

SENSORY CONTROL OF RESPIRATORY PUMPING IN *APLYSIA CALIFORNICA*

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SUMMARY

The frequency of respiratory pumping in *Aplysia* increases when CO₂ is bubbled through the bathing sea water. Air, O₂ and N₂ do not have this effect. The sensitivity to CO₂ may be mediated by receptors which are sensitive to pH changes within the range encountered during hypercapnia. In addition to the frequency change during hypercapnia, increases in the rate of pumping occur after titration to low pH with hydrochloric, acetic, nitric and sulphuric acids, thus indicating sensitivity to changes in the concentration of hydrogen ions and not to any specific anions. High pH and large deviations from normal in the tonicity of the sea water are ineffective in influencing the rate of pumping. The locus of pH sensitivity resides primarily within the mantle cavity. Lesions of the osphradium indicate that this chemosensory organ mediates a large degree of sensory control over respiratory pumping.

INTRODUCTION

Respiratory pumping in *Aplysia* has been studied extensively. It was first recognized as a spontaneously occurring behavioural pattern which was similar to the gill withdrawal reflex and which used many of the same neuronal and muscular elements (Kupfermann & Kandel, 1969; Peretz, 1969; Pinsker, Kupfermann, Castellucci & Kandel, 1970). It is a relatively fixed behavioural pattern consisting of withdrawal of the gill, siphon and mantle shelf along with closing of the parapodia. As a result of these movements, a forceful stream of water leaves the mantle cavity, mainly *via* the posteriorly directed siphon. When the muscles relax water enters the mantle cavity chiefly between the anterior edges of the parapodia. This behaviour thus serves to irrigate the mantle cavity which contains the gill. The turnover of sea water in the mantle cavity, along with simultaneous changes in haemolymph flow to the gill (Koesler, Mayeri, Liebeswar & Kandel, 1974), increase the gradients for carbon dioxide and oxygen diffusion across the gill surface (Byrne & Koester, 1978). Hence, it has been suggested that the behaviour serves primarily a respiratory function. In addition, it has been suggested that the contractions could serve to expel debris or secretory substances from the mantle cavity (Kupfermann & Kandel, 1969).

The neural circuitry underlying respiratory pumping has been described at the

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level of the siphon and gill motoneurons (Kupfermann, Carew & Kandel, 1974), the motor programme generator in the abdominal ganglion (Byrne & Koester, 1978) and the integration of the different components arising from the abdominal and pedal ganglia (Hening, Carew & Kandel, 1976). However, relatively little has yet been reported about any sensory control over the behaviour. While it is known that tactile stimulation of the siphon can elicit activity in the neuronal network responsible for respiratory pumping (Kanz, Eberly, Cobbs & Pinsker, 1979), the relationship between this stimulus and a respiratory function is not obvious. Koester, Dieringer & Mandelbaum (1979) demonstrated that long-term immersion in non-aerated sea water results in increased rates of respiratory pumping. However, it is unclear whether the rate changes were due directly to hypoxia or hypercapnia or secondarily to altered metabolic activity. The present paper demonstrates that *Aplysia* sense the pH (and thereby CO₂ levels) of sea water in the mantle cavity. The animals respond by increasing the rate of pumping to clear low pH sea water from the cavity. A lesion experiment suggests that the pH sensitivity is mediated by the osphradium. A preliminary report of these experiments has appeared elsewhere (Croll, 1984).

METHODS

Specimens of *Aplysia californica*, 250–400 ml in volume, were purchased from Sea Life Supply of Sand City, California. They were maintained in individual compartments within large troughs provided with running, fresh, filtered sea water heated to 13°C and at pH 7.8. Lighting was provided by fluorescent lamps in a 12 : 12, light : dark cycle.

All observations involved unrestrained animals. Respiratory pumping can be easily recognized by strong contractions of the parapodia coupled with funnelling and withdrawal of the siphon. The sequence of contractions usually begins at the anterior edges of the parapodia and ends with the withdrawal of the siphon. Towards the end of the sequence, which typically lasts approximately 5 s, a jet of water can usually be seen leaving the mantle cavity by the siphon. A source of ambiguity arises in that similar movements occur during locomotion in *Aplysia* (Kandel, 1979); however, during locomotion the movements are less vigorous and do not result in a noticeable exhalant current (unpublished observations). In order to eliminate the locomotory movements from the present analysis, parapodia movements were operationally defined as respiratory pumping if either (1) they consisted of strong parapodial closing accompanied by visible siphon withdrawal in the absence of locomotion, or, (2) they resulted in a visible exhalant current if the movements occurred during locomotion.

