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ADAPTATION OF FEEDING SEQUENCES IN *APLYSIA OCULIFERA* TO CHANGES IN THE LOAD AND WIDTH OF FOOD

BY ITAY HURWITZ AND ABRAHAM J. SUSSWEIN

Department of Life Sciences, Bar-Ilan University, Ramat-Gan, 52 900, Israel

Summary

In *Aplysia oculifera*, we examined how feedback from foods of differing physical characteristics produces feeding sequences that are appropriate to the food. Animals were fed strips of food systematically modified by attaching weights to the end of the strip or by changing the width of the strip. Both changes led to an increase in inter-swallow interval and a decrease in the amount of food that entered the mouth per swallow. These data indicate that the oscillator setting swallowing frequency is affected by feedback, but that the amplitude of swallowing movements may not be regulated by feedback. Some swallows led to partial or complete cutting of the food. In some cases, cutting led to loss of contact and release of the food. When cutting releases food, its function is apparently to prevent food that has already been swallowed from being pulled out of the mouth. Cutting food may also serve additional functions.

Introduction

Feeding in marine gastropods of the genus *Aplysia* has been widely used as a model system for investigating the control of a complex behaviour pattern (for a review, see Kohn, 1983; Carefoot, 1987). The behaviour pattern is relatively easily observed and quantified (Kupfermann, 1974; Schwarz *et al.* 1988), and its neural control is accessible to investigation (Chiel *et al.* 1986; Cohen *et al.* 1978; Jahan-Parwar *et al.* 1983; Plummer and Kirk, 1990; Rosen *et al.* 1989; Susswein and Byrne, 1988; Weiss *et al.* 1986). In addition, feeding is modulated by factors such as change in motivation state (Kupfermann, 1974; Susswein *et al.* 1976, 1978; Kuslansky *et al.* 1987; Weiss *et al.* 1981) and by associative and non-associative learning (Susswein *et al.* 1986; Schwarz *et al.* 1988). Neural mechanisms underlying modulation have also been characterized (Weiss *et al.* 1981; Teyke *et al.* 1990).

Aplysia feeding bouts are generally patterned into sequences consisting of a series of different motor acts. Appetitive acts consist of locomotion and head-waving movements (Preston and Lee, 1973; Kupfermann, 1974), which localize food to the region of the lips. The touching of food by the lips then leads to a series of consummatory responses, conveying food into the gut. The first consummatory

act, the bite, leads to entry of food into the mouth. Food within the mouth elicits a series of swallowing movements, which transfer food from the mouth to the oesophagus. Swallowing movements generally occur at a fixed frequency largely determined by animal size (Kupfermann, 1974). Finally, a third movement, rejection, can push food or non-food objects out of the mouth (Kupfermann, 1974; Weiss *et al.* 1986) in preparation for an additional series of biting and swallowing responses.

In nature, foods eaten are highly variable in form and in consistency (Susswein *et al.* 1984b; Carefoot, 1987). Feeding sequences on natural foods can be correspondingly complex and variable, to fit the nature of the food encountered. A question that has not been examined previously is how feeding sequences are modified by feedback from the food to provide a response that is appropriate to a particular food. This question is the focus of the present report.

Our approach was to quantify feeding responses to uniform strips of the preferred natural food *Ulva lactuca* (Susswein *et al.* 1984b). We modified the food either by attaching various weights to the end of the strip, so that animals had to exert greater force in order to consume the food, or by changing the width of the strip. We then examined how the motor strategy of the animals changed in response to altered food.

Materials and methods

Animal collection and storage

Experiments were performed on *Aplysia oculifera* Adams & Reeve collected along the Red Sea coast of Israel at sites previously described (Susswein *et al.* 1987). Until used in an experiment, they were stored in 940-l tanks of aerated, filtered Mediterranean sea water maintained at 18°C and with a specific gravity of 1.025. While in the storage tanks, animals were maintained 5–6 to a cage in plastic mesh cages immersed in the sea water. Animals were fed *Ulva lactuca* 2–3 times weekly. *Ulva lactuca* was collected at sites harbouring *Aplysia* and was stored frozen until needed. Animals were maintained on a light:dark schedule of 12h:12h.

Experimental arrangement

Experiments were performed during the winter months (January–March) over 3 years (1988–1990). Three days before the start of an experiment, animals were weighed and then transferred from the storage tanks to experimental aquaria. Experimental aquaria were 0.5-l glass beakers filled with aerated sea water at room temperature (approximately 23°C). Sea water was changed daily. During the 3 days prior to the experiment, animals were not fed. Animals were weighed again after an experiment had ended, and the mean of the two masses was used in all calculations of animal mass mentioned below. Animal mass ranged from 4 to 13 g, with a mean value of 7.4 g, and a standard deviation of 1.9 g. *Aplysia oculifera*
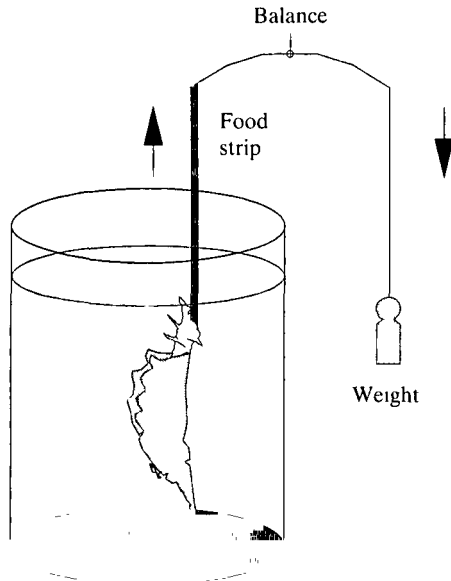
Fig. 1. The experimental arrangement. In many experiments, animals were allowed five swallows in response to a strip of *Ulva lactuca*. The strip was attached to one arm of a balance *via* a clip. The other arm of the balance was attached to a weight, which was released only after five swallows had been completed. This procedure allowed us to measure feeding responses both before and after a weight had been added.

are small animals; these were fully sexually mature, as shown by mating and egg-laying in the storage tanks.

