPa3, the FMRFamide, morphine or leu-enkephalin failed to modulate the ACh-induced depolarization, while naloxone decreased it. Furthermore, ACh showed no connection with FMRFamide or opiates when applied to the neurons RPa8, V4, V9, V11 or V12, although the membrane potential of these neurons was altered by FMRFamide (table 2).

5. Membrane characteristics of habituating neurons are modified by FMRFamide

Two types of neurons with different plasticity have been identified in the right and left parietal ganglia (fig. 10) of *Helix pomatia*[8,27]. One type (named habituating cells) was found to habituate to serial intracellular stimulation. Spike generation decreased or stopped within one or several minutes after the onset of prolonged stimulation with direct depolarizing current. Other cells were found to be non-habituating (fig. 10), these showed no decline in their response to intracellular stimulation for hours[8,27].

In this series of experiments the habituating neurons were penetrated with two microelectrodes filled with 2.5 M KCl and having a resistance of 10–20 MOhm. The membrane and action potentials of the neurons were recorded with conventional microelectrophysiological equipment. Habituation of the neurons was tested using intracellular stimulation with depolarizing current pulses of threshold value (duration 1 s, frequency 2.0 Hz). The changes in habituation of the neurons were determined after application of FMRFamide, and compared to the control value. FMRFamide was added to the bath surrounding the isolated *Helix* ganglia.

FMRFamide was found to be involved in the regulation of membrane plasticity of habituating cells. For the habituating cells the threshold concentration for FMRFamide was found to be 10^{-6} M. In order to demonstrate the effect of FMRFamide on the plasticity of the neurons FMRFamide was added to the bath for 1–2 h and the changes in responses to the intracellular stimulation were studied and compared to those in the control.

FMRFamide abolished habituation to threshold intracellular stimuli. In controls, the neurons habituated within one or several minutes of stimulation. Following FMRFamide treatment there was no habituation of the response for more than 2 h (fig. 11). In figure 11 the elimination of habituation can be seen on the 'h' cell, following FMRFamide treatment for about 1 h preceding stimulation.

In addition to preventing habituation, FMRFamide induced a slowly rising depolarization, an increase in input resistance and a decrease in the amplitude of action potentials. Both depolarization and inhibition of habituation caused by FMRFamide were found to be reversible; by washing out the peptide the cell was repolarized and again showed habituation to intracellular stimuli (fig. 11).

Inhibitory effects of FMRFamide on habitation were observed on cells LPa3, RPa2, and on e, f, g, h, i, j, cells. Habituation of cell RPa3 was not blocked by FMRFamide. The habituating cells e, f, g, h, i, j were found to be white cells; they were probably neurosecretory ones.

The blocking effect of FMRFamide on habituating neurons was antagonized by serotonin (5HT) as well as by drugs altering intracellular levels of cyclic 3′,5′-AMP[8,27]. Both imidazole and caffeine were shown to eliminate habituation to intracellular stimuli in the white cells (fig. 12).

FMRFamide had no effect on the non-habituating cells, even when perfused for more than 2 h.

The data obtained allow two conclusions: 1) on the neurons investigated the FMRFamide receptors do not show desensitization; 2) FMRFamide caused basic changes in the plasticity of the habituating cells. This effect is thought to be connected with inhibition of Ca-dependent K^+-conductance[27].

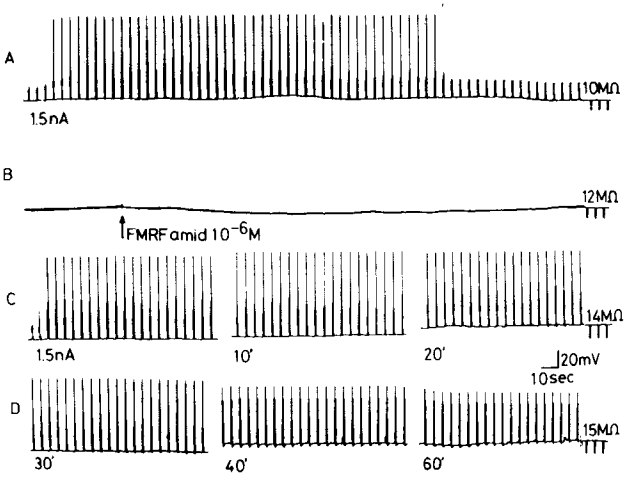

Figure 11. Inhibition of habituation with FMRFamide (10^{-6} M), versus the response of the cell 'h' to intracellular stimulation for one hour. *A* Habituation of the neuron to threshold stimuli. *B* Introduction of FMRFamide to the bath causes slight hyperpolarization then slowly rising depolarization. *C* and *D* After FMRFamide treatment the cell failed to habituate to the intracellular stimulation for more than an hour. The input resistance of the cell is shown before and 5.20 and 60 min following application of FMRFamide. The input resistance of the cell was measured using a hyperpolarizing current of 1 μA.

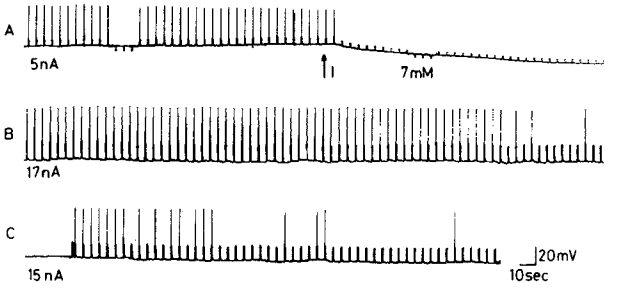

Figure 12. Effect of imidazole on inhibition of habituation of the white cell 'g'. *A* Introduction of imidazole (5 mM) to the bath 30 min after FMRFamide treatment caused inhibition of the habituation to the intracellular stimuli. *B* With simultaneous presence of FMRFamide and imidazole, habituation to threshold intracellular stimulation developed at 5 min. *C* Repeated stimulation of the cell in the presence of FMRFamide and imidazole leads to habituation at 1.5 min (the threshold was decreased to some extent here).

