J. exp. Biol. **156**, 315–334 (1991)
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RECEPTIVE FIELDS AND PROPERTIES OF A NEW CLUSTER OF MECHANORECEPTOR NEURONS INNERVATING THE MANTLE REGION AND THE BRANCHIAL CAVITY OF THE MARINE MOLLUSK *APLYSIA CALIFORNICA*

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Accepted 4 October 1990

Summary

The rostral LE cluster (rLE) is a new set of mechanoreceptor neurons of the abdominal ganglion innervating the mantle area, the branchial cavity, the gill and the siphon of the marine mollusk *Aplysia californica* Cooper. We have compared the organization of rLE cell receptive fields with that of three other clusters of sensory neurons in the abdominal ganglion (LE, RE and RF) that we have re-analysed. There is extensive overlap of receptive fields from the four populations of sensory cells, and the most exposed areas of the mantle are the most densely innervated. The sensory threshold is similar for all groups. The action potentials of the LE, rLE and RE neurons are broadened by serotonin and the peptide SCP_B and narrowed by dopamine and FMRFamide. The RF group does not show the same kind of sensitivity to these neuromodulators. The synaptic outputs of the LE and rLE neurons undergo similar synaptic depression and homosynaptic and heterosynaptic facilitation. We estimate that 100 mechanoreceptor neurons innervate the entire mantle and siphon skin, gill and branchial cavity of *Aplysia*. The degree of their convergence onto various interneurons and motor neurons mediating the gill- and siphon-withdrawal reflex and other reflexes is under investigation.

Introduction

The gill- and siphon-withdrawal reflex of the marine mollusk *Aplysia californica*, like reflexes in many other invertebrate preparations (Alkon and Nelson, 1990; Atwood and Govind, 1989; Carew and Sahley, 1986; Colebrook and Lukowiak, 1989; Crow, 1988), is a useful model system for studying the cellular basis of behavior and its modifications by experience (Byrne, 1987; Kandel and Schwartz, 1982). This reflex undergoes simple forms of learning such as habitua-

Key words: *Aplysia californica*, mechanoreceptor neurons, receptive fields, mantle region.

tion, dishabituation, sensitization and classical conditioning (Abrams, 1985; Carew *et al.* 1983; Colwill *et al.* 1988; Hawkins *et al.* 1989). The identification of some of the sensory neurons, interneurons and motor neurons mediating it has been carried out in order to analyze the sites and the cellular mechanisms underlying various types of behavioral changes. The neuronal network has two major components: first, the monosynaptic connections from the sensory neurons to motor neurons, and second, the polysynaptic pathways with their excitatory and inhibitory interneurons (Byrne *et al.* 1974, 1978; Castellucci and Kandel, 1974; Castellucci *et al.* 1978; Dale and Kandel, 1990; Frost *et al.* 1988; Hawkins, 1989; Hawkins and Schacher, 1989; Hawkins *et al.* 1981; Kupfermann *et al.* 1974; Mackey *et al.* 1989.)

A great deal of effort has been devoted to investigating the monosynaptic component of the withdrawal reflex since it is one of the major sites of the synaptic plasticity that accompanies depression and facilitation of the withdrawal reflex (Bailey and Chen, 1989*a*,*b*; Bailey *et al.* 1979; Braha *et al.* 1990; Castellucci and Kandel, 1974; Castellucci *et al.* 1970, 1978; Dale *et al.* 1988; Frost *et al.* 1985; Hawkins *et al.* 1983). During our investigation of the less studied polysynaptic component of the reflex, we discovered a new cluster of mechanoreceptor neurons (rLE) whose receptive fields complement those of the three other clusters of the abdominal ganglion (Byrne, 1980; Byrne *et al.* 1974).

The first cluster of sensory neurons of the abdominal ganglion to be described in detail was the LE one (Byrne *et al.* 1974). This cluster is located on the left ventral side of the abdominal ganglion and its neurons have their receptive fields mainly on the siphon. The RE cluster, located on the right ventral side of the ganglion, was identified in the same study. Some of its receptive fields were reported to cover the anterior part of the mantle shelf. Finally, the third group, or RF cluster, was identified by Byrne (1980) while studying the neuronal circuit for inking behavior. These cells are located mainly on the right side around the commissure of the abdominal ganglion. Their receptive fields were not described extensively.

In this report, we describe the properties of the new rLE cluster of neurons, their receptive fields, their sensory threshold and some of their physiological and synaptic properties. We also compare their properties with those of the other identified sensory clusters that we have reinvestigated.

Materials and methods

Types of preparation

Experiments were performed on *Aplysia californica* supplied by Marinus Inc. (Long Beach, CA, USA). The animals were kept in a 900 l holding tank containing artificial sea water (ASW) (Forty Fathoms, Marine Enterprises, Baltimore, MD, USA) maintained at 14°C and pH 7.8–8.1. To anesthetize the animals before surgery they were injected with isotonic $MgCl_2$ to about one-third of their weight.

Three types of preparations were used in this study. In the first type, the intact animal preparation, an incision was made in the neck region to externalize the

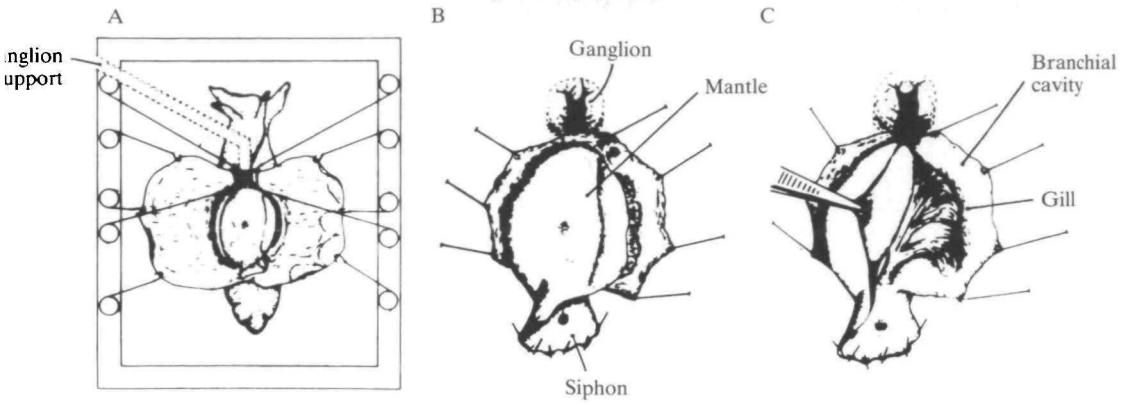

Fig. 1. Two types of preparations used in this study. (A) The intact animal preparation. The animal is suspended in a small chamber and the abdominal ganglion is externalized to permit intracellular recordings. (B, C) The mantle preparation. The abdominal ganglion is still attached to the intact mantle organs by all major nerves. To plot the receptive fields on the gill and the branchial cavity, the mantle was gently lifted with blunt forceps (shown in C).

abdominal ganglion. The ganglion was attached to a small Sylgard-coated platform and desheathed to allow intracellular recordings. All nerves and connectives were left intact except for the vulvar nerve, which was cut. The animal was then put in a 1.5 l tank and suspended by blunt hooks attached to the parapodia and around the incision (Fig. 1A). This preparation was used to search for receptive fields over the whole body.