Food and feeding procedure in experiments

Ulva lactuca was used as food in all experiments. Frozen *Ulva lactuca* was thawed and then cut into strips. In most experiments, strips were 2 mm wide by 60 mm long. In some experiments, 1 mm or 3 mm wide strips were used.

To perform the experiment successfully, it was necessary for animals to be in a relatively steady condition of food arousal (Susswein *et al.* 1978). At the start of an experiment, the lips of the animals were stimulated with food. Food was briefly withdrawn when an animal responded, and then stimulation was continued until three biting responses of maximal intensity (type 4 of Susswein *et al.* 1976, and of Rosen *et al.* 1989) were elicited. After maximal arousal had been achieved, experimental procedures were performed.

Effect of weights on feeding

To examine the effects of weight on feeding, the strips of *Ulva lactuca* were suspended above the experimental aquaria, attached to one side of a balance constructed of a 10.7 cm long metal bar (Fig. 1). The other side of the balance was attached to various weights. The weights were allowed to hang free and only affect the weight of the food at certain times during experiments (see below).

After animals had been aroused, the lips were touched with a strip of food. Animals responded to the strip and, on entering the mouth, it elicited a series of swallowing responses (Kupfermann, 1974). After the fifth swallowing response, weights were suspended from the balance, thus affecting the weight against which animals had to pull. Animals were permitted an additional five swallows against weights before the experiment was terminated. In many experiments, animals lost contact with the food before completing all 10 of the swallows permitted. Feeding variables that were recorded included (1) the time at which the bite and the subsequent swallows occurred; (2) the time at which the animals lost contact with the food, and (3) the length of the strip of food eaten.

Experiments were carried out on 10 groups with five animals in each group, giving a total of 50 animals. All animals in each group were tested for 5 days in succession. During this time the animals were fed only as required by the experimental conditions. On each day, for each animal, a different weight was applied to the food against which it had to pull. Thus, each animal was examined with five different weights. For every experimental group, all five individuals were exposed to the same five treatments, but on different days. In all, 14 different weights were used. Since every group was tested with only five different weights, not all weights were examined with all groups. Weights used were 0, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 10.0 g. At least two groups of animals were used to measure the effect of any one weight on feeding. During the experiment, five animals either died or did not respond to food during a test session. Data collected previously from these animals were not included in subsequent statistical analyses, even though the data were in no way remarkable.

Cursory examination of the data showed no consistent tendency for systematic changes in response over the 5 days during which animals were examined. For this reason, responses for each animal for each day were treated as independent measures in subsequent statistical tests. Thus, after discarding the five animals which did not complete all procedures, the values of N for most statistical tests was 225.

Effects of variability in strip width on feeding

After animals had been aroused, they were fed with strips 1, 2 or 3 mm wide. The strips were 5 cm long. The time of occurrence of every swallow was noted. After the strip had been eaten completely, animals were anaesthetized immediately with 50–100 % of their body mass of $MgCl_2$. They were immediately dissected, and the gut contents examined.

Results

Unless otherwise noted, animals were fed strips of food that were 2 mm wide. This width was used as preliminary experiments showed that narrower strips were too fragile to withstand the addition of weights without tearing and wider strips were not easily swallowed (see below).
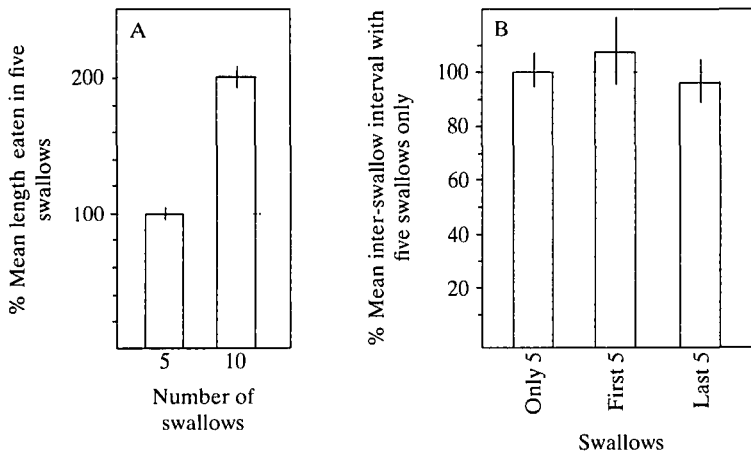
Fig. 2. Swallowing was unchanged over the first 10 responses. (A) Length of food eaten in two groups of animals. In one, animals were permitted five swallows on 2 mm wide strips of *Ulva lactuca*, while in the other group 10 swallows were permitted. Data were normalized, by expressing length eaten by each animal as a percentage of the mean length eaten by animals that were permitted five swallows. The data show that animals permitted 10 swallows ate twice as much as animals permitted five swallows. (B) Timing of inter-swallow intervals, during the only five swallows permitted in one group of animals, and during the first and last five swallows in the group of animals permitted 10 swallows. Inter-swallow intervals were normalized, by expressing them as percentages of the mean inter-swallow interval in animals allowed only five swallows, by first calculating the mean inter-swallow interval for each animal and then calculating the mean for all animals. $N=10$ for both groups. Standard errors are shown as vertical lines.

Variable features of handling an unweighted strip

To determine how the addition of weights alters feeding movements, it was first necessary to examine the feeding movements in response to 2 mm wide strips without the addition of weights.

Stability of swallowing responses

We first examined whether the responsiveness of animals to unweighted food changed over 10 successive swallows. Feeding responses to unweighted strips were measured in two groups with 10 animals in each. In one, animals were allowed five swallows, while 10 swallows were permitted in the second group.

All animals successfully completed all of the 5 or 10 swallows that they were permitted. Moreover, the length of food swallowed during five responses (Fig. 2A) was not significantly different from half the length swallowed when animals were permitted 10 responses ($P>0.8$, $t=0.26$, d.f.$=18$, two-tailed t-test). Also, in animals permitted 10 swallows, inter-swallow intervals during the first five swallows (Fig. 2B) were not significantly different from those during the second five swallows ($P>0.5$, $t=0.44$, d.f.$=9$, paired t-test) or from inter-swallow intervals when only five swallows were permitted.