Testing generally consisted of taking the animals from their home compartments and placing them into clear plastic containers (length, 30 cm; width, 18 cm; depth, 12 cm) filled with 3 l of sea water which, depending on the experiment, was adjusted as to pH, tonicity or atmospheric gas content. The cages were floated in the large home trough to maintain constant temperature. Animals were observed for between 90 and 300 s, again depending upon the experiment. The numbers of observed pumping movements were counted either directly or were marked on an event chart recorder for later analysis. Each experiment was conducted in either one or two sessions. When two sessions were employed, approximately half the animals were tested in each

session and the results were pooled for statistical analysis. Animals were routinely used for more than one experiment, but no animal was ever used more than once in any one experiment. No animal was ever used more than once in a 1-week period. No animal was ever used again after being used in experiment 6.

Details of the methods used for each experiment are given in the Results.

Statistical analyses

Within this report, pumping is usually described in terms of rate or frequency, which is meant to signify the number of pumping movements observed within the individual test periods. No attempt was made to determine instantaneous frequencies. In experiments 1–5 each subject was tested under each experimental condition and therefore an analysis of variance (ANOVA) for repeated measures was used to examine differences in the numbers of respiratory pumping movements noted during the observation periods. In Experiment 6 the site of the lesion was treated as a between subjects factor and the pH of the sea water was treated as a within subjects factor for the ANOVA. If a significant difference was found between treatments, multiple pairwise comparisons using the Newman-Keuls method (Ferguson, 1971) were performed to determine the sources of variance.

RESULTS

Experiment 1

If respiratory pumping plays an important role in enhancing gas exchange across the gill, its expression might be expected to be influenced by the partial pressures of carbon dioxide and/or oxygen in the bathing medium. The purpose of this first experiment, therefore, was to determine the effects of changes in various gas concentrations of the bathing sea water on the frequency of respiratory pumping. Ten animals were used in this experiment, which was conducted during two sessions. Either air, oxygen, nitrogen or carbon dioxide was bubbled (at a rate of 1.5 ml s^{-1}) through one each of four cages filled with sea water for at least 10 min before and also during testing. Each cage had a loose-fitting, clear, plastic cover. Gases were delivered from compressed gas canisters (Fisher Scientific) or the in-house compressed air system *via* submerged gas diffusion stones. Each animal was tested in each cage in random order. Tests lasted 300 s and animals were allowed at least 0.5 h recovery between tests.

An ANOVA revealed that there was a significant difference in the pumping rate observed in the different treatments ($P < 0.01$). Fig. 1 shows that when animals were bathed in sea water with increased contents of oxygen or nitrogen they pumped at mean rates of 0.10 and 0.00 pumps per 5-min period, respectively. These values do not differ significantly (Newman-Keuls test, $P > 0.05$) from those observed when air is bubbled through the sea water. However, when *Aplysia* are placed in sea water through which CO_2 is bubbled, they pump significantly more often ($P < 0.01$). Hypercapnia is therefore a potent condition for enhancing the pumping rate. Hypoxia, due to replacement of O_2 by CO_2 , does not adequately explain the results, since replacement of O_2 by N_2 did not have any noticeable effect. That CO_2 levels are detected as pH changes remains a possibility.

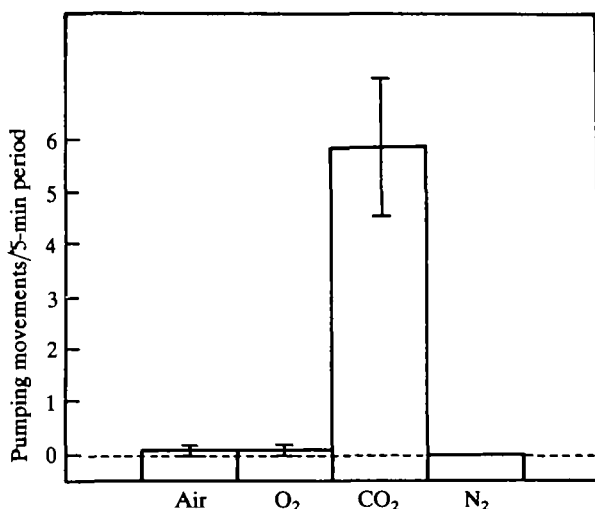


Fig. 1. Effect of different gas contents. Mean number of respiratory pumping movements ($N = 10$ animals) during 5-min test periods when either air, oxygen, carbon dioxide or nitrogen gas was bubbled through the sea water. Standard deviations are indicated for each mean.