In the second type, the mantle preparation, the mantle region was removed with the base of the parapodia left intact and pinned to the Sylgard-coated floor of a 150 ml chamber (Fig. 1B,C). This mantle preparation permitted the exploration of receptive fields on the mantle shelf, the purple gland, the siphon, the gill, the branchial cavity and the base of the parapodia. The abdominal ganglion was still attached to the mantle organs by the branchial, genital, pericardial and siphon nerves.

The third type, the isolated ganglion preparation, consisted of the isolated abdominal ganglion pinned to the floor of a 2 ml chamber. Suction electrodes were placed on the cut nerves for extracellular stimulation and the small volume of the chamber allowed the perfusion of various drugs for pharmacological tests. For the mantle and the isolated ganglion preparations, the ganglion was immersed in 0.5 % gluteraldehyde for 30 s, lightly fixing the sheath to minimize its contractions and facilitate its dissection. This was carried out in a mixture of cold (7 °C) ASW and isotonic $MgCl_2$ (1:1) to reduce synaptic activity during the dissection. This solution was then replaced by ASW (18–22 °C) and the preparation was allowed to rest for a minimum of 1 h before beginning the experiments.

Determination of receptive fields

The receptive fields of the sensory neurons were determined by tactile

stimulation with a calibrated set of von Frey hairs, ranging from 0.5 to 7 g. A 0.5 g von Frey hair was sufficient to elicit one or two spikes in the center of a receptive field (Byrne *et al.* 1974). We obtained the maximal extent of a receptive field by using a von Frey hair of 7 g. For receptive fields on the gill and the floor of the branchial cavity, the mantle edge was lifted gently with forceps to allow stimulation of less accessible parts (Fig. 1C). Response discharges were fairly independent of resting potentials and action potential amplitudes.

Electrophysiology

Standard intracellular recording and stimulating techniques were used to record from and to stimulate individual sensory or motor neurons. Motor neuron L7 (Kupfermann *et al.* 1974) or LFS siphon motor neurons (Frost *et al.* 1988) were used to monitor excitatory postsynaptic potentials (EPSPs) evoked by the sensory neurons. The follower cell was hyperpolarized by 30 mV from its normal resting potential to enhance the EPSPs and to prevent spiking. After a rest of 10 min, the sensory cell was stimulated with intracellular pulses and EPSPs were recorded in the follower cell. Three protocols were used to investigate the electrophysiological characteristics of the cells. To examine homosynaptic depression, the first EPSP was followed by nine others evoked at intervals of 30 s. Post-tetanic potentiation was examined by evoking five EPSPs at 5 min intervals to establish a baseline value, followed by five trains (500 ms, 25 Hz) of sensory cell action potentials. The intertrain interval was 5 s and the duration of each intracellular pulse was 20 ms. Sampling of the EPSP amplitudes was resumed once every 5 min for the next 60 min. In the pairing protocol, the same trains of action potentials were paired with extracellular stimulation of the left connective tract (500 ms train duration, 25 Hz, 0.5 ms pulse duration, 10 V). Each extracellular train of stimuli began when the intracellular train of action potentials ended (Walters and Byrne, 1985).

The effects of serotonin (5-hydroxytryptamine, 5-HT; 10^{-4} mol l^{-1}), small cardioactive peptide B (SCP_B; 5×10^{-6} mol l^{-1}), dopamine (10^{-4} mol l^{-1}) or Phe-Met-Arg-Phe-NH_2 (FMRFamide; 5×10^{-6} mol l^{-1}) on spike duration in sensory cells were monitored after local perfusion of these agents onto the soma. In these experiments, the ganglion was bathed in 50 mmol l^{-1} tetraethylammonium chloride (TEACl) (Klein and Kandel, 1978); this broadens the spike by blocking some K^+ channels and allows an easier analysis of the drug effects.

Results

Receptive fields of the rLE and LE cells

The new cluster of sensory cells, rostral LE or rLE, is situated in the rostro-lateral part of the left side of the abdominal ganglion, just caudal to the left bag cell cluster (Fig. 2). It is useful to turn the ganglion on its side to see the entire cluster. This contains about 25 neurons which, like the LE cells, are orange in color, have dark rims and have diameters of about 50 μm (Byrne *et al.* 1974).

The rLE neurons are normally silent and have no spontaneous excitatory or

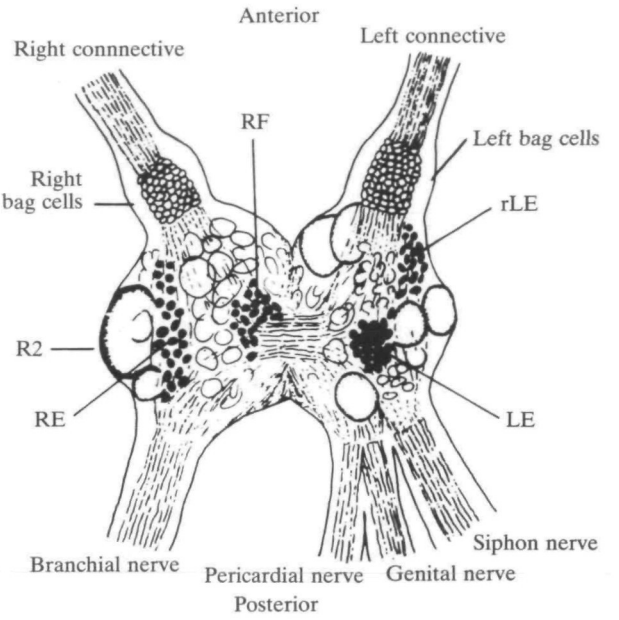

Fig. 2. Ventral view of the abdominal ganglion showing the LE, RE, RF and the new rLE cell clusters in a schematic way. Some large neurons have been drawn in for general reference.

inhibitory postsynaptic potentials. Their average resting potential is -39 ± 8 mV (20 neurons in seven preparations) and the average amplitude of their action potential is 64 ± 9 mV (16 neurons in three preparations). Some cells show a large (10–15 mV) and long-lasting (1–2 s) depolarizing afterpotential that sometimes elicits an afterdischarge.

The axons of the rLE cells have a distinctive distribution in the nerves. Action potentials were elicited by stimulating various nerves of the abdominal ganglion with suction electrodes (Fig. 3A). Compared to the LE cells, almost all of which (96 %, 20/21) have a single axon in the siphon nerve, only 56 % (50/90) of the rLE cells have a single axon in that nerve. The distribution of rLE axons is varied; we could find axons in all the nerves (except the right connective) and many cells had axons in two nerves simultaneously (Table 1).