The data also allowed us to estimate the average length of food that entered the
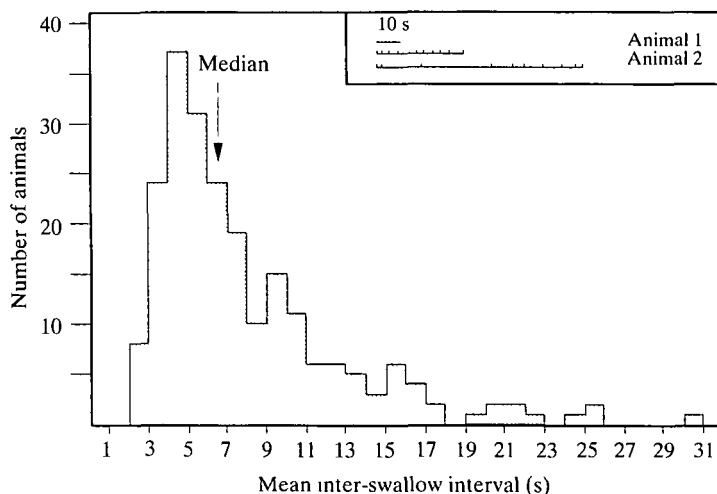
Fig. 3. Inter-swallow intervals were broadly distributed. In most experiments described below, animals were allowed five swallows of 2 mm wide strips of food before a weight was attached. The distribution of all inter-swallow intervals measured during this period is shown. For each animal, a mean inter-swallow interval was determined, and the figure shows the distribution of these means. The distribution is strongly positively skewed. Inset: two examples of 10 sequences of 10 swallows in response to 2 mm wide strips of food. Inter-swallow intervals are highly irregular.

gut per swallowing movement. We found that 4.12±0.7 mm (s.d.) enters the gut for each swallow. It is important to note that data for individual swallows were not available, and length eaten per swallow is estimated from lengths of food eaten over 5 or 10 swallows in 20 animals.

Variability in inter-swallow intervals

In all animals for which weights were attached to the food, five swallows were performed before the weights were attached. An examination of the inter-swallow intervals for the first five swallows over all experiments showed that the mean interval between swallows was 8.1 s (N=884 swallows). This is somewhat longer than the inter-swallow interval described in earlier experiments (Kupfermann, 1974; Schwarz et al. 1988).

To gain more insight into why long inter-swallow intervals were observed, a histogram was constructed (Fig. 3) of the mean inter-swallow interval for all animals over the first five swallows (those in which no weights were attached) in all the experiments described below. We found that the modal interval was 4–5 s, while the median was 6–7 s. However, in many animals the mean inter-swallow interval was much longer. In these animals, the pattern of inter-swallow intervals was highly irregular (Fig. 3, inset), with pauses between swallows. The variability in inter-swallow intervals in these animals may reflect the wide strip of food used relative to the size of the animal. It is possible that individuals with long intervals had 'trouble' with this width of food strip so that many swallows were delayed.
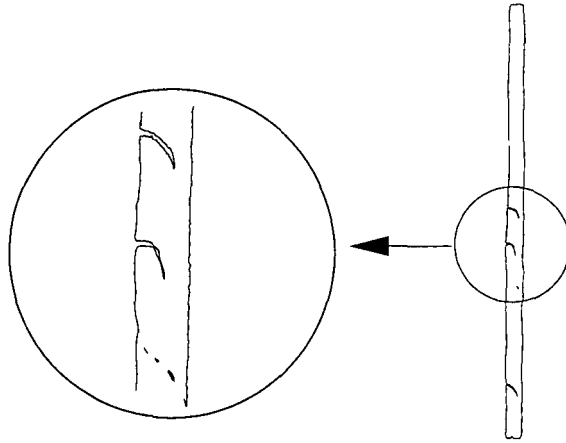
Fig. 4. Strips of food that have been swallowing are notched. After they had been fed strips of food, the animals were dissected, and the 2 mm wide strips were removed from the gut and examined. They had notches cut into them, as shown.

Weiss *et al.* (1986) have shown that an increase in the width of a food strip leads to an increase in variability of inter-swallow intervals.

Appearance of the strip after it has been swallowed

To examine how *Aplysia oculifera* handles the type of strips used in all subsequent studies, nine animals were dissected immediately after they had been fed 2 mm wide, 10 cm long strips of food. As in the animals described above, these animals succeeded in consuming the entire strip. Nonetheless, when the strips were removed from the gut and examined, it was found that animals had cut the strips. In six animals, the food was cut into smaller pieces (mean number of pieces 4.3, range 2–11 pieces). In the three other animals the strips were not cut into pieces. However, in all nine animals the strips had semicircular notches cut into their sides (Fig. 4). The notches appeared at irregular intervals, and were of variable depth into the strip. Notches were always confined to one side of the strip. The mean length of strip between notches was 7.4 mm, with a range from 2 to 30 mm.

Weights cause the animals to release the food

After quantifying how animals handle unweighted strips, we were able to determine whether feeding sequences were changed by attaching weights to the strips. Animals were permitted five swallowing responses to a strip before a weight was added. They were then permitted an additional five responses against the weight.

