J Comp Physiol (1982) 149:221–234

Journal of Comparative Physiology · A


# Chemoreception and Mechanoreception in the Gastropod Mollusc *Pleurobranchaea californica*

## I. Extracellular Analysis of Afferent Pathways

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Accepted May 17, 1982

**Summary.** 1. Sensory reception in the mollusc *Pleurobranchaea californica* was studied in whole animals and surgically reduced preparations by delivering chemical and mechanical stimuli while observing behavior or recording extracellularly the responses of the corresponding nerves. Sensory structures studied included the rhinophores, tentacles and oral veil.

2. Specimens reliably (50% or more) exhibited feeding responses to 10 of 18 amino acids tested (Table 1), including alanine and glycine. In 14 electrophysiological experiments on the rhinophore, medium to high centripetal responses to alanine and/or glycine were obtained in 7 preparations (Fig. 2), while little or no response was obtained in 7 preparations. Responses to different amino acids were sometimes mediated by the same centripetal unit (Fig. 2).

3. The rhinophore nerves showed vigorous excitatory responses to variations in the salt concentration (Figs. 3, 4), osmolarity (Figs. 5, 6), and pH (Figs. 7, 8) of sea water solutions directed onto the rhinophore sensory epithelium. The rhinophore and tentacle nerves showed strong excitatory responses to various salt solutions directed onto the corresponding sensory structure, including 1 osmolar NaCl, NaBr, NaI, $Na_2SO_4$ and KCl, but not LiCl (Table 2). Curves relating extracellular discharge to stimulus strength typically showed a minimum in the physiological range and increases to either side of this range (Figs. 4, 8).

4. All nerves studied showed excitatory responses to stimulation with mechanical stimuli (Figs. 9–11). Maps of receptive fields of different nerves (Fig. 10) delineated areas of functional innervation for each nerve and showed little overlap. The same centripetal unit(s) typically responded to mechanostimulation of a wide peripheral area (Fig. 11).

5. All nerves studied showed excitatory responses to application of liquefied food substances to the sensory structures (Figs. 12–15). Dose response curves for different food stimuli (Fig. 13) were similar except at higher stimulus strengths, where mean discharge rates were significantly different for different foods. These and other data furnish neurophysiological evidence for discrimination between different food stimuli, as suggested also by earlier behavioral studies (Davis et al. 1980).

6. For all stimuli, severing the afferent nerves leading from the peripheral sensory epithelium abolished electrophysiological responses. Therefore the responses observed were mediated by the sensory epithelium rather than by direct stimulation of peripheral ganglia or nerves.

7. It is concluded that the rhinophores, tentacles and oral veil participate not only in food detection but also have the sensory capacity to detect changes in several other environmental parameters. The data are consistent with the hypothesis that incoming afferent information is processed by peripheral ganglia before it is relayed centrally.

### Introduction

Gastropod molluscs possess well-developed chemosensory abilities that are employed in such diverse behaviors as localization of food (Audesirk 1975; Croll and Chase 1980; Frings and Frings 1965; Jahan-Parwar 1972), appetitive and consummatory responses to food (Davis and Mpitsos 1971; Jahan-Parwar 1975; Horwitz and Senseman 1981; Susswein et al. 1976), reproduction (Audesirk

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1977), localization of conspecifics (Audesirk and Audesirk 1977; Chase et al. 1980; Lederhendler et al. 1977), homing (Gelperin 1974) and escape swimming behavior (Davis and Mpitsos 1971; Willows et al. 1973). The chemosensory pathways of gastropods have been employed in studies of higher behavioral phenomena as well, including behavioral hierarchies (Davis et al. 1974a, b, 1977) and associative learning in *Pleurobranchaea* (Davis et al. 1980; Mpitsos and Davis 1973; Mpitsos and Collins 1975; Mpitsos et al. 1978), *Limax* (Gelperin 1975; Chang and Gelperin 1980) and *Aplysia* (Carew et al. 1981; Walters et al. 1980; Walters et al. 1981). Differential conditioning experiments indicate that *Pleurobranchaea* has the chemosensory ability to distinguish between different food stimuli (Davis et al. 1980).

Although chemoreception in gastropods has formed the basis of numerous behavioral studies, less is known about the underlying sensory and neural mechanisms. Such studies have been hampered by a lack of information about the peripheral sensory receptors and ganglia, and also by the small size of the afferent units (Audesirk and Audesirk 1977; Bailey and Benjamin 1968; Chase 1979), which has impeded extracellular studies of the centripetal pathways. Extracellular recordings of afferent activity have nevertheless been made in *Tritonia* (Audesirk and Audesirk 1977; Field and Macmillan 1973), *Aplysia* (Chase 1979; Jahan-Parwar 1972) and *Pleurobranchaea* (Lee and Liegeois 1974). Progress has recently been made in understanding the structural basis of chemoreception in gastropods. Electron microscopy in *Aplysia* has revealed ciliated exteroreceptors in chemoreceptive areas (Emery and Audesirk 1978). Similar recent studies in *Pleurobranchaea* have disclosed a ciliated cell whose cilia bear discoid-shaped distal expansions (Matera and Davis 1982) and whose distribution on the periphery exactly parallels the distribution of chemosensory areas (Davis and Matera 1981; Lee et al. 1974).

The present paper seeks to extend the understanding of chemoreception and mechanoreception in gastropods by means of behavioral observations and an extracellular analysis of the corresponding centripetal pathways in *Pleurobranchaea*. Electrophysiological evidence is presented for detection of a broad range of chemical and mechanical stimuli and for discrimination within the chemosensory modality. The companion paper (Bicker et al. 1982) presents a neuroanatomical and intracellular analysis of the peripheral pathways and ganglia that integrate chemical and mechanical inputs.

## Materials and Methods

*General.* Experiments were performed on *Pleurobranchaea californica* ranging in size from 150 to 450 ml. Specimens were obtained by trawling in Monterey Bay or purchased commercially from Dr. Rim Fay, Pacific Biomarine, Venice, California, USA. Specimens were maintained in fresh, running water at ambient temperatures (11–16 °C) at the Long Marine Laboratories, and fed weekly to satiation on raw squid.

Observations were made on intact specimens in fresh, running sea water, or on surgically reduced preparations. Such preparations were made by excising the sensory structure of interest (rhinophore, tentacle or oral veil) and its nerve supply, which was severed adjacent to the cerebropleural ganglion. Such preparations were pinned to the Sylgard bottom of an experimental chamber and bathed in cold sea water (12 °C) during experiments.

*Stimulus Delivery Systems.* Chemosensory stimuli were delivered to intact specimens by directing a flow of solution onto the sensory structure using a Pasteur pipette (approximate flow rate, 15–30 ml/min). The apparatus used to deliver liquid chemical stimuli to the isolated sensory structures (Fig. 1) consisted of a temperature-controlled sea water supply, a double pole double throw hydraulic valve and a stimulus reservoir, all submerged in a water jacketed cooling chamber. A peristaltic pump forced a continuous stream of cold (12 °C) sea water through the valve and onto the sensory tissue through a polyethylene tube (i.d., 3.2 mm), positioned 1 cm from the tissue, at a rate of 15 ml/min. Depression of the valve re-routed the carrier stream of sea water through the stimulus reservoir, propelling the stimulus fluid onto the tissue. The valve was designed so

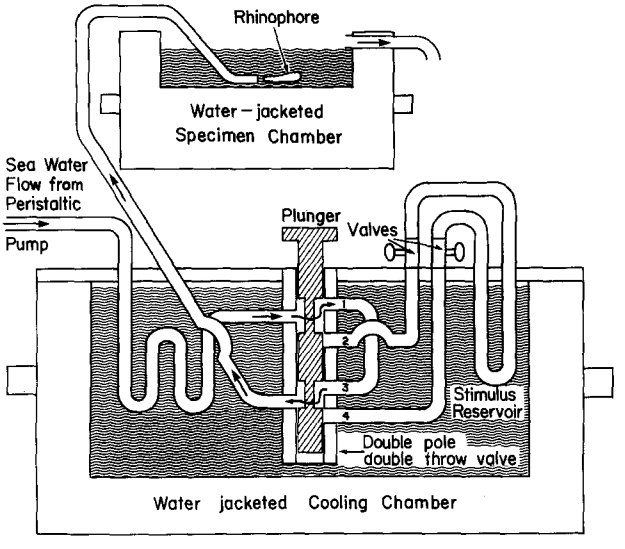


**Fig. 1.** Schematic diagram of the stimulus delivery system. The water cooled system contains a stimulus reservoir that can be filled through the valves with a test solution. A double pole double throw hydraulic valve re-routes a carrier stream through the stimulus reservoir, thereby propelling the test solution onto the tissue of interest. When the plunger of the valve is depressed, closure of the flow route 1→3 occurs continuously and simultaneously with opening of route 2→4, minimizing pressure waves caused by induction of substances into the carrier stream. Stimulus solutions and carrier stream are kept at a constant temperature of 12 °C

that the opening of the route through the stimulus reservoir was accompanied by the continuous proportionate closing of the other route, thereby minimizing transient pressure waves that could otherwise result from induction of stimulus fluid, causing mechanical stimulation of the preparations. With rare exceptions noted in the Results, the stimulus reservoir was filled with 2 ml of stimulus solution. Experiments with marker dyes showed that the stimulus reached the preparation $15 \pm 1$ s after depression of the hydraulic valve. Consequently the time of stimulus onset in all figures was defined as 15 s following valve depression, which was monitored electrically in most experiments.