The pH of an aqueous solution generally varies linearly with the partial pressure of CO_2 . However, the relationship is complex and depends on such factors as temperature, salinity, etc. (Dejours, 1975). In this set of experiments, no attempt was made to quantify changes in the gas contents of the sea water baths. Therefore, in order to determine the pH changes that accompany gas concentration changes under the conditions of this experiment, the pH was measured in the cages as the gases were bubbled through the sea water. No change in pH occurred over a 10-min period during which air was bubbled through the sea water. When O_2 or N_2 was bubbled through the sea water the pH rose slightly from 7.8 to 7.9 over a 10-min period. When CO_2 was bubbled through the sea water the pH immediately began to fall and reached a value of 5.9 after 10 min.

Experiment 2

The observations described above suggest that pH may be an effective stimulus for influencing the frequency of respiratory pumping. To test this possibility 11 animals were used in this experiment, which was conducted during two sessions. Eight cages were filled with sea water which was then adjusted to different pH values using 1 mol l^{-1} NaOH or HCl. Each animal was tested at each of the pH values in random order. Tests lasted 90 s and animals were allowed at least an 8-min recovery period between tests.

There was a significant effect of pH on the rate of respiratory pumping as revealed by ANOVA ($P < 0.01$) (Fig. 2). Using the pH 8.0 group as a near-normal control, pairwise comparisons were made to determine differences in the rates of respiratory pumping. Baths of neither pH 9.0 nor 10.0 elicited pumping significantly different from near-normal (Newman-Keuls, $P > 0.05$). However, a lowering of the pH caused a large, graded increase in the rate of pumping. Pairwise comparisons showed that

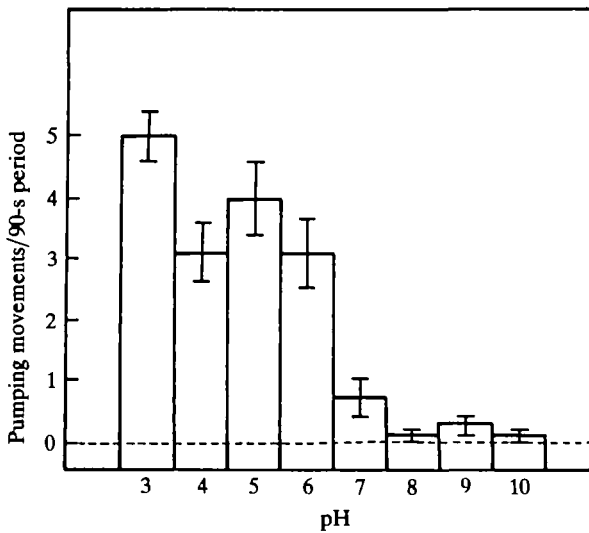


Fig. 2. Effect of pH. Mean number of respiratory pumping movements during 90-s test periods when animals ($N = 11$) were bathed in sea water adjusted to pH 3.0–10.0. Standard deviations are indicated for means.

animals in baths at pH 6.0 and below all pumped significantly more than animals at pH 8.0 (Newman-Keuls, $P < 0.01$). Changes in pH resulting from titration with HCl as well as from hypercapnia, therefore, have a strong influence on respiratory pumping.

Experiment 3

To test whether the results from the previous experiment might be explained in terms of small changes in chloride concentration instead of pH, the sea water was adjusted to pH 5.0 by either acetic acid, nitric acid, or sulphuric acid in one each of three cages. A fourth cage contained sea water at pH 7.8. Tests were 90 s long and animals were allowed a 20-min recovery between tests. Testing was conducted over two sessions.

An analysis of variance revealed a significant difference in the frequency of pumping between the different treatments as shown in Fig. 3 ($P < 0.01$). Pairwise Newman-Keuls analyses revealed that all three of the acids caused a significantly higher rate of pumping than did sea water at pH 7.8 ($P < 0.01$). Furthermore, there were no differences in the number of pumping movements elicited by the various acids used ($P > 0.05$). It therefore appears that it is pH, and not changes in the concentration of a specific anion, that is the stimulus for changing the rate of respiratory pumping.