Since some of the rLE axons travel in the left pleuroabdominal connective or the branchial nerve, it is possible that the cells may have receptive fields in areas other than the mantle organs. For this reason we began our study of the receptive fields by using the intact animal preparation (Fig. 1A), allowing us to explore the whole surface of the animal with all nerves intact. We used mechanical, chemical (crystals of NaCl) and thermal (hot, 55°C, or cold, 0°C, water) stimuli to determine the sensory class of the rLE neurons. Since the rLE cells were only activated by tactile stimulation of the siphon, mantle shelf or branchial cavity (eight neurons in five preparations), we continued our analysis on the more

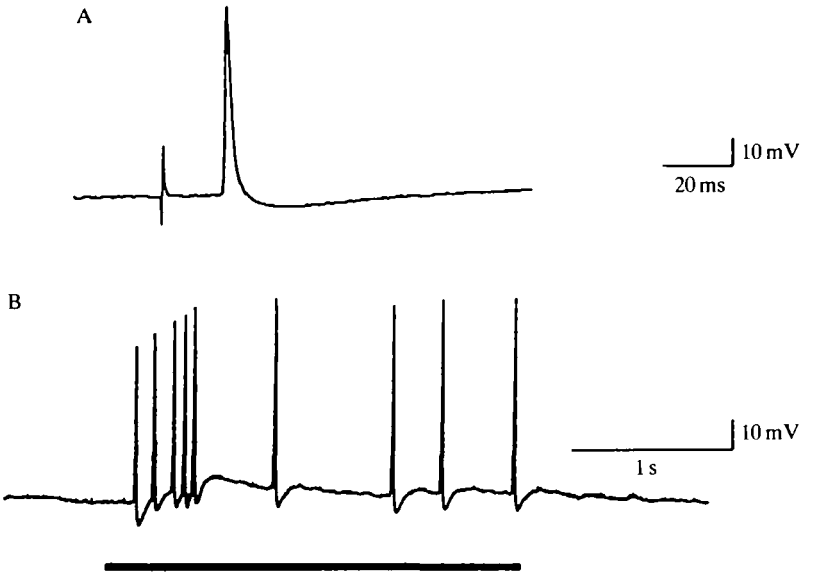

Fig. 3. Intracellular recordings from rLE neurons. (A) An action potential was evoked in an rLE mechanoreceptor cell body by electrically stimulating the siphon nerve with a brief shock. There is an all-or-none action potential with no prepotential. The action potential had a constant latency and followed one-for-one repetitive shocks (15 Hz). (B) Sample response from an rLE neuron to tactile stimulation of the siphon skin with a von Frey hair (7 g). The bottom trace indicates the duration of the stimulation. There is an initial high-frequency discharge which decays to a slower rate.

reduced and simpler mantle preparation (210 neurons in 53 preparations) (Fig. 1B,C).

Some receptive fields of the rLE neurons, like those of the LE neurons, are located on the siphon itself, but in a pattern that complements the fields of the LE cluster. A large proportion of fields (81/210) are found outside the siphon; some receptive fields are on the mantle and the purple gland (33), some are on the floor of the branchial cavity (33) and others are on the gill itself (15), on the pinnules, on the veins or on both. Like the LE cluster organization, there is an overlap of the fields that have very different shapes and sizes. Despite a certain variability, similar receptive fields could be found from one preparation to the other. After considering the regular occurrence of these, we suggest that the rLE cluster consists of 26 cells with receptive fields as shown in Fig. 4. The number of easily visible cells in most preparations is only 15 since the other cells in the group are covered by larger neighboring neurons.

We tested the rLE and LE neurons in the same preparation with von Frey hairs. The activation thresholds of the rLE cells were similar to those of the LE cells; these were generally less than 0.5 g (Byrne *et al.* 1974). A mechanical stimulus to the receptive field of an rLE cell elicits a response like that of the LE cells with an

Table 1. *Axonal distribution of neurons from each cluster*

Number of axons per neuron	Axon location	Cluster			
		LE (N=21)	rLE (N=90)	RE (N=30)	RF N=27)
One	Siphon nerve	96% (20)	56% (50)	–	–
	Branchial nerve	–	13% (12)	30% (9)	19% (5)
	Other nerves	–	12% (11)	3% (1)	–
Two	Siphon+branchial nerves	–	6% (5)	3% (1)	–
	Siphon+other nerves	4% (1)	12% (11)	8% (2)	–
	Branchial+other nerves	–	1% (1)	30% (9)	22% (6)
	Other nerves	–	–	–	4% (1)
Three	Siphon+branchial+other nerves	–	–	10% (3)	7% (2)
	Siphon+other nerves	–	–	–	7% (2)
	Branchial+other nerves	–	–	10% (3)	11% (3)
Four or more	Siphon+branchial+other nerves	–	–	3% (1)	11% (3)
	Branchial+other nerves	–	–	3% (1)	15% (4)
	Other nerves	–	–	–	4% (1)

The percentages of neurons with one or more axons are given in the various boxes.
The siphon and branchial nerves contains most axons from these clusters, but axons also pass into other major nerves; the genital nerve, the pericardial nerve and the left and right connectives.
Numbers in parentheses indicate numbers of neurons.

initial high-frequency discharge followed by a slowly adapting phase that persists until the end of the stimulus (Fig. 3B).

We did not observe any somatotopic organization within the rLE cluster itself; there was no precise correlation between cell body position in the cluster and receptive field position in the periphery. A similar conclusion was also reached for the LE cluster (Byrne *et al.* 1974).

We have confirmed in our study the observation of Byrne *et al.* (1974) that the LE cells have their receptive fields on the siphon and the caudal part of the mantle (43 neurons in nine preparations). However, since our mantle preparation was less reduced than their siphon preparation, we found, in addition, that certain LE cells had their receptive fields on the floor of the branchial cavity (see Fig. 11). These cells were restricted to the rostral edge of the LE cluster.

Receptive fields of the RE cells

The RE cells were first described by Byrne *et al.* (1974). They reported the existence of some receptive fields on the anterior part of the mantle region. We have confirmed and extended their original observation by using the mantle preparation (177 neurons in 28 preparations). We found new receptive fields on the gill itself and in the branchial cavity. We tested the idea that a cluster 'rRE' symmetrical to the rLE might exist on the right side of the abdominal ganglion, but found none. We observed, however, that the RE cluster is more spread out than the LE or rLE cluster taken separately.