When weights were added, animals did not complete all the swallows

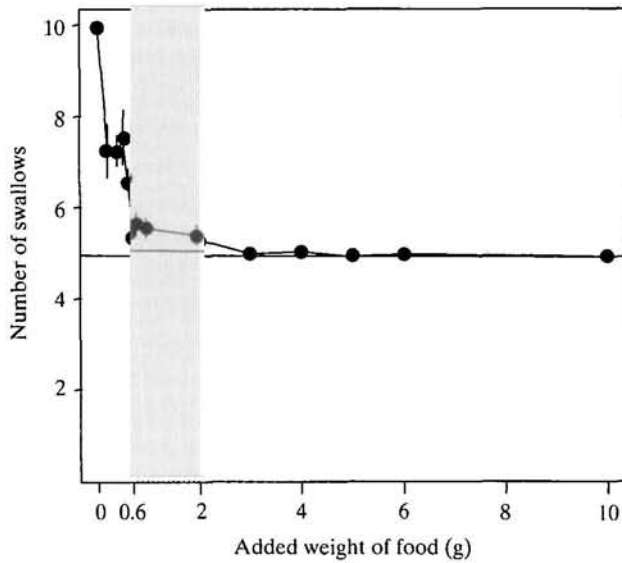In most cases after weights had been added the strip was released from the

Fig. 5. Addition of weights leads to a decrease in the number of swallows. The number of swallows performed is shown for different added weights. All animals performed five swallows before weights were added. Animals were then permitted up to five swallows after weights had been attached to the food. A horizontal line is drawn to represent the five swallows that were always performed. The data show that the number of swallows performed falls into three ranges. These ranges are marked by differences in shading. From 0 g (animals that were allowed an additional five swallows in the absence of added weight) to 0.6 g, the number of swallows gradually decreases. From 0.6 g to 2 g, the mean number of swallowing responses remains at approximately 5.5 swallows. Above 2.0 g, virtually no swallows were attempted beyond the five performed before the weight had been added. Standard errors are shown as vertical lines.

mouth before all 10 swallows were completed (Fig. 5). The number of swallows performed before releasing the food decreased progressively in the range of weights from 0 to 0.6 g, reaching a mean value of approximately 5.5 swallows. From 0.6 to 2.0 g, the mean number of swallows remained constant at approximately 5.5 swallows and from 3 to 10 g, only 5 of 90 animals attempted even a single swallow after the weight had been attached.

Animals lost contact because they cut the food

After weights had been added, animals lost contact with the food before completing 10 swallows. In most cases, the strip of food was cut, with some of the food remaining inside the mouth or gut after the animal had disengaged from the food. It is possible that the loss of food occurred passively: that is, the strip tears because of the weight pulling on it. Alternatively, it is possible that animals actively cut the food. To test whether loss of food could be due to passive breakage, we measured the weight needed to tear a strip of *Ulva lactuca* identical to that used to feed the animals. The mean mass needed to tear a strip was

92±3.4 g (s.d., *N*=20), well beyond the range of weights against which the animals were tested.

When strips were removed from the gut, semicircular notches were found on the strips (see Fig. 4). It is possible that the notches decrease the weight needed to tear a strip, so that the strip tears passively along a notch. To test this possibility, 2–3 notches similar to those made by the animals were cut into 20 strips, and the weight needed to cut the strip was measured. All 20 strips tore along the notches, but required more force than that used in our experiments. The weight needed to tear the strip along a notch was 30±3.0 g (s.d.). Thus, these data suggest that animals lose contact with strips of food because they actively cut the food, rather than because the food is passively torn by the addition of the weight. However, it is possible that the cuts observed occurred prior to the addition of a weight, since, as shown above, animals cut food strips even before a weight is added.

Strategies used in response to added weight before the food is cut

When weights of up to 2 g were attached to strips of food, some animals responded with swallowing responses before cutting the food. We explored whether this strategy was successful, i.e. whether animals gained food as a result of the additional swallowing responses made before the food was cut.

When weights were added, animals lost food already eaten

We investigated the effect of the addition of weights on the length of the food strip eaten. Length eaten was compared to that in the control animals allowed only five swallows in the absence of any weights, to determine whether swallowing movements against the weights were successful in pulling additional food into the mouth or whether animals lost food already swallowed because it was pulled out of the mouth by the weight.

Data on lengths of food eaten were similar to those on number of swallows attempted, in that differing degrees of success in handling the food were seen over three ranges of weights (Fig. 6). In the range of from 0.2 to 0.6 g, animals succeeded in swallowing additional food before losing contact with the strip. From 0.6 to 2.0 g, the food that entered the mouth in the first five swallows remained, but animals did not succeed in swallowing additional food. From 2.0 g onward, the length of food eaten decreased below the length of food consumed in the first five swallows, presumably because animals lost some of the food that had already entered the mouth.

To test statistically whether the length of the strip eaten is affected by weight on the food, we combined data for each of the ranges (0.2–0.5 g; 0.6–2.0 g; >2.0 g) and used a one-way analysis of variance to test whether length eaten differed between the three ranges. A significant difference was found between the three groups (*F*=63, 2 and d.f.=218, *P*=0.009).

Nature of swallows in response to weight

Animals differed in their response strategy to weights ranging from 0.2 to 2.0 g.
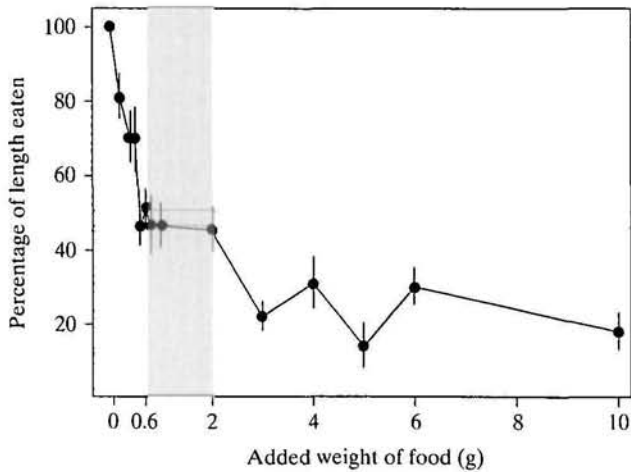
Fig. 6. Addition of weights leads to loss of food consumed before the weight was added. The length of food eaten was measured after animals had been allowed five swallows of unweighted food and five swallow after weights had been added. In most cases, animals did not complete the second five swallows (see previous figure). The length eaten was normalized and expressed as a percentage of that eaten when animals were allowed 10 swallows in response to unweighted food (0 g). For comparison, the length eaten when only five swallows were permitted is shown as two dotted lines depicting one standard error above and below the mean. The data show that lengths eaten fall into three ranges. The ranges are marked by changes in shading. From 0 to 0.6 g, animals succeed in consuming more than the quantity eaten during the first five swallows. However, the length eaten decreased in this range as a function of the added weight against which animals had to pull the food. From 0.6 to 2.0 g, animals succeeded in holding on to food already consumed during the first five swallows, but no additional food was consumed. Above 2.0 g, the length eaten was less than that seen when only five swallows were permitted, presumably because some of the food was lost as the weight pulled it out of the gut after it had been swallowed. Standard errors are shown as vertical lines.