Amino acid solutions (1 molar) were prepared (in all cases the l-amino form), diluted 1:9 with sea water, and adjusted to pH 7.5 using concentrated NaOH. To assess the effects of pH, HCl and/or NaOH were added as appropriate to sea water to produce test solutions of desired pH. The salt concentration and osmolarity of sea water test solutions were varied by mixing Instant Ocean at desired concentrations or dilutions, and/or adding a 2 osm sucrose solution to sea water. Food stimuli consisted of solutions of homogenized squid or sea anemone (*Corynactis*) mixed 1:1 with sea water and filtered to produce a standard solution (concentration 1 × or 10 °). The standard solution was diluted with sea water in logarithmic steps to produce more dilute solutions as required. Also used as a food stimulus was a solution of vegetable protein hydrolysate (4.5% in distilled water, equivalent to 1 osmolar, and diluted 1:1 with sea water) sold commercially as Dr. Bronner's Vegetable Protein Seasoning (no preservatives added). Osmolarity of all test solutions was measured using a Wescor 5100B vapor pressure osmometer. The pH of all food stimuli was 6.5 and the osmolarity of all food stimuli was approximately 1,000 mosm/kg. For all chemical stimulus applications a 10 min interstimulus interval was utilized to permit flushing of the specimen chamber and recovery of the preparation.

To deliver mechanical stimuli, the sensory epithelium was typically stroked gently with a fine-tipped glass rod or the tips of dissecting forceps. In some experiments more quantitative control over stimulus intensity was achieved by attaching the probe to a diaphragm of a loudspeaker which was activated by a pulse generator.

*Recording.* Feeding responses of intact specimens were observed directly and recorded as no response, orientation to the food stimulus, extension of the feeding proboscis, or the bite/strike (Davis and Mpitsos 1971). Extracellular recordings were made from the severed trunks of the rhinophore, tentacle, large oral veil, small oral veil and mouth nerves (see Davis et al. 1973 and Fig. 10 of this paper for identification of nerves) using glass capillary suction electrodes. Action potentials were amplified, displayed on a Tektronix oscilloscope and photographed with a Nihon-Kohden oscilloscope camera. Action potential recordings were usually processed in parallel by a spike counter with a variable threshold for quantification of responses. The threshold of the spike counter was set just above the noise level such that spontaneous background discharge (approximately 10–50 spikes/min) was counted. Responses to applied stimuli were thus measured against the background level. Data were typically analyzed for 1 min periods beginning 15 s prior to the contact of stimulus solutions with the sensory epithelium. In some experiments the movement of the sensory structure was monitored with a photocell which was sufficiently sensitive to detect any visible movement. In some instances visible movement of the appendage was observed more or less coincident with the spike response to the applied chemostimulus. In these cases we cannot eliminate the possibility that a portion of the centripetal spike response was causally associated with the stimulus-induced movement.

Because ganglia and sensory epithelia were bathed by a common solution, direct contact of stimulus solutions with nonsensory neurons cannot be fully excluded. To minimize such contact, ganglia were shielded from the direct flow of the stimulus solution by the overlying flap of sensory epithelium. To control for the possibility of direct stimulation of neurons by chemosensory stimuli, electrophysiological responses were measured before and after severing the nerve between the sensory epithelium and the peripheral ganglion (rhinophore or tentacle) or cerebropleural ganglion (in the case of the large oral veil nerve). This control was performed at least once in experiments involving all nerves studied and each chemosensory stimulus used. Without exception the responses induced by chemical stimulation were abolished by severing the nerve. Therefore the responses reported here were caused not by direct stimulation of peripheral ganglia or nerves, but rather by transduction of stimuli by the sensory epithelium.

*Statistical Methods.* Conventional statistical procedures and tests were used as indicated in the Results. In some cases where discharge frequency data from several experiments were pooled, the data were first normalized. To normalize data the maximum mean discharge frequency in any single trial on a given preparation was assigned the value of 1.0, and all other values were expressed as a decimal fraction of this maximum. In some cases all plotted points were less than 1.0 because these points represented means of several trials within a single preparation.

## Results

Several components of a food stimulus could in principle be utilized for both detection and discrimination of the stimulus, including individual or combined amino acids, salt concentration, osmolarity, pH, and the mechanical stimulus associated with the delivery of the chemostimulus. As a first step toward assessing the responses to food stimuli, responses to each of these individual components in isolation were examined.

### *Amino Acids*

Behavioral responses of 10 specimens to several l-amino acids are summarized in Table 1. Specimens were selected for this experiment only if they exhibited feeding responses to a solution of protein hydrolyzates (Dr. Bronner's solution) both before and after application of all amino acid stimulus solutions. None of the specimens tested exhibited any of the three appetitive or consummatory feeding responses to a solution of sea water, showing that mechanical stimulation associated with stimulus application did not evoke feeding response. Of 18 amino acids tested, 10 elicited feeding responses reliably (i.e., in 50% or more of the 10 specimens tested). The strongest and most frequent responses were obtained from alanine, as-

**Table 1.** Responses of 10 intact specimens to sea water and 1 ml of 1 molar amino acid solutions diluted 1:9 with sea water and directed onto anterior sensory structures. *NR* no response; *Orient.* orienting response to the stimulus; *Exten.* extension of the feeding proboscis; *Bite/Strike* the biting feeding response. Total Feeding Responses show percent of specimens exhibiting orientation, extension and/or the bite/strike response to the indicated solution

| Substance | % Response (10 animals) | | | | |
|---|---|---|---|---|---|
| | NR | Orient. | Exten. | Bite/ strike | Total feeding responses |
| Sea water | 100 | 0 | 0 | 0 | 0 |
| Alanine | 10 | 90 | 0 | 0 | 90 |
| Arginine | 50 | 20 | 30 | 0 | 50 |
| Asparagine | 10 | 60 | 30 | 0 | 90 |
| Aspartic acid | 20 | 70 | 10 | 0 | 80 |
| Glutamic acid | 100 | 0 | 0 | 0 | 0 |
| Glutamine | 20 | 70 | 10 | 0 | 80 |
| Glycine | 10 | 10 | 60 | 20 | 90 |
| Histidine | 60 | 20 | 20 | 0 | 40 |
| Isoleucine | 40 | 60 | 0 | 0 | 60 |
| Leucine | 60 | 40 | 0 | 0 | 40 |
| Lysine | 90 | 10 | 0 | 0 | 10 |
| Methionine | 50 | 40 | 10 | 0 | 50 |
| Phenylalanine | 10 | 40 | 30 | 20 | 90 |
| Proline | 80 | 0 | 20 | 0 | 20 |
| Serine | 60 | 40 | 0 | 0 | 40 |
| Threonine | 70 | 20 | 10 | 0 | 30 |
| Tryptophane | 30 | 50 | 20 | 0 | 70 |
| Valine | 80 | 20 | 0 | 0 | 20 |

paragine, aspartic acid, glutamine, glycine, phenylalanine and tryptophane. 1 osmolar combinations of more than one amino acid yielded greater responses than single amino acids (not shown), demonstrating a synergistic or additive effect on the feeding behavior.