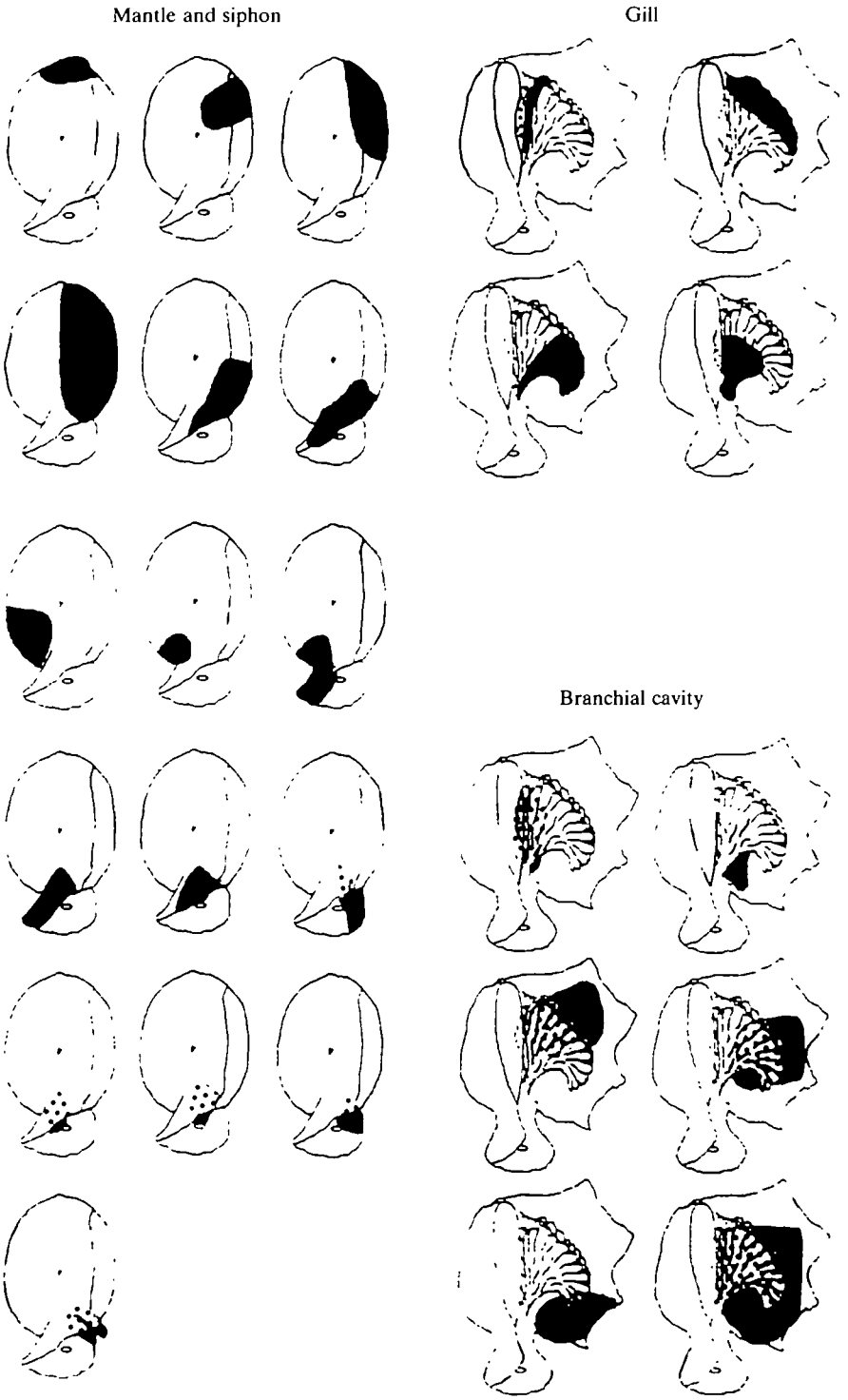

Fig. 4

Fig. 4. Summary of the receptive field organization of the rLE cluster cells. The information was collected from 210 neurons in 53 preparations. The cluster is estimated to contain 26 cells. All these fields were observed, with minor variations, in several preparations. A black area indicates that a receptive field is on the external visible surface, a dotted area indicates that the receptive field is under the illustrated organ. The fields are found on the mantle and the siphon skin, the gill itself and on the floor or the ceiling of the branchial cavity (see text for further details).

Like the LE and rLE cells, the RE cells have a characteristic dark rim and the same fundamental electrophysiological properties. The RE cluster is located on the right ventral side of the abdominal ganglion just medial to the giant neuron R2 (Fig. 2). It begins in the more caudal part of the ganglion and often extends rostrally past R2. This cluster is not compact, allowing other cell types to intrude into it. The axons of RE neurons have a wider distribution than those of the rLE cells (Table 1) and often enter more than one nerve, although one of the axons is almost always in the branchial nerve (90%; 27/30).

Using the same method as for the rLE cluster, we estimate that the RE cluster is made up of 27 cells with the receptive fields illustrated in Fig. 5. Like the rLE fields, the RE fields located on the gill are on the pinnules, on the veins or on both.

Receptive fields of the RF cells

The RF cluster, about 20 cells according to Byrne (1980), is situated on the right side of the abdominal ganglion around the commissure (Fig. 2). The cells are located deep in the ganglion and are often hidden by larger cells. Byrne (1980) described briefly some of the receptive fields at the base of the gill and on the mantle skin. Since they are less accessible, we did not make an exhaustive investigation of their receptive fields, as we did for the rLE and the RE cells. In our re-examination of the RF cells (31 cells in six preparations) we found receptive fields not only on the veins of the gill but all over the gill itself, inside the branchial cavity, and on the mantle in a region poorly covered by the other clusters. More than 50% of these cells had three or more axons in the various nerves (Table 1). An example of a particularly successful experiment is illustrated in Fig. 6.

Comparison of the synaptic plasticity of the rLE and LE neurons

Homosynaptic depression, homosynaptic facilitation and heterosynaptic facilitation have all been reported at the monosynaptic junction between the LE neurons and the motor neurons or interneurons of the gill- and siphon-withdrawal reflex (Abrams *et al.* 1984; Castellucci and Kandel, 1974; Castellucci *et al.* 1970). We wanted to know if the same types of synaptic plasticity occurred at the synapses of the rLE cells.

We first compared the synaptic depression of the LE and rLE EPSPs and found a great similarity between the two populations (Fig. 7). The homosynaptic and