However, alteration in Na- and Ca-conductances following FMRFamide treatment cannot be excluded either. Multiple action of FMRFamide has also been shown on the giant metacerebral cells of *Helix*[6,7].

In Aplysia two cardioactive peptides, different from FMRFamide, have been shown to modulate withdrawal reflexes through a cAMP mediated closure or a specific K^+-channel[1,20]. Our results suggest that the same intracellular unit which is commonly used for behavioral facilitation[1,15] can be involved in the elimination of habituation by FMRFamide. Here, the suppression of a Ca-activated K-current might be a target site.

Elementary forms of learning have been attributed to serotonergic synapses in several molluscan species[2,15,19]. For further studies of this, the method of selective labeling of the serotonerigc soma and endings by 5,6-DHT or 5,7DHT seems likely to be valuable[29]. It means that the serotonergic endings can now be directly studied using a microelectrophysiological method in the semi-intact preparation.

Conclusion

In the subesophageal ganglion complex of *Helix pomatia*, a network consisting of a number of identified neurons was found to regulate the visceral organs, including the cardiorenal, respiratory and genital systems. This network is organized around the multifunctional interneurons and functions as a coupled system. The central elements of the network receive an extremely wide input and show variation in the fine structure of synaptic contacts. Furthermore, great variability in sensitivity to transmitters and to pharmacological treatments has been shown. The multifunctional interneurons (cells V21, V22) are capable of selecting and analyzing peripheral information and also of fulfilling both afferent and efferent roles. The same interneurons are sensitive to a wide range of transmitters or transmitter candidates such as ACh, 5HT, DA, octopamine, glutamate and certain oligopeptides (tables 1 and 2). The results proved that the overlapping neural network, which is involved in the regulation of the visceral organs in *Helix pomatia* L., can be characterized as a temporary combination of regulatory units. No one single unit of the network regulates only one function and the neurons can commonly substitute for each other in the regulation of visceral functions. As a result, the regulatory role should be attributed to temporarily organized networks rather than to single command neurons. The variable cooperation of the subsystems and prohibition of or preference for certain connections will decide whether one or another part of the network is responsible for the regulation of certain functions at a given moment. In this system, the selection of the information is carried out with simultaneously liberated neurotransmitters. The peptides, like FMRFamide, are involved in both the modulation of the membrane effect of classical neurotransmitters and the habituation of the neurons.

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Neural control of the circulatory system of *Aplysia*

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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-

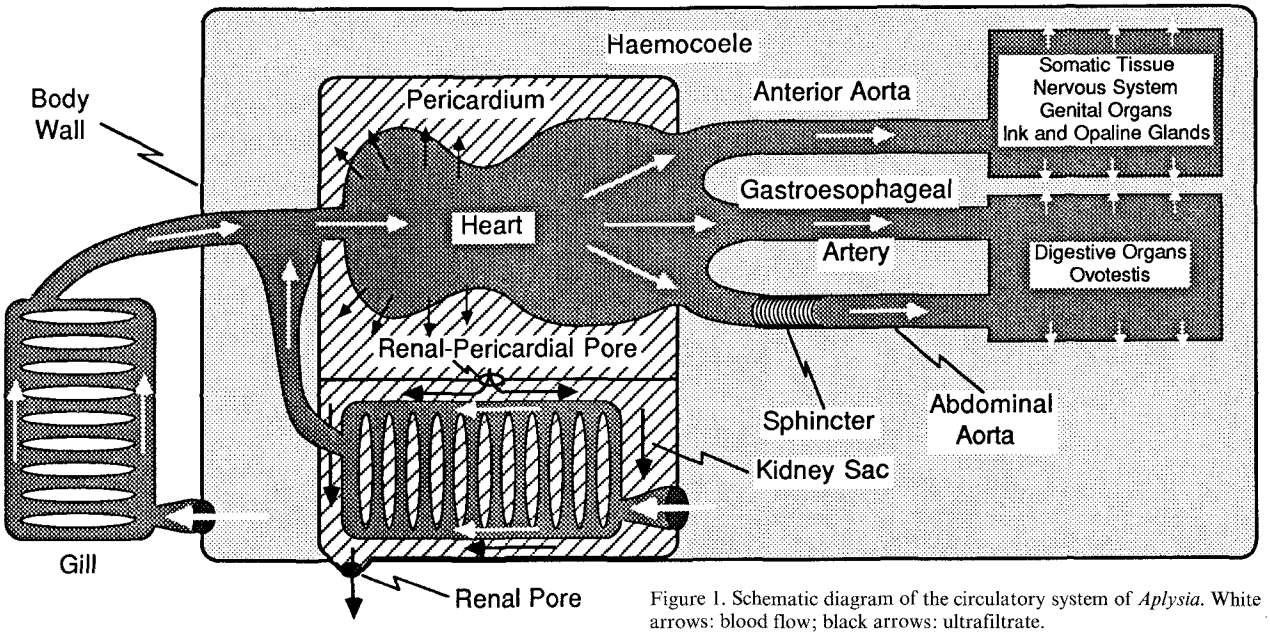

Figure 1. Schematic diagram of the circulatory system of *Aplysia*. White arrows: blood flow; black arrows: ultrafiltrate.