Experiment 4

Ten animals were used in two sessions in this experiment, which was designed to test whether the tonicity of sea water influences the frequency of pumping. Three cages were used. One was filled with normal sea water (isotonic); one filled with a 3:1 (v:v) solution of sea water to distilled water (hypotonic); and the final one was filled with sea

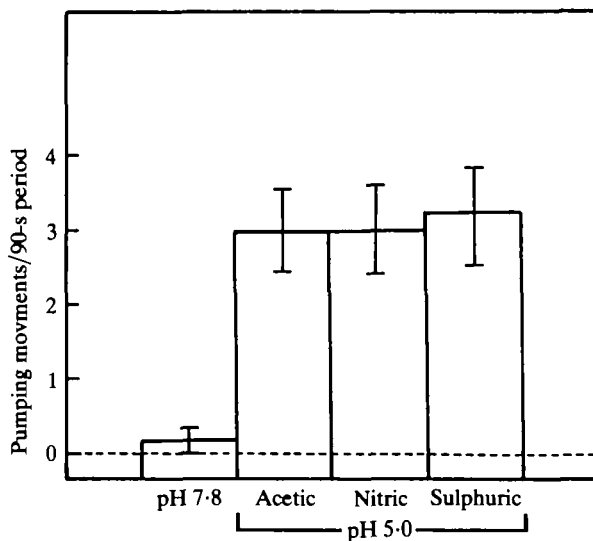


Fig. 3. Comparison of the efficacy of different acids in eliciting respiratory pumping. Animals ($N = 10$) were tested for 90 s in normal sea water and in sea water adjusted to pH 5.0 with acetic, nitric and sulphuric acid. Standard deviations of the means are indicated.

water to which 9.5 g of artificial sea salts (Tropicarium Buchschlag) was added, making the final solution approximately 125 % the salinity of normal sea water (hypertonic). Each animal was tested in each solution in random order. Tests lasted 90 s and animals were allowed at least 8 min recovery between tests. When each animal had been tested, it was given a final test of 90 s in sea water which had been adjusted to pH 5.0.

An analysis of variance for the data presented in Fig. 4 revealed a significant

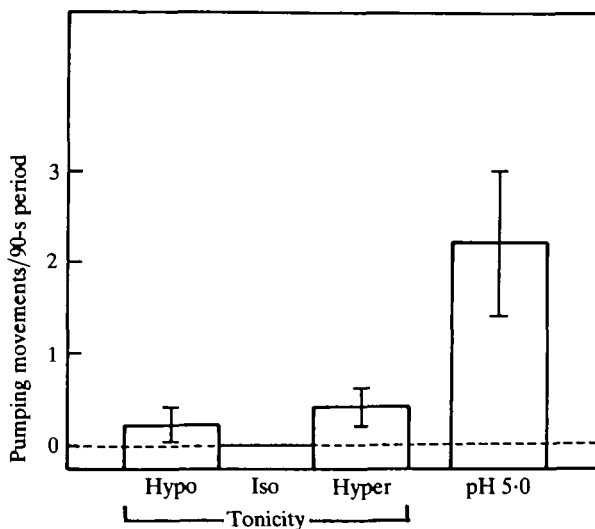


Fig. 4. Effect of variations in tonicity. Mean number of respiratory pumping movements for 10 animals tested in hypotonic, isotonic and hypertonic salt water and in isotonic sea water adjusted to pH 5.0. Tests were 90 s in duration. Standard deviations are indicated for the means.

difference between the means ($P < 0.01$). However, immersion in neither hypotonic nor hypertonic sea water results in significantly different rates of pumping from immersion in normal (isotonic) sea water (Newman-Keuls, $P > 0.05$). All three of these treatment means differed significantly from the mean rate occurring when the animals were placed in sea water (normal tonicity) adjusted to pH 5.0 ($P < 0.01$), indicating that all subjects were capable of high rates of pumping.