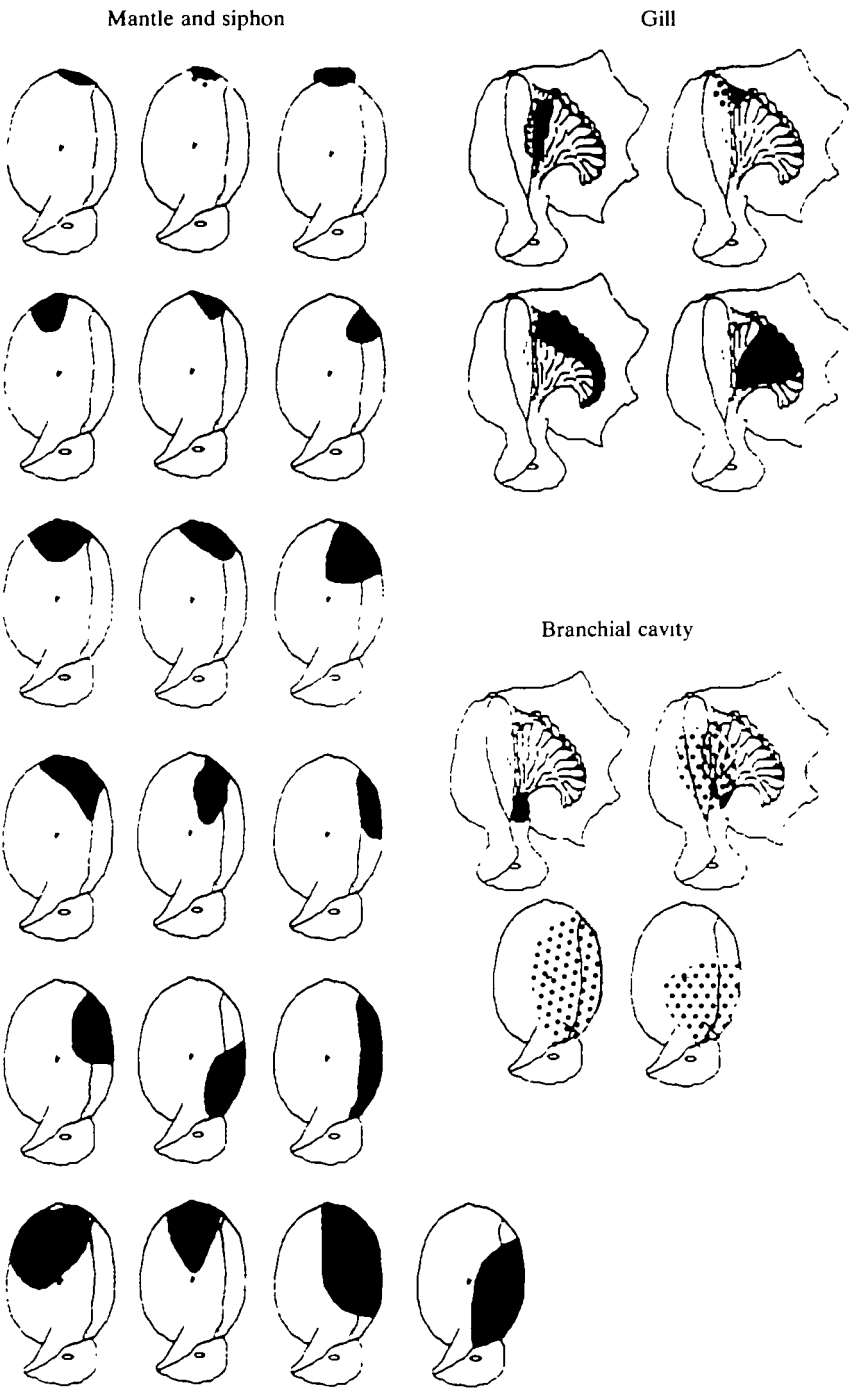

Fig. 5

Fig. 5. Summary of the receptive field organization of the RE cluster cells. The information was collected from 177 neurons in 28 preparations. The cluster is estimated to contain 27 cells. All these fields were observed, with minor variations, in several preparations. The fields are found on the mantle and the siphon skin, the gill itself and on the floor or the ceiling of the branchial cavity (same conventions as for Fig. 4; see text for further details).

paired heterosynaptic protocols (see Materials and methods) also produced similar EPSP facilitation in both groups (Figs 8 and 9). We have not explored all possible protocols, but the three we have chosen indicate that these clusters share many physiological properties. This idea is supported by the additional observations that these cells have a similar threshold of activation, a similar type of receptive field and similar pharmacology (see below).

Effects of serotonin, SCP_B, FMRFamide and dopamine on the duration of the LE, rLE, RE and RF action potentials

Previous studies have shown that LE action potentials are broadened by serotonin (5-HT) and SCP peptides and narrowed by FMRFamide and dopamine (Abrams *et al.* 1984; Klein and Kandel, 1978). We tested whether these neuromodulator agents had a similar action on the neurons of all four clusters (Fig. 10). We found that the action of these transmitters was essentially identical for the LE, rLE and RE clusters (Table 2). To our surprise, two different features were observed in the RF cells. First, these cells seemed to be less sensitive to TEACl and their action potentials did not broaden as much as the action potentials of the

Table 2. *Percentage increase or decrease of action potential duration in cells from different clusters in the presence of modulators*

	Cluster			
Drugs	LE	rLE	RE	RF
5-HT	+146±24 %*	+86±12 %*	+107±18 %*	+40±13 %**
(10^{-4} mol l^{-1})	N=16	N=12	N=15	N=11
SCP_B	+53±10 %*	+78±19 %*	+40±8 %*	+6±4 %†
(5×10^{-6} mol l^{-1})	N=10	N=10	N=12	N=9
DA	−19±2 %*	−28±6 %*	−23±4 %*	0±0 %†
(10^{-4} mol l^{-1})	N=16	N=10	N=14	N=9
FMRFamide	−19+4 %*	−22±4 %*	−16±3 %*	−1±1 %†
(5×10^{-6} mol l^{-1})	N=9	N=10	N=10	N=7

In each box, the mean values and the standard error of the mean are indicated: N, number of neurons; *$P<0.01$; **$P<0.05$; †, not significant for a paired t-test comparison.

Notice that for DA and FMRFamide the values of the percentage of diminution may have been underestimated because of the 'shunting effect' of these drugs. In fact, in 8 % of the DA application and 61 % of the FMRFamide application action potentials could not be triggered with the constant pulse we used 30–60 s after the beginning of application.

DA, dopamine; 5-HT, serotonin.