Some individuals performed one or a number of swallowing responses before releasing food, while others released food without even a single attempt to swallow weighted food. Beyond 2 g, very few animals attempted even a single swallow before releasing food.

It is possible that differences in strategy in response to weighted foods from 0.2 to 2.0 g reflect differences in animal size. Larger animals may be able to pull harder than smaller animals and, therefore, continue responding to weighted food. Support for this possibility comes from a recent finding by H. J. Chiel, N. Weiner, M. Bamburger and D. Morton (personal communication) that animal mass is a major determinant of the force exerted by animals during a newly described feeding movement, the 'tug'. Larger animals are able to exert more force when tugging at food.

No significant difference in mass was found between animals that attempted to swallow weighted food and animals that abandoned the food (mean mass of
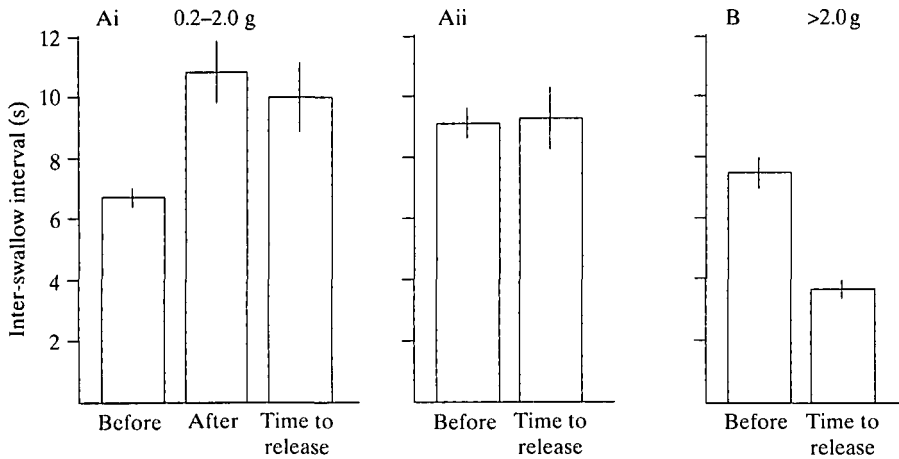
Fig. 7. Effect of addition of weight on inter-swallow interval and time before cutting the food. (A) Animals that were challenged with added weights from 0.2 to 2.0 g were divided into two populations, based on their response to the food. (Ai) The responses of animals that responded to weighted food with additional swallows. The three columns show the inter-swallow intervals before and after addition of weight and the time from the last swallow to the release of the food. (Aii) The responses of animals that immediately released weighted food without trying to perform even a single swallow. The two columns show inter-swallow intervals before the addition of weight and the time from the last swallow to release of the food. (B) Inter-swallow intervals before the addition of weight and the time from the last swallow to the release of the food in animals challenged with weights of more than 2.0 g. Standard errors are shown as vertical lines.

animals that attempted to swallow food was 7.92 g; mean mass of animals that did not attempt to swallow food was 7.58 g; $P>0.5$, $t=0.77$, d.f.$=73$).

It is possible that differences between individuals challenged with 0.2–2.0 g added weights can be predicted from their responses to food before the weights are added. Specifically, one might predict that animals not responding to weighted food might already have been having difficulty in handling 2 mm strips of food even before the weights were added. Their responses to unweighted food would then be characterized by longer, more-variable inter-swallow intervals than in animals responding to weighted food. To test this possibility, animals challenged with weights in the 0.2–2.0 g range were divided into two group: those that responded with at least a single swallow after the weight had been added, and those that immediately cut and released the food (Fig. 7A). We calculated the mean inter-swallow interval before weight was added for both groups and the mean inter-swallow interval after weight had been added for the group that responded. We also calculated the time from the last swallow to the release of the food.

For animals that responded after weight had been added, we found no tendency for inter-swallow intervals to change progressively as the weight was increased.

For this reason, these values were grouped and regarded as a single unit in all analyses.

A one-way analysis of variance showed there were significant differences between some of the five values shown in Fig. 7A [$F(4, 645)=5.34$, $P=0.003$]. A post-hoc statistical analysis (Tukey-test, $\alpha=0.05$) was performed to determine which of the values were significantly different from one another. Two significant differences were found. The mean inter-swallow interval before the addition of a weight was significantly shorter in animals that subsequently responded to weighted food than in animals that released the food immediately when weight was added. Also, in animals that continued responding to the weighted food, the addition of a weight significantly lengthened the inter-swallow interval.

These data indicate that the difference in strategy between individuals responding to the 0.2–2.0 g weight range can be predicted from their behaviour before weight was added. Animals that did not respond to weighted food were apparently already having difficulty in handling the 2 mm strips of food, as shown by their slower responses, which were comparable to the responses of the other individuals after the weight had been added. Animals that immediately released weighted food apparently were able to recognize the food as being beyond their ability to handle successfully and so responded in a manner that prevented loss of the food. In contrast, animals responding to the weighted food modified their swallowing responses by increasing the inter-swallow interval, enabling them to cope with the food more successfully, before finally releasing it.

These data also indicate that the time needed to release a strip of food is similar to the inter-swallow intervals that immediately precede the release. This similarity was found in animals that did and those that did not respond to the weighted food, suggesting that the cut occurs instead of a swallow, at the time expected for the next swallow.

It is illuminating to compare responses to added weights of 0.2–2.0 g and responses to greater weights (Fig. 7B). In animals challenged with weights greater than 2.0 g, the mean inter-swallow interval before the addition of the weight is intermediate in value to those of the two groups shown in Fig. 7A. This is because these animals are not selected on the basis of their subsequent response: in this weight range all animals failed to respond. Of more interest is the observation that the time from the last response until the release of the food was much shorter than in the other two groups. It is likely that this reflects a different process underlying release. In animals challenged with less weight, release of food represents an active cut, while in animals challenged with a greater weight the release probably reflects passive release of the food.