Experiment 5

Thirteen animals were used in this experiment, which was designed to locate the general region of pH sensitivity on the animal. The experiment was conducted during a single test session. Testing was performed in a 6-litre Plexiglas box through which fresh sea water flowed at a rate of 1 l min^{-1} . Animals were placed individually into the box and after 5 min each animal was observed for 2 min to obtain a baseline frequency of pumping. After another pause of 5 min the animals were presented with either: (1) a stream of sea water adjusted to pH 3.0 played over the head region, including the rhinophores and tentacles, or (2) a stream of sea water adjusted to pH 3.0 channelled into the opening to the mantle between the anterior edges of the parapodia, or (3) a stream of normal sea water (pH 7.8) also channelled into the opening to the mantle between the anterior edges of the parapodia. The streams of sea water flowed from tubing leading from a Mariott bottle at a rate of 10 ml min^{-1} . After one of these presentations, another 5 min lapsed before the animal was tested with one of the two remaining stimuli. After another 5 min the animal was tested with the last of the three presentations. The order of the three presentations was randomized for each animal. The test periods each lasted 2 min.

An analysis of variance revealed that there is a significant difference in the rate of

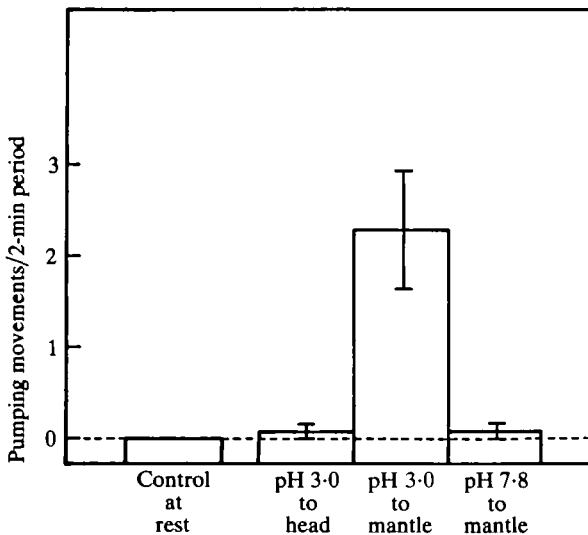


Fig. 5. Localization of pH sensitivity within the mantle. Mean number of respiratory pumping movements (with standard deviations indicated) for animals at rest, with pH 3.0 sea water presented to head region, with pH 3.0 and pH 7.8 sea water introduced into mantle cavity. Tests lasted 2 min and 13 animals were used.

pumping between the different treatments ($P < 0.01$). (Fig. 5). Application of the Newman-Keuls method revealed that when the mantle cavity is irrigated with low pH sea water the animals pumped significantly more than they did at rest ($P < 0.01$). Irrigation of the mantle cavity with normal sea water does not result in higher rates of pumping nor does presentation of low pH sea water to known chemosensory structures (Croll, 1983) on the head of the animal ($P > 0.05$). The animals are not insensitive to low pH sea water on the head, however; surprisingly, they reliably started biting at a mean rate of $1.33 \text{ bites min}^{-1}$. No animals were observed biting under any other conditions in this experiment except when low pH sea water was presented to the head region. These results indicate that although other regions of the body may be sensitive to low pH, only stimulation of structures within the mantle cavity results in increased rates of pumping. Tactile components of the stimulus appear to be inadequate, since irrigation of the mantle with normal sea water does not affect the pumping rate.

Experiment 6

The final experiment was designed as a means of further locating the pH receptors responsible for influencing the rate of respiratory pumping. Twenty-two animals were used in this study. Three days before testing all animals were anaesthetized by injections of $0.5 \text{ mol l}^{-1} \text{ MgCl}_2$ equal to 10–15 % of their body volumes. Using a small vessel cauterizer (Fine Science Tools, North Vancouver, BC, Canada) the osphradia of 11 of the animals were lesioned. In the other eleven animals, a lesion was made in the body wall approximately 5 mm anterior to the osphradium. The subjects appeared to have fully recovered from the effects of the anaesthetic by the next day.

Testing consisted of placing each subject into containers filled with either normal sea water (pH 7.8) or sea water adjusted to pH 5.0. The order of presentation was randomized between subjects. Following testing for pH responses all animals were also tested for responses to tactile stimulation of the siphon. A single nylon bristle from a scrub brush (approximately 4 cm long and $500 \mu\text{m}$ in diameter) was mounted on the end of a wooden applicator stick. The tip of the bristle was carefully placed about 1 cm into the lumen of the siphon. It was then briskly brushed forward to stimulate the edge of the siphon. The duration of the siphon withdrawal was measured with a stopwatch from the time of stimulation until the siphon returned to its resting position. The presence of parapodial movements were also noted at this time. Testing was conducted in two sessions.