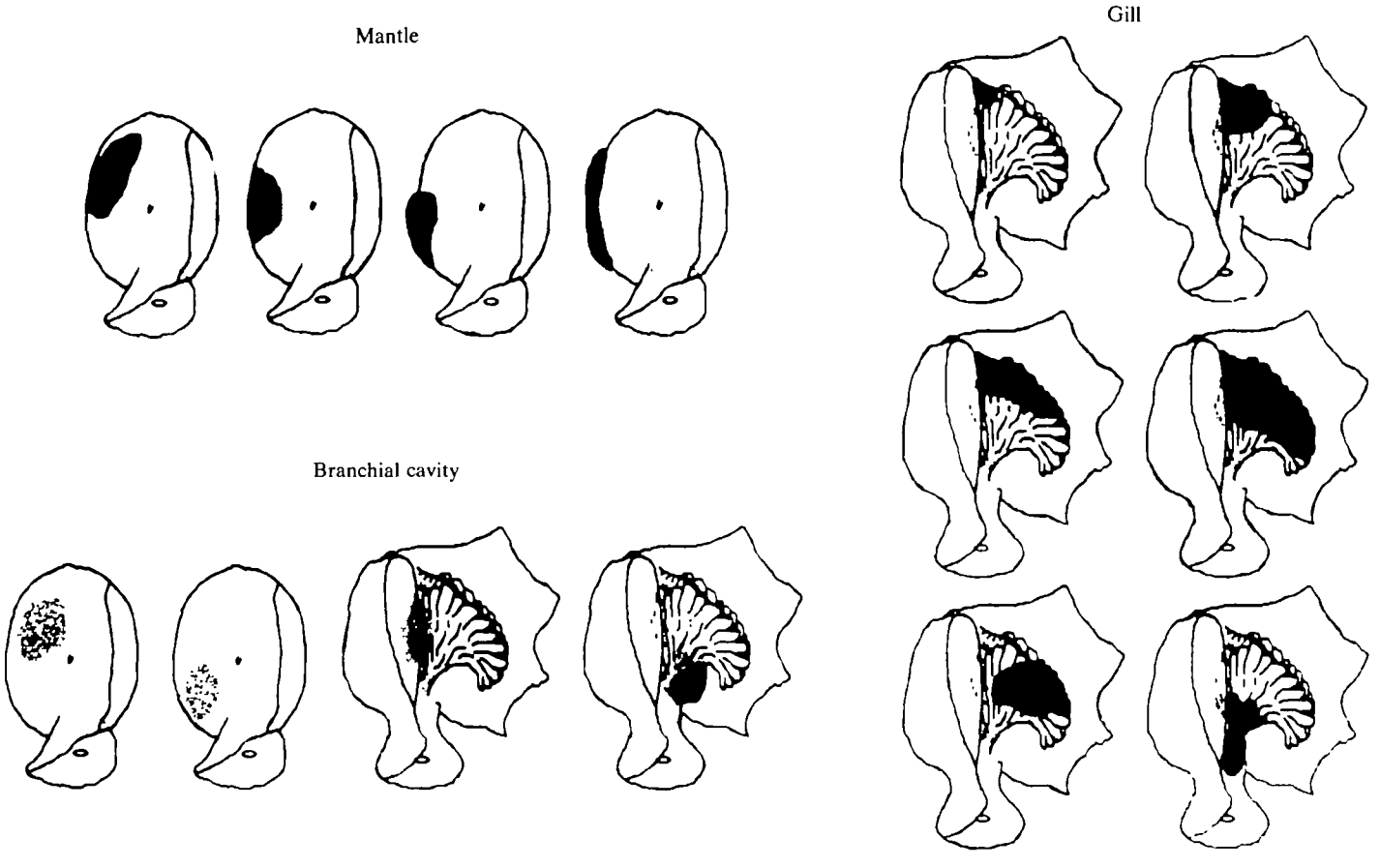

Fig. 6. RF cells receptive fields observed in a single experiment. A similar receptive field organization was found in five other experiments. A black area indicates that a field is on the external visible surface. The two fields that are lightly shaded were on the ceiling of the branchial cavity.

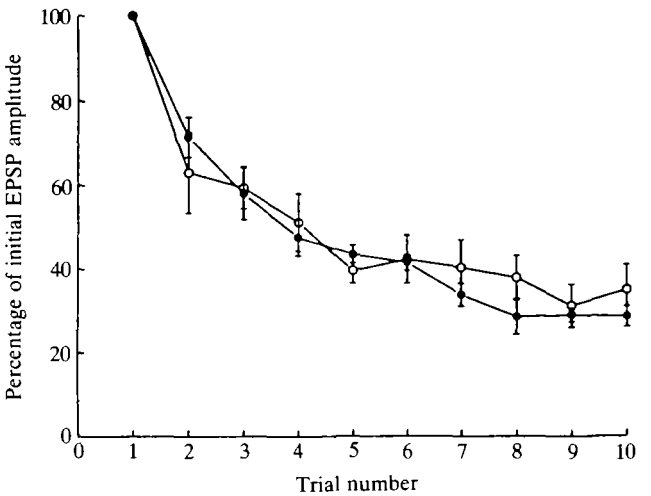

Fig. 7. Similarity of synaptic depression from the rLE (○) and LE (●) cells. Intracellular recordings were obtained from rLE or LE neurons and motor neuron L7. Single action potentials were obtained by intracellular stimulation of the rLE or LE cells once every 30 s. Data are expressed as a percentage of the initial EPSP amplitude; values are mean±S.E.M. The mean initial EPSP amplitudes were not significantly different; 6.9±3.1 mV (S.E.M.), N=5, for the LE cells and 5.7±2.6 mV, N=5, for the rLE cells.

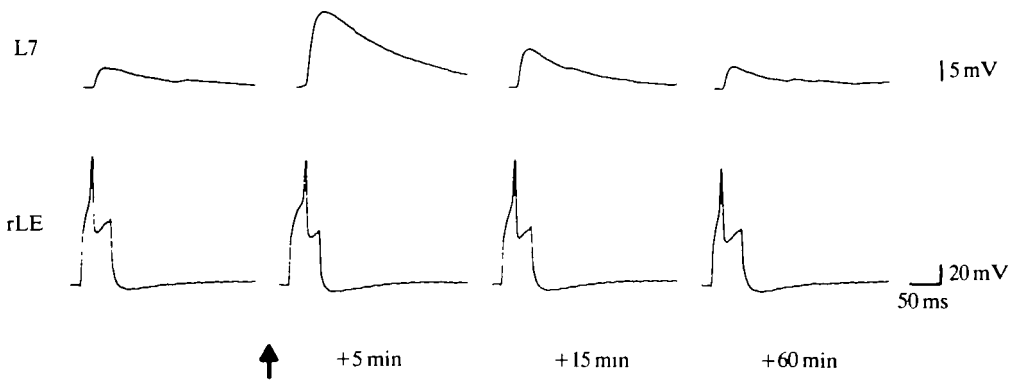

Fig. 8. Facilitation of rLE EPSPs in motor neuron L7. Intracellular stimulation of an rLE cell (lower trace) evoked an EPSP in cell L7 (upper trace). In this particular protocol, (paired protocol, at the arrow), five EPSPs were evoked at 5 min intervals to establish a baseline value, then five trains (500 ms, 25 Hz, intertrain interval 5 s) of sensory cell action potentials were elicited. These action potentials were paired with extracellular stimulation of the left connective tract (500 ms train duration, 25 Hz, 0.5 ms pulse duration, 10 V). Each extracellular train of stimuli began when the intracellular train of action potentials ended. Sampling of the EPSP amplitudes was resumed once every 5 min for the next 60 min. Sampled EPSPs taken before the trial and after 5, 15 and 60 min are illustrated.