Electrophysiological responses to alanine and glycine were studied in 43 trials on reduced preparations of the rhinophore taken from 14 preparations. Responses were quantified automatically with a spike counter (6 preparations) or manually by counting spikes (8 preparations). Responses were scored as the number of spikes in response minus the background level, and divided on this basis into three categories; high (40 or more spikes/min), medium (10–39 spikes/min) and low (0–9 spikes/min). Of the 14 experiments, none yielded responses to sea water solutions. Seven showed high responses to amino acids (Fig. 2), and five of these seven preparations showed repeatable high responses in several sequential trials. Discharge frequencies in response to the diluted amino acid solutions ranged to a maximum of 100 spikes/min. The same centripetal unit (based on amplitude and waveform of the extracellular spike) frequently responded to both alanine and glycine (Fig. 2). Seven of the 14 preparations showed 'low' responses, i.e., little or no discharge in response to amino acids. The causes of this unresponsiveness are unknown.

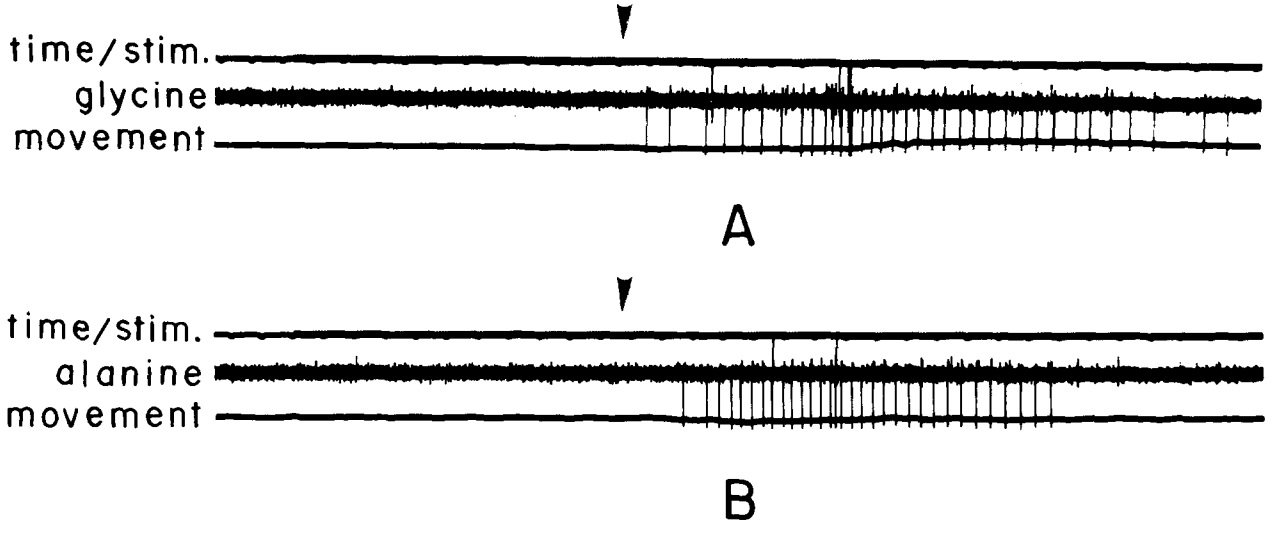


**Fig. 2.** Extracellular responses of the rhinophore nerve of a single preparation (middle traces) to application of two different amino acids, glycine (**A**) and alanine (**B**). In each case 1 ml of 0.1 molar amino acid was applied (1 molar diluted 1:9 with sea water). The upper trace is a time marker, with each downward deflection corresponding to 1 s. In this and subsequent figures, the *arrow* indicates the time of stimulus onset $\pm 1$ s, i.e., the time when the test solution first contacted the sensory epithelium. Lower trace: output of a photocell configured to record movement of the rhinophore

*Salt Stimuli*

To examine responses to specific ions, a range of different salts were prepared as 1 osmolar solutions, approximately isosmotic to sea water, and each was delivered to 5 rhinophore and 4 tentacle preparations (Table 2). The mean response to LiCl was not significantly different from the mean response to isosmotic sea water. In contrast, vigorous spike responses were elicited to various sodium salts and to KCl. The mean responses to the different sodium salts were approximately the same, while the mean response to KCl was about twice that of sodium salts. This electrophysiological result is consistent with the greater sensitivity to KCl evident also in the behavior. Eight out of

**Table 2.** Spike response of rhinophore and tentacle nerves to stimulation of the corresponding sensory structure with 1 osmolar salt solutions. The response was measured for each preparation after stimulation with 2 ml test solution delivered in a randomized sequence. This discharge frequency was averaged across $n=5$ (rhinophore) and $n=4$ (tentacle) preparations. The table lists the mean discharge frequency (spikes/min) $\pm$ the standard error

| | Rhinophore | Tentacle |
|---|---|---|
| Sea Water | 48± 9 | 40± 16 |
| LiCl | 39± 15 | 46± 22 |
| NaCl | 430±132 | 483±238 |
| NaBr | 386±134 | 398±135 |
| NaI | 449±141 | 459±192 |
| $Na_2SO_4$ | 484±133 | 533±201 |
| KCl | 1,028±279 | 1,158±479 |

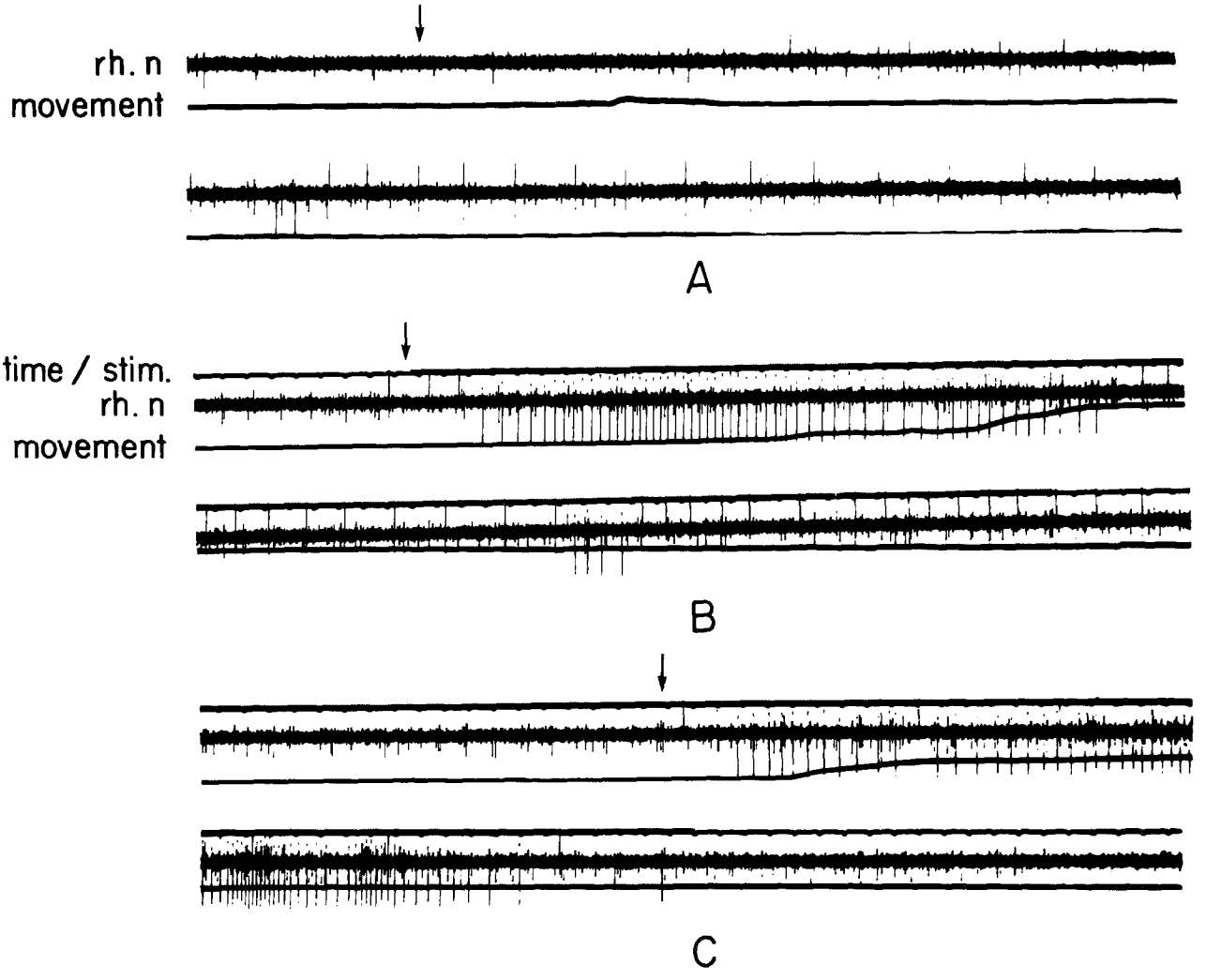


**Fig. 3A–C.** Extracellular recordings obtained from a rhinophore nerve (*rh.n*) of a single preparation during stimulation with artificial seawater solutions. **A** shows a control experiment with artificial seawater (1.0 × Instant Ocean). **B** with a solution of high salt concentration (1.2 × Instant Ocean), and **C** with distilled water. Time mark and stimulus onset as in Fig. 2