Two to three days after testing all animals were again anaesthetized with MgCl_2 and the lesions were confirmed by visual inspection under a dissecting microscope. All attempts to lesion the osphradia resulted in complete or near complete (approx. 90 %) destruction of the pigmented sensory epithelia. Both the osphradial lesions and the control lesions resulted in loss of pigmentation from the body wall for a distance of about 1 mm from the site of cauterization. No damage to the osphradium was observed in any of the control animals.

An ANOVA revealed that there were significant main effects both of pH and of lesioning the osphradium within this experiment ($P < 0.01$) (Fig. 6). Furthermore, it indicated a significant interaction between pH and lesioning ($P < 0.01$), reflecting a decreased responsiveness of lesioned animals to low pH sea water. Pairwise

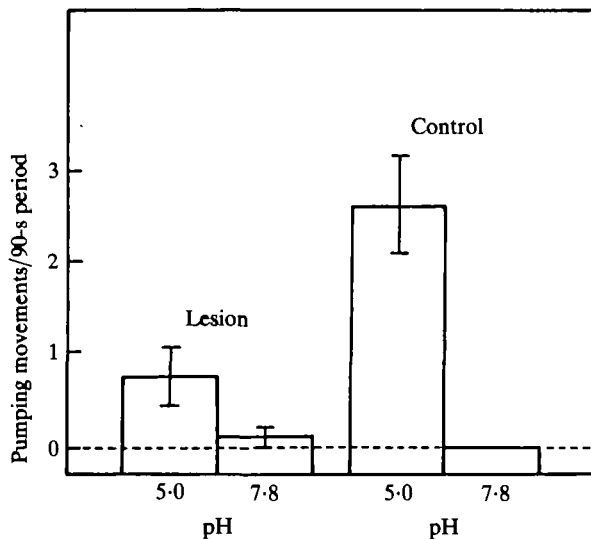


Fig. 6. Effect of osphradial lesion. Half the animals ($N = 11$) had osphradial lesions and the other half ($N = 11$) had control lesions to the body wall near the osphradium. Animals were tested for 90 s in sea water at pH 5.0 and pH 7.8. Standard deviations are indicated for the means.

comparisons (Newman-Keuls) revealed no significant difference between pumping rates in pH 5.0 and pH 7.8 for the lesioned animals ($P > 0.05$). However, in the control group, a significant difference was found in pumping rates at the different pH values ($P < 0.01$).

Tactile stimulation of the siphon resulted in withdrawal durations that were not significantly different for the two groups (ANOVA, $P > 0.05$). The mean siphon withdrawal lasted 23.38 s (s.d. = 9.28 s) for the osphradial lesion group and 21.53 s (s.d. = 8.33 s) for the control lesion group. Parapodial contractions were noted in all animals of both groups in response to the tactile stimulation of the siphon. Therefore, it appears that while the rate of respiratory pumping in response to low pH sea water decreased in animals with osphradial lesions, major components of the motor response were not significantly altered when siphon withdrawal was elicited by other means.

DISCUSSION

Functional significance of respiratory pumping

Earlier hypotheses on the function of respiratory pumping emphasized the importance of increasing the gradients for gas exchange across the gill (Koester *et al.* 1974; Byrne & Koester, 1978). More recently, Koester *et al.* (1979) reported that hypoxic and/or hypercapnic conditions can alter respiratory pumping rates. Evidence contained within the present report strengthens the argument for an important respiratory role since it has now been demonstrated that changing the CO_2 levels or decreasing the pH in the bathing sea water greatly influences the rate of pumping. To understand the significance of these findings, it is essential to consider the morphology of the mantle of *Aplysia*.

The mantle cavity is delineated ventrally by the animal's dorsal body wall and

laterally and dorsally by the parapodia. Numerous organs (such as the purple gland, opaline gland and gill) communicate with the exterior *via* the mantle cavity. The quality of the sea water within the confines of the mantle cavity, therefore, depends greatly upon the animal's own behavioural and metabolic activity. Factors such as pH, for example, may be expected to vary considerably within the mantle cavity due to differences in CO₂ efflux across the gill. Thus, when an *Aplysia* senses low pH water within the mantle cavity, an appropriate response might be to flush the mantle with fresh sea water.