In animals that continued to respond to the weighted food, the length per swallow was examined as a function of the increase in weight. As weights were added, there was a tendency for the mean length of strip eaten per swallow to decrease ($P<0.05$, $F=2.29$, 7, d.f.=66, one-way analysis of variance) (Fig. 8). From 0.2 to 0.6 g, mean length eaten per swallow decreased, reaching a value not significantly different from zero. We have no way of distinguishing whether length
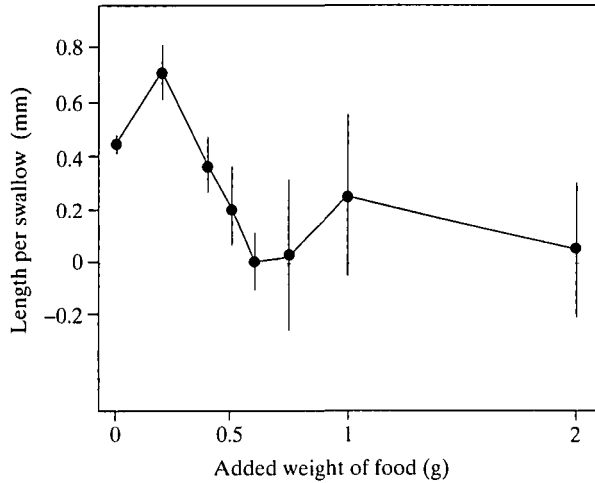
Fig. 8. Addition of weights decreased the length of food eaten. In the same animals shown in the previous figure, length consumed per swallow was calculated for each animal. The data show that, as weight increased, length per swallow decreased. Standard errors are shown as vertical lines.

decreased because some swallows were successful, while some failed, or whether every swallow led to entry of less food into the mouth.

The width of strips modulated the feeding pattern

The data above suggest that addition of weight to the food profoundly modulates feeding sequences. The data also suggest that the 2 mm widths of food used may not be easily swallowed by the experimental animals, even before weights have been attached, since distribution of inter-swallow intervals is so broad. Also, animals make cuts or notches in the food, even when not releasing it.

To determine how a second treatment that altered the food affected feeding sequences, animals were fed 5 cm long strips of food that were 1, 2 or 3 mm in width. A change in width has been shown (Weiss *et al.* 1986) to modulate feeding movements. To allow comparison with changes caused by the addition of weight, we measured inter-swallow intervals and the number of swallows needed to consume the strip, the same variables that were measured before. After food had been consumed, the animals were dissected and the gut contents examined.

For animals fed 1 mm and 2 mm strips, all the food was consumed. However, in three of five animals fed 3 mm wide strips, the strip was cut and released before it was completely eaten. All three of these animals performed at least 10 swallows before releasing the strip. The animals that released food did so after consuming 1, 2.5 and 4 cm of the strip.

Inter-swallow intervals were significantly increased as the strip width was increased ($P<0.01$, $F=13.6$, d.f.$=2$ and 12; one-way analysis of variance), primarily due to a large increase in inter-swallow intervals in animals fed 3 mm
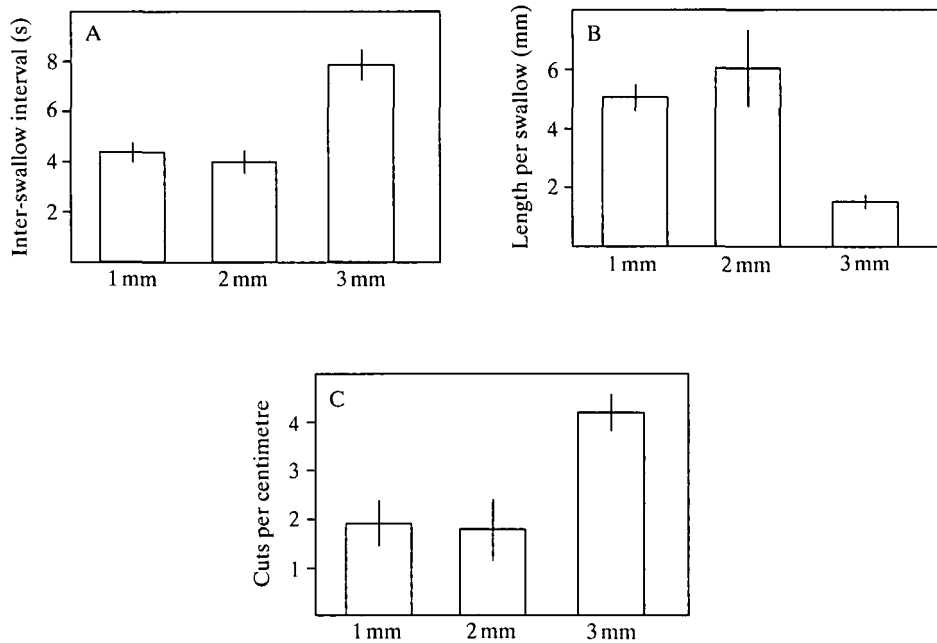
Fig. 9. Strips of differing widths are treated differently. As the width of the strip was increased from 1 to 3 mm, there were changes in a number of feeding variables. The changes occurred as width was increased from 2 to 3 mm. (A) An increase in inter-swallow interval was seen as strip width increased. (B) There was a decrease in the length of food that entered the animal per swallow as strip width increased. (C) The number of cuts or notches per centimetre of food also increased as width increased. Standard errors are shown as vertical lines.

wide strips (Fig. 9A). A significant difference was also found in the length of food taken in per swallow ($P<0.025$, $F=5.5$, d.f.$=2$ and 12, one-way analysis of variance). This difference was primarily due to a large decrease in length per swallow in animals fed 3 mm wide strips (Fig. 9B).