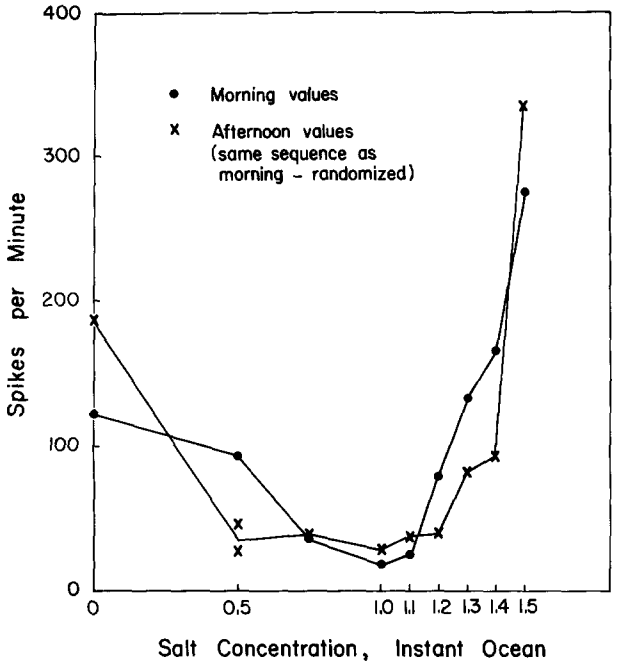


**Fig. 4.** Dose response curve obtained from one rhinophore preparation during stimulation with artificial seawater (Instant Ocean) of different concentrations. Points for each curve were obtained in a randomized sequence. The two curves were obtained from the same preparation 3 h apart

15 animals showed escape swimming to 1 ml of a 1 osmolar KCl solution applied to the tentacle and oral veil. No escape swimming could be elicited by NaCl and LiCl solutions of this concentration.

*Osmolarity/Salt Concentration*

The osmotic pressure of test stimuli was varied independently of salt quality (but not quantity) by varying the concentration of Instant Ocean artificial sea water. Experiments were performed on 16 rhinophore preparations. Application of isosmotic sea water control solutions had little or no effect on the discharge recorded from rhinophore nerves (Fig. 3A). In contrast, application of a solution of 1.2× Instant Ocean suppressed the discharge of one centripetal unit in Fig. 3B, and induced intense discharge in a second centripetal unit. The recorded movement of the appendage began several seconds after the centripetal discharge, suggesting that the spike response was not caused by the movement itself. Application of distilled water to the rhinophore also elicited intense centripetal discharge in the same unit that was activated by 1.2× Instant Ocean (Fig. 3C). These results thus suggest that osmotic deviations in either direction from normal sea water can be signaled by the same centripetal unit(s).

Dose response curves of the summed responses of several centripetal units show a minimum of response at salt concentrations isosmotic with normal sea water, and increases in discharge to either side of this value (Fig. 4), in accord with observations on single units (Fig. 3). Such curves, prepared for several rhinophore preparations, were always steeper with increasing concentration than with decreasing concentration, indicating a greater sensitivity to hypertonic than hypotonic solutions. Repetition of measurements within a single preparation produced curves that closely paralleled the first measurements, illustrating the low variability of such data within a single preparation.

*Osmolarity*

To examine the response to osmotic change independent of salt concentration, solutions of different concentrations of sugar (glucose and sucrose)

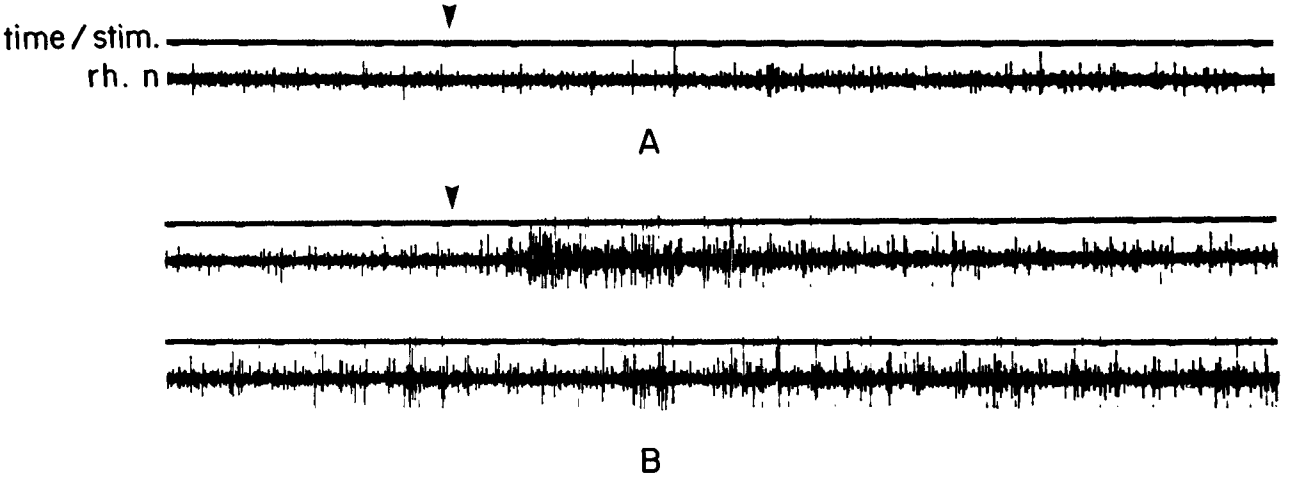


**Fig. 5A, B.** Extracellular recordings obtained from a rhinophore nerve during stimulation with sucrose solutions of different osmolarity. **A** 1 osm sucrose solution; **B** 2 osm sucrose solution diluted 1:1 in seawater (osmolarity 1.5)

were tested in 6 rhinophore preparations. Solutions of 1 osmolar sugar caused little or no change in the spontaneous discharge rate (Fig. 5A). In contrast, a 1.5 osmolar glucose or sucrose solution, prepared by diluting a 2 osmolar solution 1:1 with normal sea water, induced strong discharge in the same rhinophore nerve (Fig. 5B). This hypertonic solution had the same sugar concentration as the isosmotic solution and hence the response to the hypertonic solution was presumably induced primarily by the increased osmolarity. Dilution of a 2 osmolar sucrose solution 1:4 with sea water also caused a strong response. In this case, the salinity level was 80% normal sea water, presumably insufficient to generate a response based exclusively on the change in salinity (see Fig. 4).

Such results are quantified in Fig. 6, which summarizes data from experiments on 4 rhinophore preparations. Sea water control solutions and 1 osmolar sucrose solution in distilled water induced similar weak responses, while the response to 1.5 osmolar solutions of sucrose and sea water were approximately four times greater (Fig. 6).