Aplysia range into littoral zones (Kandel, 1979) where they also must adapt to changes in water quality within the mantle cavity that result from environmental rather than metabolic causes. Thus, when trapped in hypoxic and/or hypercapnic tidal pools, increased rates of pumping would appear to be inappropriate, as they may also be within the context of the present series of experiments. However, it must be remembered that the major focus of these experiments was on respiratory pumping and that other responses to changing pH were not examined in detail. The animal may use other mechanisms to adapt to situations such as low pH of the surrounding sea water. During these experiments, it appeared that when immersed in moderately low pH sea water (pH 5.0–6.0) the animals crawled more than they did when immersed in normal sea water. In nature increased locomotion could take the animals to areas with better quality sea water. Analogous movements appear to be widespread within the animal kingdom (Tenny & Bartlett, 1981).

It should also be remembered that the results only indicate that the stimuli studied influence the *short-term* control of respiratory pumping. Tests generally lasted only for 90 s. Thus, increased irrigation of the mantle cavity may be thought of as a 'best, first guess' as to the appropriate response to decreased pH of the sea water in the cavity. While the findings of Koester *et al.* (1979) suggest that long-term hypoxia and/or hypercapnia result in elevated rates of respiratory pumping, it is possible that long-term changes in the bathing sea water might also result in other changes in respiratory pumping and/or other behaviour patterns. When *Aplysia* are kept in stagnant water for several days (as occurs during shipments from California to Nova Scotia) they do not exhibit rhythmic respiratory pumping movements but rather the parapodia remain fully open, thus continuously exposing the mantle shelf and the everted gill (unpublished observations). Eales (1921) described a similar response to hypoxic water.

Comparative physiology of control of respiration

Aquatic animals show a variety of respiratory responses to changes in the concentration of oxygen, carbon dioxide and hydrogen ions (Dejours, 1975). For example, oxygen levels generally have a fairly strong effect on ventilation in seawater and freshwater teleosts. Hypercapnia, on the other hand, has a moderately facilitatory effect on only about one-third of the teleost species tested and a weakly excitatory effect on another one-third. The rest of the species appear to be relatively unresponsive to hypercapnia as do most species to decreases in pH. The importance of oxygen levels in influencing respiration may be generalized to numerous other aquatic species and appears in sharp contrast to the control of respiration in terrestrial vertebrates which depend heavily on CO₂ and pH levels in the blood (Dejours, 1975; Tenny & Bartlett,

1981). Thus the control of respiratory pumping by the osphradium of *Aplysia* is more similar to the control of ventilation by the carotid bodies of higher vertebrates (Dejours, 1975; Tenny & Bartlett, 1981).

It remains unclear why some aquatic animals (such as *Aplysia* and certain cephalopods, Winterstein, 1925) are sensitive to hypercapnia while others are not. The answer probably lies in differences in the natural environments of the various species and in the morphology of accessory respiratory structures, such as the mantle cavity of molluscs, which may restrict the diffusion of gases to and from the gill in a manner unlike that occurring in many other aquatic animals.

It is possible that oxygen levels can also affect the rate of respiratory pumping in *Aplysia*. Since the rate of spontaneous respiratory pumping was low, any decrease in the rate due to increased oxygen levels may not have been detectable within the present set of experiments. Furthermore, although slight hypoxia may have occurred when nitrogen was bubbled through the sea water, the response to anoxia was not specifically tested. Finally, it must be noted once again that only short-term effects of the different stimuli were examined in the present report. Oxygen levels may have more long-term effects.

Base level of pumping

The present study indicates that under conditions which involve relatively little trauma to the animals, the rate of pumping is lower than generally reported in the literature. The mean spontaneous rate of pumping from pooled data for control groups from normal or near-normal conditions in experiments 1–5 was $0.03 \text{ pumps min}^{-1}$ (s.d. = $0.13 \text{ pumps min}^{-1}$). This is similar to the value of $0.14 \text{ pumps min}^{-1}$ reported for unrestrained animals by Kanz *et al.* (1979), but is much lower than the rate of 0.20 – $1.50 \text{ pumps min}^{-1}$ described by Kupfermann & Kandel (1969). However, these authors are imprecise about the conditions under which the animals were observed. It is possible that the rate of pumping may increase with trauma caused by restraining the animals, as described by Pinsker *et al.* (1970) for the study of habituation of the gill withdrawal reflex. In fact the work of Kanz *et al.* (1979) suggests that the neural circuitry underlying respiratory pumping may be influenced to a considerable degree in restrained and semi-intact preparations. Caution should therefore be exercised in the study of respiratory pumping or of its motor components in preparations other than unrestrained animals.