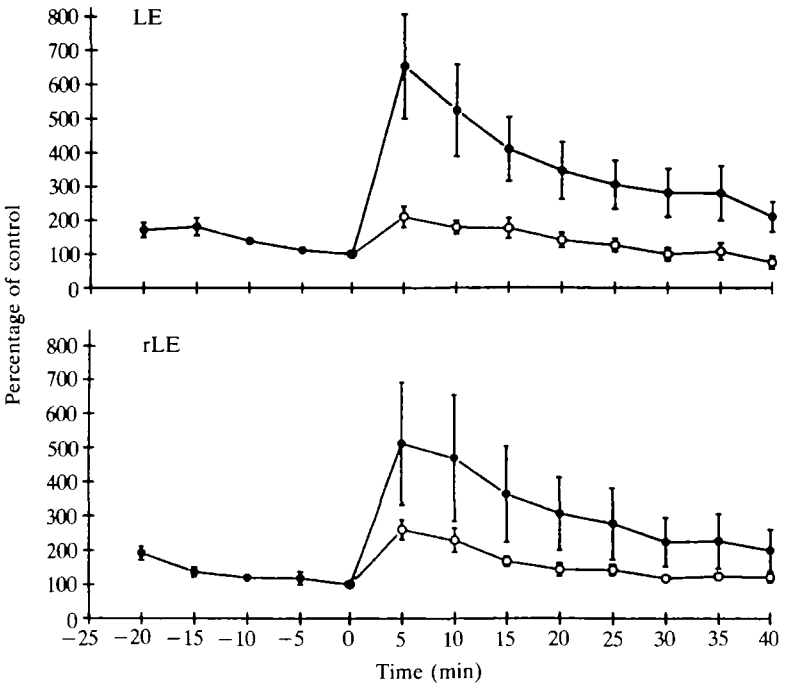

Fig. 9. Homosynaptic and paired heterosynaptic facilitation of EPSPs from rLE and LE cells. Homosynaptic (○) or paired protocols (●) were used for rLE and LE cells (see Fig. 8 and Materials and methods for details). Samples of the EPSP amplitudes were taken every 5 min. The 100% control value was the amplitude of the last EPSP before the facilitating procedures; LE homosynaptic $N=6$, paired $N=6$; rLE homosynaptic $N=9$, paired $N=7$. There is no significant difference in the magnitude of the effects for the two protocols in the two clusters. Values are mean±S.E.M.

other neurons when exposed to TEACl. The spike duration of the RF cells, when measured to one-third of the height of the action potential, was 12±2 ms (27 neurons in seven preparations). This duration was significantly different from the durations of the other groups (t-test, $P<0.001$): 19±6 ms (±S.D.) (16 neurons in four preparations) for the LE, 21±4 ms (12 neurons in five preparations) for the rLE and 19±9 ms (26 neurons in eight preparations) for the RE. Second, the effects of 5-HT were more modest, whereas SCP_B, dopamine and FMRFamide showed no significant effects (Table 2). We retested these last two drugs with another protocol to confirm these observations. Instead of using TEACl to broaden the spike, we took advantage of the inactivation kinetics of some potassium channels to broaden the action potentials by repetitive firing in ASW (15 Hz, 1.5 s) (Abrams, 1985). We compared the duration of the last action potential in the train before and after exposure to dopamine or FMRFamide. There was no effect; the results were similar to the ones obtained with these two transmitters in the presence of TEACl (six neurons in three preparations). In contrast, we carried out parallel experiments with LE or RE cells (12 neurons) in

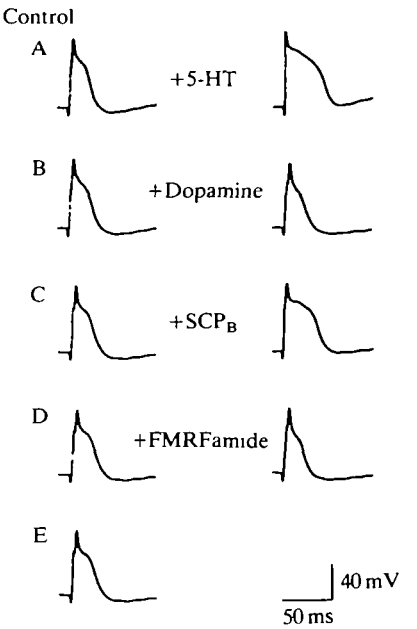

Fig. 10. Changes in an rLE action potential duration caused by 5-HT (10^{-4} mol l^{-1}), dopamine (10^{-4} mol l^{-1}) SCP_B (5×10^{-6} mol l^{-1}) and FMRFamide (5×10^{-6} mol l^{-1}). Traces are from a single experiment in which action potentials were evoked by a short (3 ms) constant pulse stimulation every 10 s. Drugs were perfused locally and sequentially (A–D) with control wash (ASW only) between drug applications (left-hand column). The experiment was carried out in the presence of 50 mmol l^{-1} TEACl. (E) The action potential after FMRFamide had been washed out. The results of several experiments are summarized in Table 2.

the same three preparations and found effects comparable to those with TEACl. The duration of the action potentials of the RF cells (4.8±0.3 ms, standard error of the mean, $N=14$) in the control situation was again significantly shorter (t-test, $P<0.001$) than those of the LE and RE cells (7.7±0.4 ms, $N=12$).

Discussion

The mantle preparation left many organs intact (gill, floor of the branchial cavity and the whole mantle) and also retained all the small nerve collaterals of the principal nerves. This allowed us to maintain a more intact organization of the receptive fields than in previous preparations (Byrne *et al.* 1974) and to identify new receptive fields. Several features emerged from the mapping of receptive fields of the newly found rLE cluster. Some areas innervated by the rLE cells are regions that are either not covered or are covered poorly by the other identified groups. For example, a large proportion of the rLE receptive fields are located on the siphon but cover a region rostral to the anus which is poorly innervated by the LE cells (Fig. 4). Large rLE fields cover parts of the mantle skin and purple gland

that are already partially covered by the RE cells. The receptive fields on the gill are organized in such a way as to cover practically the whole organ. Finally, a significant number of rLE cells innervate the floor of the branchial cavity, a very distinctive characteristic of this group.

The partitioning of axons into peripheral nerves is different for each group of sensory cells and reflects the diversity of their receptive fields. For instance, the LE cells, which have their fields almost exclusively on the siphon, have the great majority of their axons in the siphon nerve. The other clusters (rLE, RE and RF) have a much more varied distribution and often even have axons in the connectives. This latter characteristic also distinguishes them from the pleural VC mechanoreceptors (Walters *et al.* 1983) that do not send any axons into the connectives, a pathway to the other ganglia and their peripheral nerves. Thus, axons in the connectives are probably efferent to other motoneurons or interneurons, since we were unable to find fields outside the mantle area in the intact preparation.

The VC sensory cells (Walters *et al.* 1983) of the pleural ganglia that innervate the tail, and the cerebral J and K sensory cells (Rosen *et al.* 1979, 1982) that innervate the buccal mass, are found in two symmetrical clusters in each hemiganglion. The abdominal ganglion does not have such a clear and well-defined symmetry; there is no symmetrical equivalent of the rLE cluster on the right side of the ganglion. However, since the RE cluster sometimes extends from the caudal to the rostral end of the right hemiganglion, one can speculate that it might correspond to the LE and rLE combined, which are, in fact, sometimes in close apposition. This hypothesis would leave the RF cluster without a homologous cluster, an idea that is reinforced by the different pharmacological properties of the RF cells. The lack of a clear symmetry in the abdominal ganglion may be due to the asymmetrical character of the innervated structures themselves (the mantle, the gill and the siphon). It may also be due to the ontogeny of this particular ganglion, which is formed by the fusion of three main components; a right, a left rostral and a left caudal entity (Kriegstein, 1977).