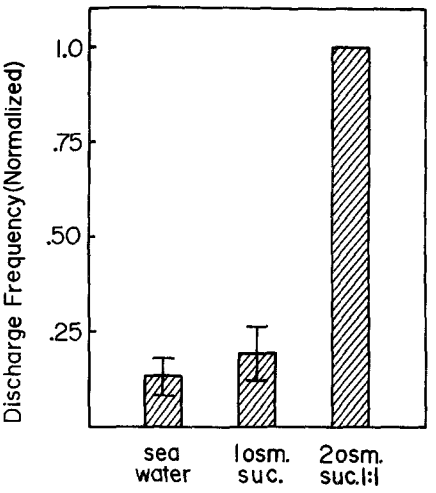


**Fig. 6.** Spike response of the rhinophore to stimulation with seawater and sucrose of different concentrations. The response was measured once for each preparation as spikes/min after stimulation with test solution in a randomized sequence. The histogram shows the normalized mean discharge frequency ± standard error ($n=4$ preparations). Data were normalized for each preparation by converting the maximum discharge rate to 1.0 and expressing lower discharge rates as a fraction of the maximum

*Responses to pH Variation*

Experiments were performed on 10 rhinophore preparations to assess the effects of pH. Application of sea water adjusted to pH 8.0 did not alter ongoing spontaneous discharge (Fig. 7A). Progressively higher pH values, however, caused proportionate increases in discharge (pH=8.5, Fig. 7B; pH=9.0, Fig. 7C). Dose response curves for summed units from individual preparations showed the same type of curve as seen in the case of salt concentration curves, i.e., a minimum at normal pH values and increases to either side of normal (Fig. 8).

*Mechanical Stimuli*

All sensory nerves studied showed responsiveness to tactile stimulation of the corresponding sensory

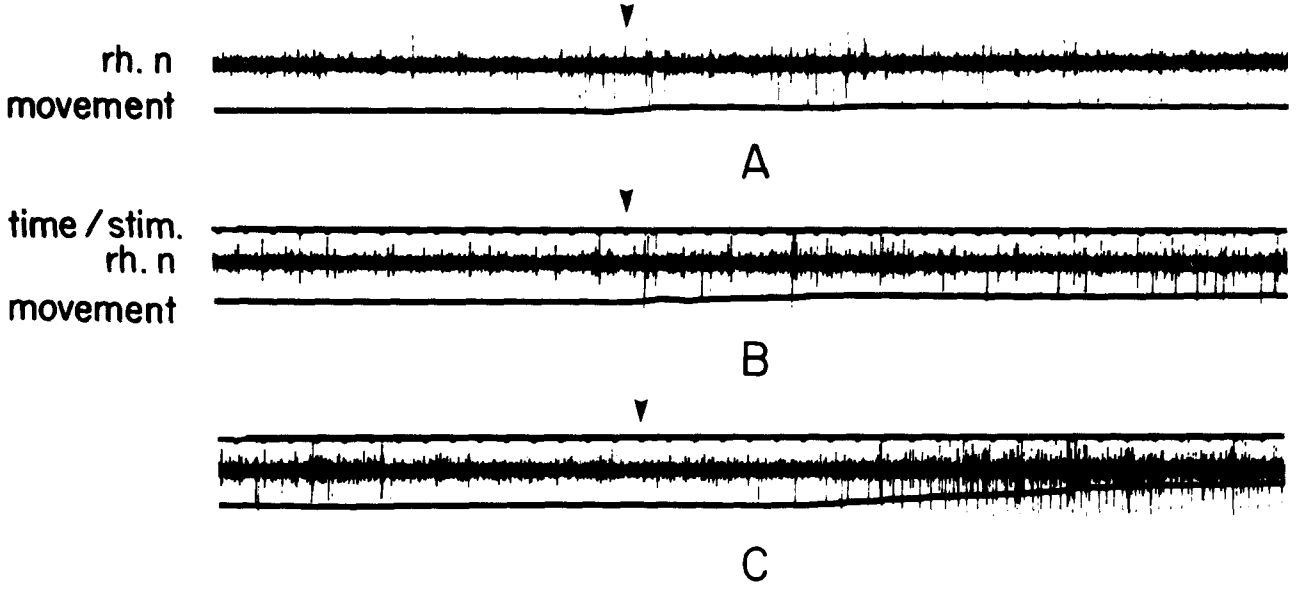


**Fig. 7.** Extracellular recordings obtained from a rhinophore nerve during stimulation with seawater solutions adjusted to pH=8 (**A**), 8.5 (**B**) and 9 (**C**). Time marker and stimulus onset as described in Fig. 2

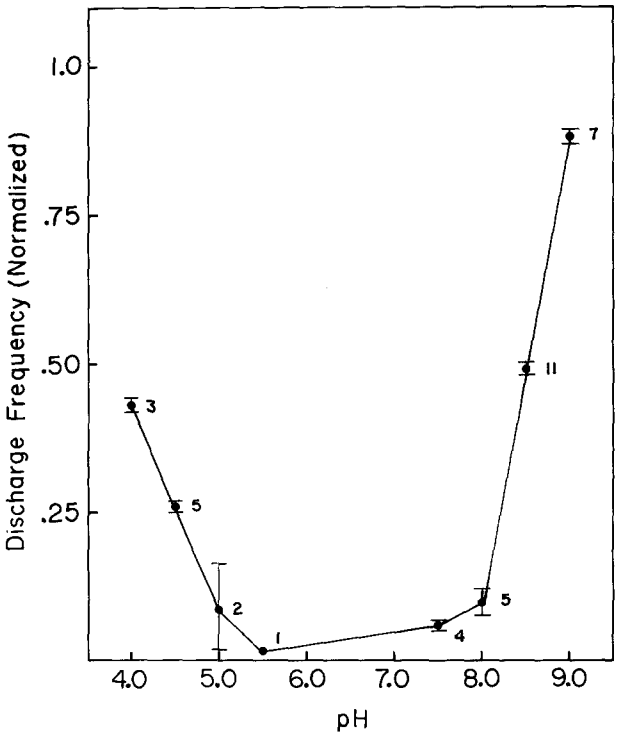


**Fig. 8.** Spike response of the rhinophore to stimulation with seawater adjusted to different pH's. The response was measured several times for each preparation as spikes/min after stimulation with 10 ml of test solution in a randomized sequence. This discharge frequency was normalized by considering the maximum discharge frequency (for pH = 8.5) as 1.0 and averaged across 3 preparations. The graph shows the normalized mean discharge frequency ± standard error. For each data point the number of measurements are given. The standard error reflects both the variance within and between preparations

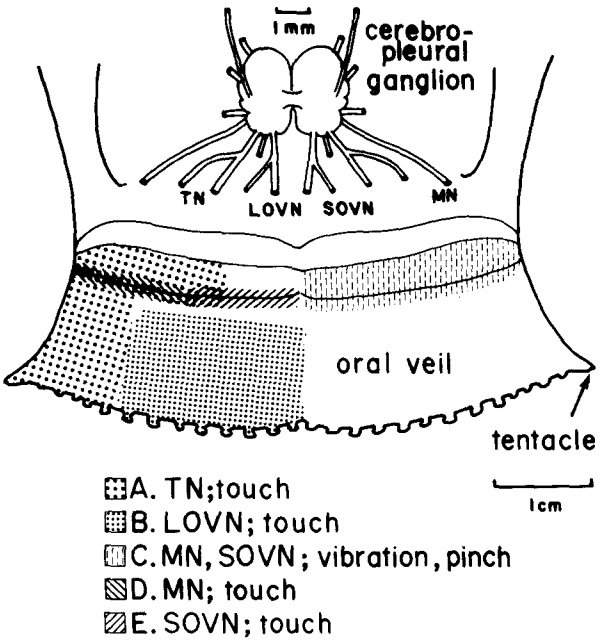


**Fig. 10.** Receptive field map of the ventral oral veil to mechanical stimulation. The drawing shows the areas that respond to stimulation of different sensory nerves. Responses to stimulation could only be recorded for the ipsilateral side, although for the sake of clarity the corresponding receptive field of the mouth nerve was drawn on the contralateral side. *TN* tentacle nerve; *LOVN* large oral veil nerve; *SOVN* small oral veil nerve; *MN* mouth nerve

epithelium. Figure 9, for example, shows the response of the rhinophore nerve to repeated tactile stimuli of the sensory epithelium in the rhinophore at a frequency of 1 Hz. Bursts of activity followed short (< 10 ms) individual stimuli to a frequency of about 10 Hz, at which point the bursts fused into a continuous discharge.