We also determined whether an increase in width increased the number of cuts and notches in the strip. For this purpose, animals were dissected and food was removed from the gut. The numbers of cuts and notches in the strip were counted, and the combined number of both cuts and notches was normalized to the length of the strip eaten. A significant increase in cuts per centimetre was found as width increased ($P<0.025$, $F=5.14$, d.f.$=2$ and 12, one-way analysis of variance), primarily due to an increase in those animals fed 3 mm wide strips (Fig. 9C).

Cursory examination of the data suggests that most swallows were accompanied by cuts or notches. For animals given 1 and 2 mm wide strips, animals made just under 2 cuts per centimetre, which yielded about 10 cuts for the 5 cm length given to animals. For these animals, length per swallow was about 5 mm, which led to 10 swallows per strip. However, in other experiments it was clear that many swallows were not accompanied by cuts or notches. In another series of experiments, a

number of animals were fed compound strips in which the first 5 cm was 1 mm wide and the strip widened to 2 mm for an additional 5 cm. After these animal had been dissected, it was found that relatively few cuts or notches had been made in the 1 mm wide portion, while both cuts and notches were found in the 2 mm wide portion.

Discussion

In many organisms, cyclic repetitive movements are controlled by central pattern generators (Delcomyn, 1980). Motor systems controlled by central pattern generators are often active during a number of different behaviour patterns (Getting and Dekin, 1985). Also, the intensity of behavioural activity must be modulated as the nature of a movement varies, in accordance with the behavioural needs of the animal. Motor systems controlling alimentary movements are particularly appropriate for examining how a small number of different activities can be coordinated and varied to provide a response appropriate for a given physiological state. For this reason, control of cyclical alimentary movements has been examined in molluscs (Benjamin *et al.* 1985; Croll and Davis, 1987), arthropods (Selverston and Moulins, 1987; Weimann *et al.* 1991) and mammals (Byrd, 1985; Lund and Olsson, 1983). Although species differences clearly exist, a common theme emerges from many studies in different organisms. Cyclic, repetitive feeding movements are controlled by a central pattern generator, while intensity and timing of movements are modulated by feedback from the food.

In this study, we have examined how feeding sequences in *Aplysia oculifera* are modified by feedback arising from the food. We have found that, as food became more difficult to consume, feeding sequences were systematically changed to allow the animals to overcome the difficulty and to consume the food. In response to attaching weights to the food and to increasing its width, inter-swallow intervals became longer and more irregular, and the length of food consumed per swallow became shorter. Also, some swallows cut the food. The various changes in feeding sequences were apparently adaptive in allowing the animals to consume difficult food.

Feedback affects the frequency and amplitude of swallows

Our data suggest that feedback from swallowing performance affects the frequency, but not the amplitude, of swallowing movements. Making food more difficult to swallow increased inter-swallow intervals and made them more variable, because of pauses between swallows. These effects were seen both in response to the addition of weight and in response to an increase in the width of the food (Figs 7, 9). Even under control conditions inter-swallow intervals were much more variable and were longer (Fig. 3) than those previously observed (Kupfermann, 1974; Schwarz *et al.* 1988), suggesting that the width of seaweed used in these experiments was causing difficulties to the animals, probably because

the *Aplysia oculifera* used in this study were an order of magnitude smaller than the *Aplysia* spp. examined previously.

Feedback apparently has relatively little effect on the amplitude of swallowing movements. Increases in the weight against which animals must pull and increases in the width of food led to decreases in the mean length of food consumed per swallow (Figs 8, 9), as would be expected if a similar force were exerted against increasing loads. However, since values for length per swallow were calculated by measuring the overall length consumed over a number of swallowing movements, it is possible that the data represent a combination of some swallowing movements that compensated for the increase in weight, coupled with some loss of food after the swallowing movements had been performed.

Pauses between swallows may have a number of causes. One possibility is that a pause represents the time needed to move complex or large pieces of food in the mouth to a central position, allowing them to be subsequently swallowed. If this explanation is correct, one would expect that muscles and motor neurones would be active between swallows, as these would function in producing movements that re-position food so that it can be swallowed. A second possibility is that gaps represent the central processing time needed to integrate information about food that is difficult to handle and then to 'decide' on the proper response to the food. If this suggestion is correct, one would expect muscles and motor neurones to be inactive between swallows, but one would expect that command neurones and/or central pattern generators for swallowing would receive considerable synaptic input during this time. Also, the muscles and motor neurones activated should vary considerably from swallow to swallow as animals 'decide' on the different strategies that are appropriate. A third possibility is that gaps represent rest periods between swallows that are relatively energetically costly. This hypothesis suggests that circuitry generating swallows should be relatively quiescent between swallows and that swallowing movements should be relatively stereotyped, even when separated by long gaps. The different predictions made by the three hypotheses may allow us to differentiate between them in future experiments directly examining the neural circuitry underlying feeding movements.

Data on increased variability and length of inter-swallow intervals are consistent with recent findings by Chiel *et al.* (1990). In that study, swallowing was elicited by dipping canvas into seaweed extract. This stimulus led to irregular inter-swallow intervals, with many long intervals interspersed among the more-normal, short intervals.

Introduction of cuts

A major finding of our study is that feeding sequences in *Aplysia oculifera* sometimes lead to cutting of the food. A previous study reported that *Aplysia punctata* cut food (Howells, 1942), but subsequent studies on *Aplysia californica* were unable to confirm this observation (Kupfermann, 1974). Three types of cuts were identified.

Notches. When animals were dissected after being fed, cuts in the form of

semicircular notches were sometime seen. Notches were always seen along just one side of the strip and were probably made by the radula cutting into the food. The function of notches may be to make the food somewhat narrower, so that it can be swallowed more easily.

Severed strips. Cuts that completely sever the strip were also seen in material taken from dissected animals. It is important to note that these cuts could not be identified without dissecting the animals, as they did not lead to release of food and its loss. It is likely that these cuts are also made by the radula cutting into the food. Animals may prevent loss of the food after it has been severed by holding the food in place with the jaws, which are anterior to the radula. These cuts may represent the equivalent of chewing movements in other animals, which increase the surface area of food prior to digestion. It is not clear whether these cuts represent a more powerful activation of the same movement that produces a notch or whether this represents a qualitatively different movement.