pH response

Despite the possibility that general trauma may increase the spontaneous rate of pumping, several factors suggest that general trauma cannot fully explain the present results. The first is the magnitude of the increase in the rate of pumping. In the present study, several animals immersed in sea water of low pH pumped at rates of 4 – 5 pumps min^{-1} . These rates are far higher than any others reported so far. Second, the response is specific to low pH. While a decrease in pH from normal to 6.0 results in a significant increase in the rate of pumping, an increase in pH of a similar magnitude has no effect on the pumping rate. Also, very large changes in the salinity of the bathing sea water were ineffective in increasing the rate of respiratory pumping. Generalizing from the few stimuli that were tested it appears that stimuli other than

low pH are relatively ineffective in influencing the pumping rate. Finally and probably most persuasively, the sensitivity to pH is not distributed generally over the body surface but, is limited to within the mantle cavity and can largely be accounted for by a 1 mm² patch of epithelium, the osphradium.

Internal vs external receptors

In a similar manner there are several reasons that make it unlikely that the increases in the pumping rate are the result of internal changes such as a deviation in the haemolymph pH. First, as mentioned above, the response was greatly diminished by lesioning the osphradium. Second, the tests were generally of short durations (90 s) and in fact the latency to the first pump was considerably shorter than 90 s, since up to seven pumps were frequently noted within the test period. If most diffusion is across the gill, it seems unlikely that the latency period was sufficiently long to permit enough permeation of sea water into the mantle cavity and diffusion of H⁺ ions across the gill to cause any appreciable change in the haemolymph pH. Furthermore, it has been suggested that the haemolymph probably has a considerable buffering capacity (Dieringer, Koester & Weiss, 1978, Bevalacqua, Kim, Kumarasiri & Schwartz, 1975).

This evidence does not preclude the possibility of other pH detectors (or selective O₂ or CO₂ detectors) located elsewhere. In fact, evidence from experiment 5 suggests that pH receptors are located in the head region. This finding is consistent with a report by Bicker *et al.* (1982) of pH sensitivity in the rhinophores of another opisthobranch, *Pleurobranchaea*. While other receptors did not have any demonstrable effect on respiratory pumping, they may mediate behavioural responses such as locomotion to more favourable locations since rhinophores (or homologous structures) appear to play important roles in orientation to chemical stimuli in numerous gastropod species (Croll, 1983).

The osphradium

The osphradium – located just in front of the gill – is an ideal candidate as a principle chemosensory organ involved in the regulation of respiratory pumping. It has long been hypothesized to be chemosensory (Croll, 1983). Eales (1921) even stated that it might sample the quality of the water within the mantle cavity of *Aplysia*. More recent studies suggest a role in osmoregulation in *Aplysia* (Jahan-Parwar, Smith & Von Baumgarten, 1969; Stinnakre & Tauc, 1969, Bablanian & Treistman, 1983). In the present report, the localization of the pH sensitivity to within the mantle cavity and the large reduction in the response upon lesioning the osphradium make it very likely that it plays a further homeostatic role in monitoring the pH of sea water near the gill.

The results from lesion experiments are often subject to misinterpretations. The control lesion in experiment 6 was included to evaluate the effects of general trauma or the lingering effects of the anaesthetic. Comparisons of parapodial movements and siphon withdrawal in response to tactile stimulation suggest that gross motor dysfunctions were not caused. Despite these reasons for thinking that the effects of the lesions of the osphradium on respiratory pumping were primarily due to selective destruction of pH receptors, caution must still be exercised. Most importantly it should be noted that destruction of the osphradium, no matter how carefully it may

be performed, can easily lead to damage of the entire branchial nerve which lies just below the osphradium and osphradial ganglion (Stinnakre & Tauc, 1969). This nerve innervates the gill and may influence respiratory functions in numerous ways.

A direct test of the pH sensitivity of the osphradium and its involvement in the regulation of respiratory pumping awaits the recording of the responses of respiratory pumping command neurones (Byrne & Koester, 1978) during selective stimulation of the osphradium, perhaps using the methodology of Stinnakre & Tauc (1969) and Jahan-Parwar *et al.* (1969).

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