Receptive fields for mechanical stimuli were determined for all of the anterior nerves using the entire anterior end of the animal (Fig. 10). All receptive fields were ipsilateral to the recorded nerve. The tentacle nerve receptive field included not only the tentacle but also the lateral region of the oral veil and the lip region, which are also innervated by the tentacle nerve. The large oral veil nerve exhibited the largest receptive field, covering more than half the ventral oral veil. The only overlap observed in receptive fields was between the tentacle and mouth nerves. The receptive fields of individual units in the centripetal nerves, identified by the constancy of the amplitude and waveform of their action potentials, frequently extended over several cm (Fig. 11).

*Food Stimuli*

Experiments were conducted on 46 rhinophore and 16 tentacle preparations to assess the responses to liquefied food stimuli (Figs. 12–15). Responses to food stimuli (Fig. 12A, B) were invariably larger than responses to sea water (Fig. 12C), showing that the responses were induced by chemical rather than any possible mechanical component of the

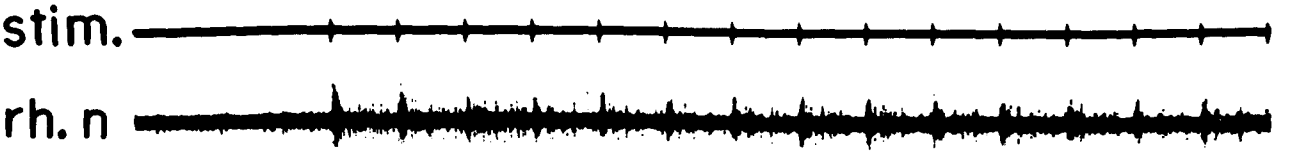


**Fig. 9.** Extracellular recordings obtained from a rhinophore nerve during mechanical stimulation. Pulses (upper trace) were delivered at a frequency of 1 Hz causing the discharge in the nerve (lower trace)

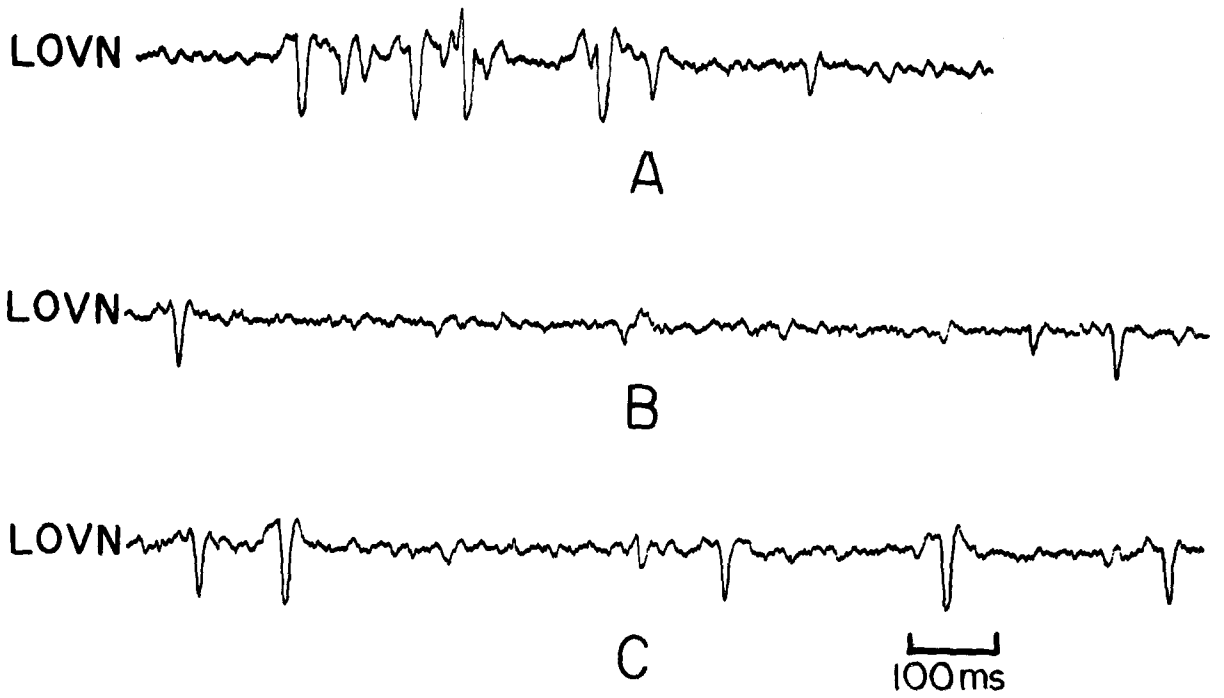


**Fig. 11A–C.** Extracellular recordings obtained from the large oral veil nerve. **A** Response to a rapid stroke of the oral veil. **B, C** Response to mechanical stimulation of the medial ventral oral veil (**B**) and a lateral papillus (**C**). Note activation of the same centripetal unit by both stimuli

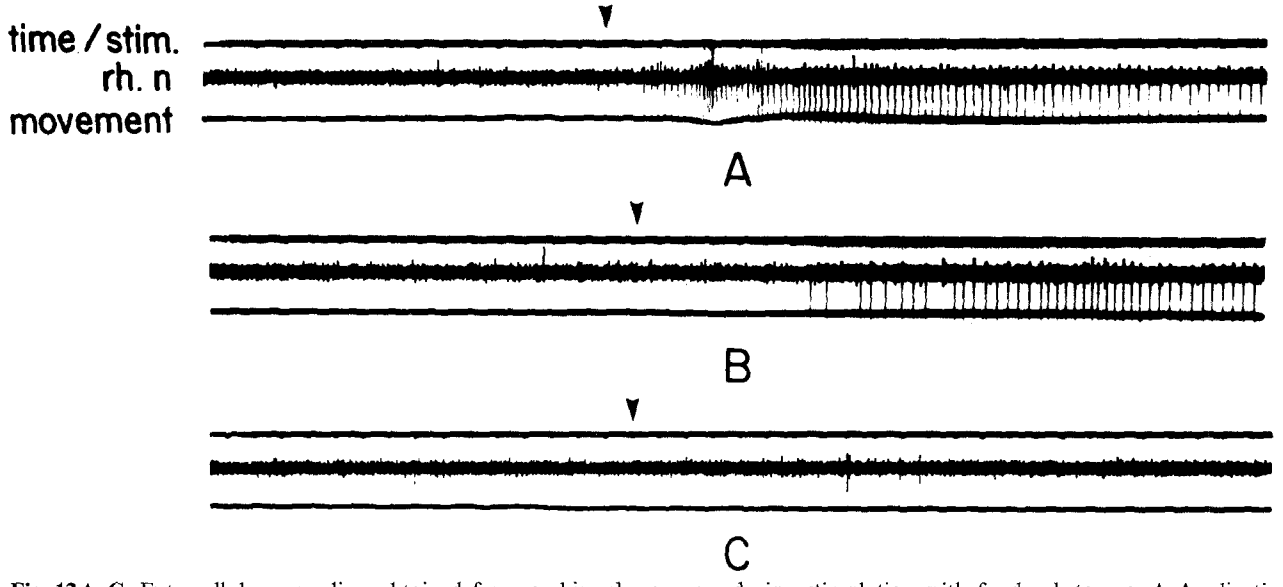


**Fig. 12A–C.** Extracellular recording obtained from a rhinophore nerve during stimulation with food substances. **A** Application of 10 ml of $10^{-1}$ squid homogenate; **B** application of 1:1 seawater dilution of a 4.5% protein hydrolyzate; **C** seawater control. Time mark and stimulus onset as in Fig. 2

food stimulus. The same centripetal units, based on the amplitude and waveform of the action potential(s), frequently fired in response to different food stimuli (cf. Fig. 12A with 12B). Discharge frequencies in response to concentrated (10 °) homogenate of the sea anemone *Corynactis* ranged up to 800 spikes/min in some preparations.