Release of food. Cuts that severed the food and led to its release before all of it was swallowed were seen when both the weight and the width of the food strips were increased. The cuts are apparently initiated in response to these stimuli and serve to prevent loss of food already consumed.

Cuts that release food were completely effective in preventing food loss only when animals were challenged with weights up to 2.0 g (Fig. 6). The time taken to release the food was shorter when animals were challenged with weights greater than 2.0 g (Fig. 7), indicating that a different process is responsible for the release of food in this weight range. We suggest that when *Aplysia oculifera* are challenged with added weights of over 2.0 g, the food is passively pulled out of the mouth, rather than being actively severed. However, even with these added weights, only some of the food eaten is lost. Total loss of the food is usually prevented, since some of the cutting movements that occur before weight is added sever the food, without releasing it. The addition of weight leads to a passive release of the food from the jaws, which hold the severed food in place. Thus, 2.0 g may represent the maximum weight that the jaws can hold.

All three types of active cuts apparently occur as part of the swallowing movements. In some animals, examination of food that had been eaten showed that the number of cuts corresponded quite well with the number of swallows. Also, the time from the last swallow to a cut that released food was equal to the inter-swallow interval (Fig. 7). These data suggest that the same oscillator is responsible for the timing of swallows and cuts.

In some animals, the number of swallows far exceeded the number of cuts, indicating that many swallows are not accompanied by cuts. To determine whether swallows that do not lead to any type of cut take less time to perform than do swallows leading to notches, we carried out a preliminary experiment in which we attempted to relate the position of notches along a strip to especially long inter-swallow intervals. No such relationship could be found.

H. J. Chiel, N. Weiner, M. Bamburger and D. Morton (personal communication) have suggested that cuts leading to the release of food are often

accompanied by a tugging movement, which consists of a contraction of the foot and a backward movement of the whole animal, while the jaws are clamped on the food. Tugs were observed in response to *Ulva lactuca*, the same food used in our experiments. In our experiments the overwhelming majority of cuts were not accompanied by tugs. The difference can be attributed to the different shapes of foods used in our protocol and by H. J. Chiel and collaborators. We used narrow strips of food that could easily be cut without a tug, while they used much wider pieces of *Ulva lactuca*.

Comparison with previous data

Feedback modulation of feeding in Aplysia

Our finding that swallowing movements are systematically altered by the load on the food are consistent with previous data on *Aplysia* feeding. Weiss *et al.* (1986) showed that an increase in width of food strips in *Aplysia californica* leads to an increase in variability of swallowing movements. Chiel *et al.* (1986) also showed that a brief pull on food leads to an increase in amplitude of electromyograms recorded from muscles active during swallowing. Jahan-Parwar *et al.* (1983) showed that proprioceptors in the buccal ganglion sense stretching of the buccal muscles, and feedback from muscle activity can affect the output of the muscles generating feeding movements.

H. J. Chiel, N. Weiner, M. Bamburger and D. Morton (personal communication) have found that the amplitude of tugging movements and the peak compressive force exerted on food are greatly increased by an increase in animal mass. In contrast, we found no difference in mass between animals that continued responding to weighted food and those that did not. Preliminary analyses were also made of whether animal mass affects the length of the strip eaten, the number of swallows made and inter-swallow intervals before and after weights have been added. For these analyses, animals were divided into two roughly equal groups on the basis of weight. No significant differences were found. The difference in results can be explained in two ways: (1) H. J. Chiel and collaborators examined a much wider mass range of animals (from 6 g to over 350 g). Thus, modulation of a movement by added weights would more easily have been found by them. (2) 'Tugging' is a fundamentally different movement from cutting and swallowing, and animal mass is of more importance in modulating this movement.

Feedback modulation of feeding in other gastropods

The hardness of the food affects bite frequency in *Limax maximus* (Reingold and Gelperin, 1980). An increase in hardness leads to a decrease in bite frequency, as was observed above for an increase in load in *Aplysia*. In a semi-intact preparation, attaching weights to the buccal mass led to a decrease in frequency of the feeding motor programme recorded from the nervous system (Reingold and Gelperin, 1980). In *Ariolimax californicus*, an increase in the hardness of food leads to a decrease in the quantity of food eaten, but no change in bite frequency

(Senseman, 1978). Feedback from feeding muscles onto the central pattern generators for feeding has also been reported in *Pleurobranchaea californica* (Siegler, 1977) and in *Helisoma trivolis* (Kater and Rowell, 1973). In *Helisoma trivolis*, feedback modulates the amplitude of feeding patterns, but not the frequency, while in *Pleurobranchaea californica* frequency is also modulated. Finally, in *Navanax inermis* the nature of feeding sequences is changed by increasing the size of the food (Susswein and Bennett, 1979; Susswein *et al.* 1984*a*). As food size increases, different feeding acts are elicited that are appropriate for the capture of larger food.

Feedback modulation of chewing in mammals

In cats, bite-to-bite variation has been examined in response to foods of different consistency, during chewing sequences that reduce food to a form that can be swallowed (Gans *et al.* 1990). Variability in the magnitude of EMGs from bite to bite is very large, with a general tendency for magnitude to decrease over a sequence of bites. Magnitude is larger, and sequences are longer, for foods of tougher consistency. Thus, the control of complex chewing sequences in mammals is apparently quite reminiscent of the control of swallowing sequences in *Aplysia*.

Aplysia *feeding as a model preparation*

Our data, as well as data from others, indicate that *Aplysia* is a particularly useful preparation for examining how a small number of different feeding activities are coordinated and varied to provide a response appropriate to the physiological state. Command neurones (Rosen *et al.* 1988), as well as elements of central pattern generators controlling feeding movements (Kirk, 1989; Plummer and Kirk, 1990; Susswein and Byrne, 1988), are accessible to study, as are neurones that apparently carry information from muscles and central pattern generators to higher control centres (Jahan-Parwar *et al.* 1983; Chiel *et al.* 1988). Qualitatively different movements in response to different food stimuli can also be studied systematically. Future studies can be addressed to linking the properties of neural circuits to changes in feeding sequences in response to different foods.

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