

Plasticity of Olfactory Orientation to Foods in the Snail *Achatina fulica*

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Summary. Snails were tested in an olfactometer for their orientation to food odours. Measures of odour choice, latency to make a choice and percentage of upwind orientations were obtained after experimental manipulations of dietary experience and hunger motivation. Juvenile snails, given an exclusive diet of a certain food for 12 h or less, oriented preferentially toward the odour of that food when subsequently presented with a choice between it and a novel food odour (Fig. 2). A 48 h period of exposure to a food resulted in a 21 day retention of the odour preference (Fig. 3). There was no evidence for an especially sensitive period for conditioning during the first 50 days of age (Fig. 4); however, adult snails (minimum 1 year old) required longer periods of dietary exposure to establish equivalent food odour memories (Fig. 5). Measures of latency and percentage of upwind orientations confirmed the choice data in demonstrating an effect of dietary experiences on olfactory orientation. Hunger motivation increased the selection for familiar food odours, and reduced the latency (Fig. 6 and Tables 3 and 4). Sensory adaptation appears inadequate to explain the conditioning phenomenon since formation of the preferences was contingent upon ingestion of the foods; exposure to the food odours alone was insufficient (Table 2). The data suggest that orientation preferences derive from a learned association between the odour of a food and its nutritional value.

Introduction

A great deal of attention has recently been focused on the feeding responses of gastropod molluscs as model systems for the study of cellular mechanisms

underlying complex behaviours. Examination of the consummatory components has proven particularly fruitful in this regard. These stereotyped responses are easily monitored while at the same time they are influenced by a number of controllable internal and external variables. The initiation of biting in *Aplysia*, for example, has been shown to be influenced by the concentration of chemical food cues, the time of day, and the degree of satiation of the animal (Kupfermann, 1974; Susswein et al., 1976). Numerous other studies have shown that learning can affect whether an animal will start feeding on a substance or whether it will withdraw or avoid it (Gelperin, 1975; Kupfermann and Pinsker, 1968; Mpitsos et al., 1978). These reports are especially relevant to the neurobiological study of behavioral plasticity in light of the recent studies on central nervous control of buccal movements in gastropods (Gillette et al., 1978; Kaneko et al., 1978; Cohen et al., 1978). They provide a promising model for examining how the central nervous system processes complex sensory information to produce an appropriate behavioural response.

In nature, however, food ingestion is usually only the final response following a series of temporally coordinated behavioural choices that begins with a decision to orient to the odour of a potential food. Choices made early in the sequence restrict the range of later choices. Any complete account of feeding must be based on an understanding of the factors controlling each response in the sequence. The present study investigates whether the initial appetitive phase of feeding, like the consummatory phase, is influenced by past experience and motivation. It is expected that the results of this study will shed light on the nature of the neuronal mechanisms which link together both appetitive and consummatory decisions to yield the total feeding behaviour.

Preliminary report of some of these findings have appeared elsewhere (Croll and Chase, 1976, 1977b).

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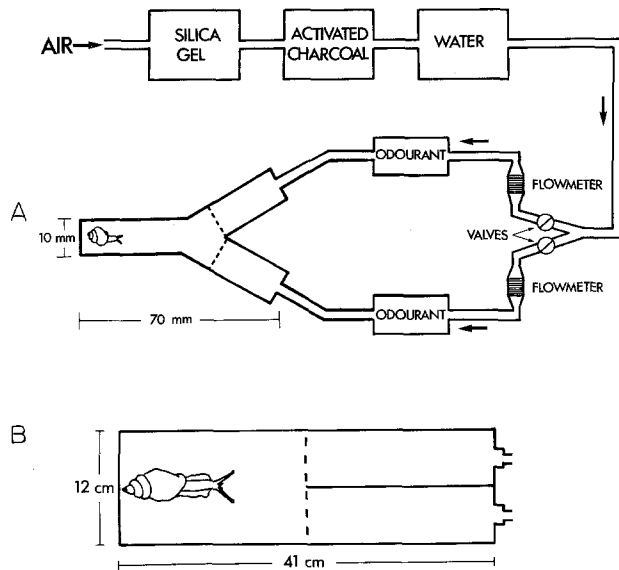