Dose response curves (Fig. 13) were obtained by measuring spike activity for matched pairs of *Corynactis* and squid homogenate at increasing concentrations within one preparation, and averaged for 8 preparations. Responses were greater than the control level (sea water) for all concentrations of food substances studied, and increased with increasing concentration of food stimuli. The response curves for different food stimuli were sim-

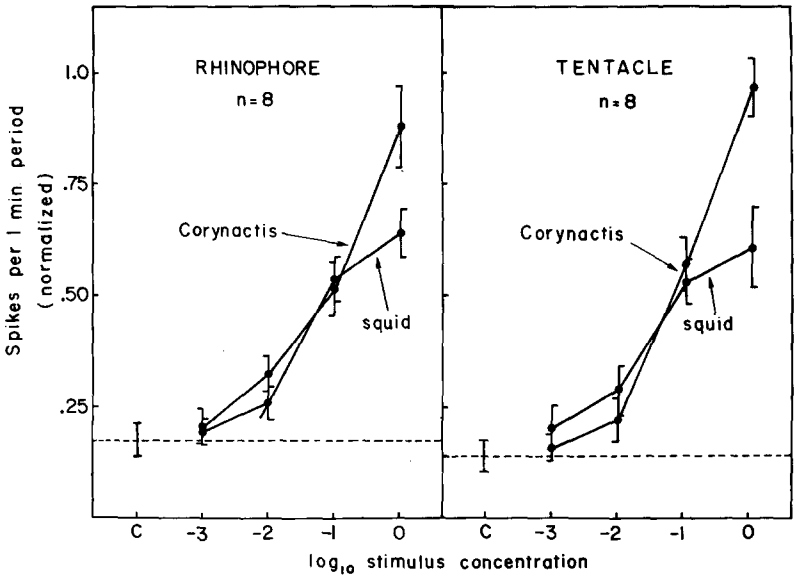


**Fig. 13.** Dose response curves for stimulation with *Corynactis* and squid homogenate. The homogenate was diluted with seawater in log steps. Concentration of this homogenate is plotted against the resulting spike response in the corresponding sensory nerve. Curves show the mean normalized discharge frequency ± standard error ($n=8$). Control level (*dashed line*) gives the discharge level with seawater stimulation

ilar except at the highest concentrations, where the mean discharge frequency of the responses differed significantly (Wilcoxon Ranked Sum Test, $P \leqq 0.05$ for the rhinophore, $P \leqq 0.025$ for the tentacle).

The dose response curves (Fig. 13) are based upon summed action potentials from all responding units. To extend this analysis to the unit level, the responses of individual centripetal neurons to different food stimuli was examined over repeated trials. Two or three separate units could usually be identified within a given preparation on the basis of action potential amplitude and waveform, and in a few cases up to 5 units were discernible (Fig. 14A). Such records were analyzed for responses not only to different food substances but also for responses to food substances versus salts, pH and osmotic variations, and tactile stimuli. In many such instances, different centripetal units displayed different responses (action potentials per unit time) to the different stimuli. An example of a replicable response is shown in Fig. 14. Here unit b (Fig. 14B(1)) fired twice as many action potentials in response to squid compared with *Corynactis*, and this difference persisted on replication (Fig. 14B(2)). The other units in this preparation, in contrast, did not show a clear differential response to the two food stimuli. In most experiments, however, the differential responses, when observed, were not replicable from one trial to another.

Although attention has been focussed mainly on the rhinophores and tentacles, which exhibit the greatest density of putative chemoreceptor cells (Davis and Matera 1982), the oral veil has also been examined for chemoreceptive capacity. The

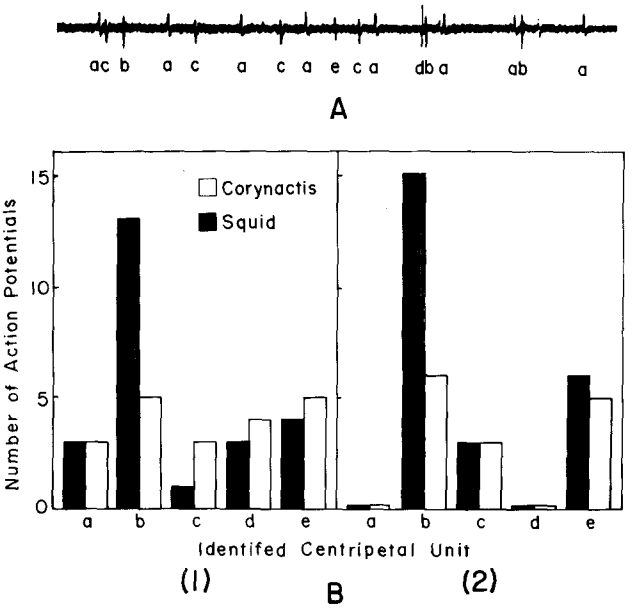


**Fig. 14.** Unit analysis of the discharge in the rhinophore nerve during stimulation with $10^{-1}$ *Corynactis* and squid homogenate in one preparation. The recording shows different units responding to 2 ml of $10^{-1}$ squid homogenate. Five units were distinguished as judged from waveform and amplitude and counted during a 10 s interval after stimulus onset. The histogram shows the mean discharge frequency of 5 units in a different preparation for *Corynactis* and squid homogenate in two repetitions separated by 10 min. Control levels (response to sea water) were zero for all units

net number of centripetal spikes per 2 s interval, defined as the recorded spikes minus the mean background in absence of stimulation, was examined for a 1 min period beginning 30 s before stimulation of the oral veil for a total of 31 trials on 6 preparations. Analyses were performed on data

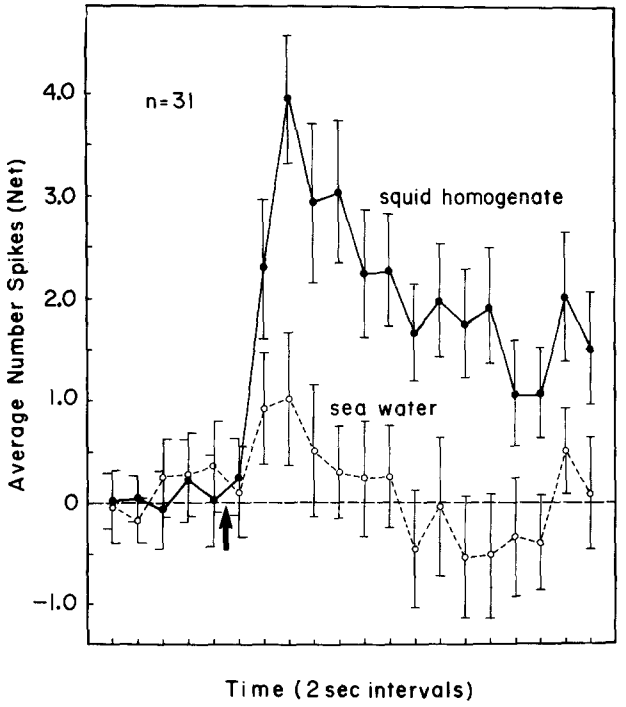


**Fig. 15.** Spike response of the large oral veil nerve to stimulation with 1 ml of squid homogenate. *Arrow* marks time of stimulus delivery. Averaging was performed across 31 experiments in 6 preparations. *Bars*: standard errors

from individual animals (not shown) and grouped data (Fig. 15) with the same results. The mean number of experimental (food induced) post-stimulus spikes was greater than the pre-stimulus experimental (food) and control (sea water) means (Mann-Whitney *U*-test, $P<0.001$), and also greater than the post-stimulus control (Mann-Whitney *U*-Test, $P<0.001$). The post-stimulus control mean was not significantly different from the pre-stimulus control means, nor was the pre-stimulus experimental mean significantly different from the pre-stimulus control mean (Mann-Whitney *U*-test, $P>0.10$). These results illustrate that the large oral veil nerve also mediates chemosensory responses, as would be anticipated from extensive behavioral data showing that the oral veil is responsive to chemical stimuli (e.g., Davis and Matera 1982).

## Discussion

We have examined the sensory responses of *Pleurobranchaea* to several variables, including amino acids, salt concentration, osmolarity, pH and mechanical stimulation. There are three reasons for examining this broad range of variables. First, in an aquatic environment, each may represent a component of a food stimulus that underlies food detection. Second, differences in one or more of these variables could underlie discrimination between different food stimuli. Third, responsiveness to one or more of these variables would indicate the necessity of controlling them in experiments involving food stimuli. The results show that the nerves innervating the chief sensory structures, namely the tentacles, rhinophores, and oral veil, respond to each of these stimuli. In the case of each stimulus, cutting the nerve adjacent to the sensory epithelium abolished the response. Therefore, the observed effects were not caused by a direct effect of stimulus solutions on peripheral ganglia or nerves, but were instead mediated by the sensory epithelium.