From the summaries of the receptive field organizations (Figs 4, 5, 6 and 11) one can make the following observations. First, each cluster innervates a preferred region but no one cluster has a regional monopoly; there is good receptive field overlapping from the cells of all the groups. The right-hand column of Fig. 11 illustrates the total superposition of all the receptive fields of all the clusters made by taking the organization summary of each cluster. This shows that there is no gap in the receptive organization of the mantle organs and it would appear that the four known sensory clusters are sufficient to mediate the mechanoreception for the whole mantle region. Second, the smallest receptive fields are localized to the caudal (siphon) or rostral extremities of the mantle. These parts of the mantle are the most exposed areas and it is conceivable there may be a need to enhance spatial resolution in these regions. Third, there is considerable overlap among these small receptive fields; these are then covered by medium-sized fields that are in turn covered by a few larger fields. We presume that sensory neurons with small

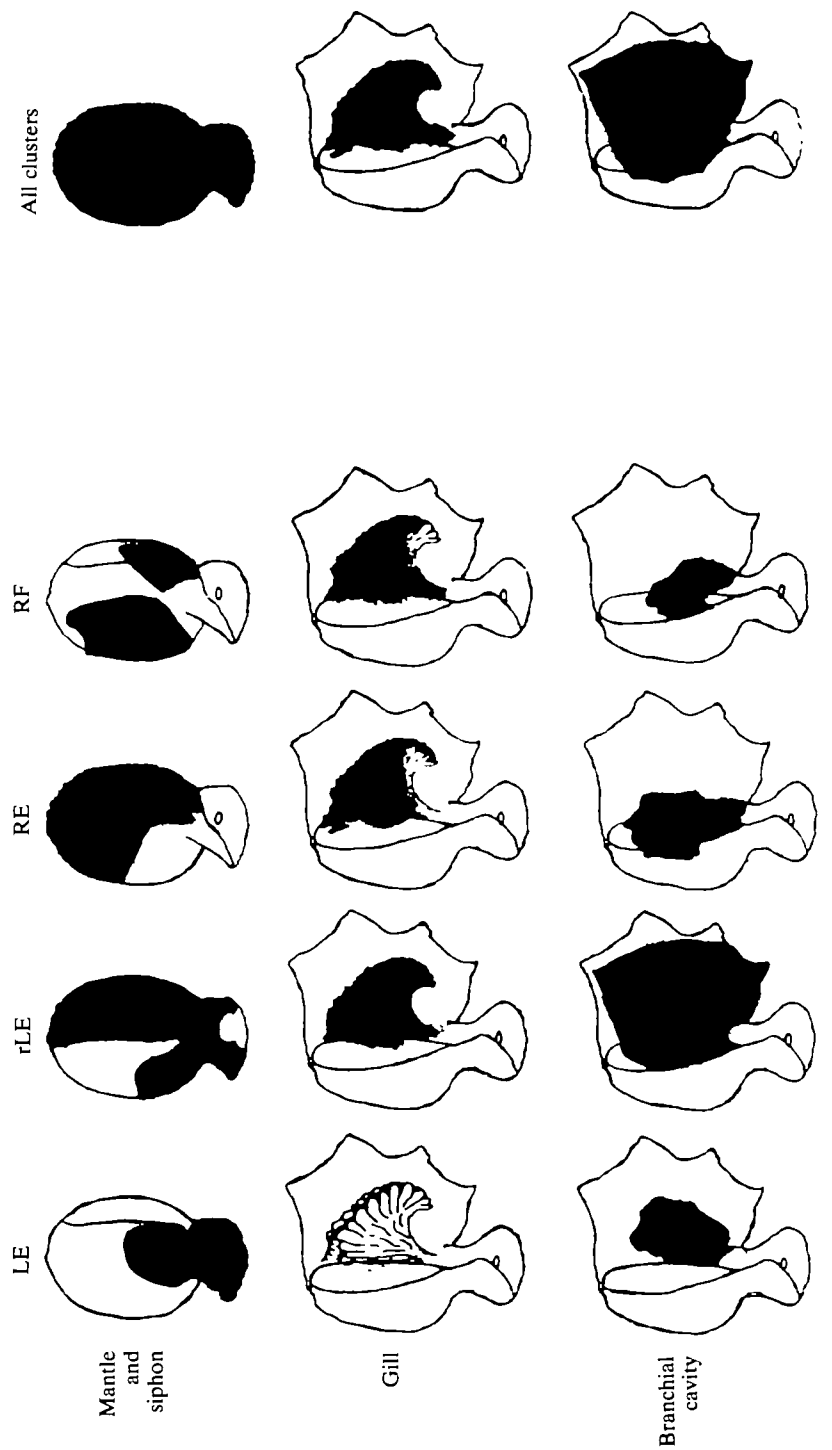

Fig. 11. Relative contribution of the four mechanoreceptor cell clusters, LE, rLE, RE and RF, to the innervation of various mantle organs. Each column summary was obtained by adding the receptive fields of all the cells of an individual cluster. In the right-hand column, the contributions of all the receptive fields of each cluster are added (see text for discussion).

fields connect preferentially with motor neurons and interneurons having a more local range of action, while sensory neurons having larger fields may connect to major motor neurons, like L7, that can move many organs. This idea should be explored in more detail. Similarly, the divergence of the connections of the various clusters on the interneurons and motor neurons of the withdrawal reflex, and of other reflexes, needs to be studied (Altman and Kien, 1990; Zecevic *et al.* 1989). There is a need to re-evaluate the contribution of all the elements of the reflex in the intact animal, since the withdrawal reflex may be the sum of a variety of distinct peripheral and central elements (Bailey *et al.* 1979; Colebrook and Lukowiak, 1988; Jacklet and Rine, 1977; Kurokawa and Kuwasawa, 1988; Kurokawa *et al.* 1989; Leonard *et al.* 1989).

It is possible that the sensory cells innervating the gill and the mantle cavity will synapse with peripheral neurons of the gill (Kurokawa and Kuwasawa, 1988; Kurokawa *et al.* 1989), as is the case for the LE cells and the local motor neurons in the siphon skin (Bailey *et al.* 1979). The apparent difference in the pharmacology of the RF cells may be a clue to differential modulation of the reflex components. This result is similar to observations made in the cerebral ganglion (Rosen *et al.* 1989). One can ask, for example, why there are so many different modulatory substances and if there are other types yet to be found. Is there a need to modulate some components selectively? Could those differential modulations last for varying times? If such differences do exist, the reflex could be used to study the cellular and molecular mechanisms of short-term, intermediate and long-term changes in the same subject (Castellucci *et al.* 1986, 1988, 1989).

We thank Marc Klein and Tanja Ouimet for their critical comments on the manuscript, Isabelle Morin for preparing the illustrations and Nicole Guay for typing the manuscript. We acknowledge support by the Medical Research Council of Canada (grant MA-10047), the National Institute of Neurological Disorders and Stroke (grant R01 NS19595) and the Richard and Edith Strauss Canada Foundation.

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