Although the spike responses were mediated by the sensory epithelium, they could in some experiments have been causally related to movement induced by the chemostimulus (e.g., Fig. 7C). In many experiments, however, centripetal discharge was recorded in absence of visible movement of the sensory appendage, or several seconds preceding visible movement (e.g., Fig. 3B). In these cases it seems likely that the response represented sensory afference, mediated probably by sensory interneurons (Bicker et al. 1982).

### *Amino Acids*

Studies of *Pleurobranchaea novaezelandiae* have shown that it normally grazes on sea anemones (Ottoway 1977). Field studies on other members of the genus indicate that *Pleurobranchaea* also eats other molluscs, including *Aplysia* (Hirsch 1915), and is also a scavenger (Hirsch 1915, and unpublished field observations by Michael Morris of Sealife, Inc., Sand City, California, USA). Therefore, amino acids represent one of the possible components of *Pleurobranchaea*'s normal diet. Of 18 amino acids tested here, specimens responded reliably to 10 with components of feeding behavior (Table 1). Strong behavioral responses were elicited by alanine and glycine. Individual amino acids were generally less effective than food stimuli in eliciting feeding behavior, even though the concentration of individual amino acids was probably abnormally high (100 mmol/l). This fact is also reflected in the electrophysiological recordings from the peripheral afferent nerves. Even in preparations in which we stimulated with undiluted 1 molar amino acids we obtained a smaller response than for food stimuli diluted 1:9 in sea water. This suggests that only a small number of receptor cells driving centripetal units are tuned to a specific amino acid, whereas a food stimulus,

which contains a broad spectrum of components, is more likely to drive more units at higher frequency.

In many instances we observed that the same centripetal unit responded to two different amino acids (Fig. 2). Such a finding could imply that the same primary afferent sensory neurons respond to different amino acids. As shown in the following paper, however, sensory interneurons in the rhinophore and tentacle ganglia collect primary afferent input from the periphery. Rhinophore nerve units responding to different amino acids could therefore be interneurons integrating inputs from different populations of primary afferents.

*Salt Stimuli*

Salt solutions applied to molluscs are known to induce escape swimming behavior (Davis and Mpitsos 1971; Willows et al. 1973). Behavioral data reported here indicate that *Pleurobranchaea* reacts differently to NaCl and KCl at concentrations isosmotic to sea water. This differential avoidance to a 1 osmolar KCl and NaCl solution implies a differential response to the two salts in the receptor cells. Electrophysiological data provide support for this hypothesis (Table 2). We have investigated the response of the rhinophore and tentacle nerve to several 1 osmolar salt solutions (isosmotic to sea water). The response to sodium cations was apparently independent of the accompanying anion (chloride, iodide, bromide, sulfate). When chloride was used as the anion, the magnitude of the response was determined by the cation, and was strongest for potassium, intermediate for sodium and weak or absent for lithium (Table 2). The response to potassium is not unexpected, since externally applied potassium would presumably depolarize the receptor cells, but the response to sodium might require a sodium-specific recognition mechanism. Based on this limited sample, it would appear that the differential response to salts is cation specific. Similar conclusions have been reached for other molluscs (Crozier and Arey 1919) and other invertebrates and mammals (Dethier 1955, 1956; Kohn 1961; Maes and Bijpost 1979). The behavioral significance of *Pleurobranchaea*'s demonstrated capacity to detect specific salts and distinguish between them is unknown. It is conceivable that specific cations and anions are found in different concentrations in different potential predators or prey, permitting sensory distinctions among or between them, but this explanation seems unlikely (see below, Food Detection and Discrimination).

*Osmolarity/Salt Concentration*

The primary osmoreceptive organ of molluscs has been thought to be the osphradium (Jahan-Parwar et al. 1969; Stinnakre and Tauc 1969). It is not known whether *Pleurobranchaea* possesses an osphradium, but the present studies establish that the sensory epithelium of the rhinophores respond to changes in osmotic pressure. Although dose response curves were not produced for osmolarity data independent of changes in salt concentration, the response to 1.2× Instant Ocean was substantially larger than the response to 1.0×, and the response to 1.5 osmolar sucrose was more than four times that to 1.0 osmolar sucrose, suggesting a high sensitivity to osmotic variation. Independently it has been found that the cilia of receptor cells within the sensory epithelium of the tentacle and rhinophore undergo specific and reproducible configurational changes in response to osmotic variation (Matera and Davis 1982).

*Response to pH Variation*

The response to pH variation was likewise sensitive, especially in the basic range, where an increase of 1 pH unit (from 8.0 to 9.0) resulted on the average in more than a quadrupling of the mean centripetal discharge frequency (Fig. 8). In contrast, the response curve between pH 5.0 and 8.0 was low and relatively flat, indicating a weak sensitivity to pH variation in this intermediate range (Fig. 8). Response levels again increased for pH values less than 5.0. The mean pH of sea water measured at the surface is $8.1 \pm 0.2$ (Martin 1970), and hence the observed sensitivity to slight increases above this value may have adaptive significance to the organism.

The inverse, approximately U-shaped dose response curve obtained for variation in both osmolarity and salt concentration (Fig. 4) and pH variation (Fig. 8) is typical for other sensory systems as well, e.g., the lobster statocyst (Cohen 1960) and mammalian temperature receptors (Dodt and Zotterman 1952). Figure 3 furnishes evidence that such a U-shaped curve applies not only to the summated responses of all centripetal units (Figs. 4, 8) but also to the discharge of single centripetal units. Such a result implies that single centripetal units can detect variations in the appropriate stimulus to either side of normal. In all cases studied here, however, i.e., salt concentration/osmolarity and pH, the response sensitivity is greater (i.e., the slope of the input/output curve is greater) for deviations toward higher stimulus values.

*Mechanical Stimuli*

Weak tactile stimuli have been shown to have an excitatory effect on feeding behavior in *Pleurobranchaea* (Davis et al. 1977). In the present studies strong responses to mechanical stimuli were obtained from all nerves examined. The observation that receptive fields for individual centripetal units extend over wide areas (several cm) may imply that these units branch extensively on the periphery. This interpretation most likely applies to the large oral veil nerve, which has no known peripheral ganglion. Alternatively, however, such an observation may be interpreted as evidence for extensive peripheral integration. As shown in the next paper (Bicker et al. 1982), the rhinophores and tentacles possess peripheral ganglia, and convergence ratios of 1,000/1 (primary afferents/sensory interneurons) characterize the throughput of these ganglia (Matera and Davis 1982). Thus mechanoreceptors covering a wide body area could converge upon single centripetal interneurons, accounting for the broad receptive fields of recorded units.

*Food Detection and Discrimination*

Behavioral experiments have shown that *Pleurobranchaea* not only detects food but also can distinguish between different types of food, i.e., squid and the sea anemone *Corynactis* (Davis et al. 1980). Differences in amino acid or protein composition could underlie this discrimination, but it seems unlikely that differences in salt concentration, osmolarity or pH contribute significantly. The cation/anion composition of the two food substances is presumably similar, since the ion content of marine invertebrate tissues is similar and since even the most concentrated food stimuli (10 °) were mixed 1:1 with sea water. The measured osmolarity of fresh 10 ° squid and 10 ° *Corynactis* solutions were similar, 1,013 and 1,024 mosm/kg, respectively (means of 3 measurements each). Finally the pH's of the two food stimuli were similar, both approximately 6.5, which lies near the center of the flat portion of the pH dose response curve (Fig. 8).

Although the components of food stimuli that underlie detection and discrimination remain unknown, at least two possible mechanisms can be envisioned. First, different food stimuli may generate different intensities of response, irrespective of the particular centripetal units involved in the response. Thus for concentrations of food stimuli that approach those seen in nature, the summed centripetal response to *Corynactis* was significantly greater than to squid (Fig. 13). Second, different food stimuli may be discriminated by activation of a different population of centripetal units. Thus, in the present experiments centripetal units which responded preferentially to one food substance but not another were sometimes detected (Fig. 14). Therefore, although the cellular mechanisms of food discrimination are not conclusively elucidated by the present experiments, the data suggest that such discrimination could occur at least in part in the peripheral ganglia.

*Acknowledgements.* We thank Dr. Roger Croll for helpful discussion and critical evaluation of the manuscript. This research was supported by NIH grant NS09050 to W.J.D. and by a postdoctoral fellowship of the Deutsche Forschungsgemeinschaft to G.B. We thank Ms. Shan Crete for technical assistance.

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