Fig. 1A and B. Olfactometers used for testing juvenile (A) and adult (B) snails. Animals were individually placed at the base of the apparatus and scored for odour choices after passing the criterion line (dotted)

Methods

Animals. Specimens of *Achatina fulica* were obtained either from Honolulu, Hawaii, through the courtesy of Mr. F.J. Olson and Mr. S.S. Miyake of the United States Department of Agriculture, or from Bangkok, Thailand through the Friendship Trading Birds and Animals Co., Ltd. These snails were collected in the field where their diets were unknown. Measuring 5–10 cm in shell length, they were estimated to be at least one year of age, and were classified as “adult”. Immediately upon arrival in Montreal the animals were placed on conditioning diets of cucumber, carrot, or lettuce as detailed for each experiment. Groups of six to ten snails were housed together in glass containers with approximately 5 cm of moist sand. They were watered and supplied with fresh food three times weekly.

Snails designated as “juveniles” or “hatchlings” were derived from clutches of 50–200 eggs that were collected from laboratory cages of lettuce fed *Achatina*. The eggs were washed, placed in small glass containers and covered with about 2 cm of moist sand. At 7–10 days after hatching (except in Experiment IV where a wider range of ages were used), the clutch was washed free of sand and then divided into two groups. One half of the animals was placed into containers with sand and diced carrot, while the other half was put into similar containers with diced cucumber. Upon termination of these experimental diets the snails were again washed and placed into clean containers with sand alone or, in some experiments, with sand and lettuce. The animals were maintained in this manner until testing.

The subjects were kept at a room temperature of between 22 °C and 26 °C, with a 12 h light, 12 h dark cycle using overhead fluorescent lighting. Testing was generally performed between the last hour of darkness and the fourth hour of the light cycle.

Apparatus. The orientation of the snails to food odours was tested in a two-armed airway, or olfactometer. For the juvenile snails, the olfactometer was a Nalgene Y-connector (Fig. 1A). The adult snails were tested in a larger Plexiglas apparatus (Fig. 1B).

Compressed air was purified through silica gel and activated charcoal filters and was then rehumidified before being split into two separate airstreams, each having a flow rate of either one liter/min for the smaller olfactometer or 4 l/min for the larger one. Before entering the olfactometer, each airstream was blown through a vial containing either shredded vegetables or a small amount of water. The odourant vials were hidden from the view of the snails during testing to minimize the use of visual cues in orientation.

Snails were individually placed at the base of the olfactometer with an initial upwind orientation. For the adult snails, a choice was scored once any portion of the shell entered either arm of the olfactometer within the time criterion of 30 min. For the juvenile snails, a choice was scored once an animal’s shell had completely entered either arm; the time criterion was 20 min. After each trial the olfactometer was disconnected from the airstream, washed thoroughly with detergent, and rinsed in tap water before the next trial.

Design and Analysis. Two types of experiments were conducted with the olfactometers. In one type (Experiments I–V, IX and X) the purpose was to determine the animals’ selection between two different food odours. The other experiments (VI–VIII) assessed the strength of orientation to a single food odour. In all the experiments, relatively large day-to-day variations were evident in orientation preferences, latencies and percentage of upwind orientations. Thus, in order to more effectively evaluate the consequences of the experimental manipulations, the experiments were conducted in multiple sessions and the data were pooled.

In the two-odour design, one airstream was odourized with cucumber, the other with carrot. Placement of the odours on either side was randomized. If the snail backed out of the olfactometer during testing it was immediately placed back in the apparatus with the same initial orientation. If no choice was made within the time criterion, the animal was removed from the olfactometer and retested at a later time.

Relative preferences of juvenile snails for cucumber and carrot odours were evaluated by comparing the performances of groups of 25–40 animals. Each animal was scored for one food odour choice. The difference in group orientation was evaluated using the χ^2 -test for independence. Because of a smaller number of available adult snails, each of these was tested four or five times, depending on the experiment. The orientation score for each adult equalled the number of cucumber choices made by the individual divided by the total number of completed trials. The group means of the orientation scores were compared using the *t*-test for one-way classifications, or an analysis of variance (ANOVA) for multi-dimensional classifications. Since our earlier study (Croll and Chase, 1977a) showed that *Achatina* orients preferentially to familiar food odours, the existence of orientation preferences in the present study was tested by means of one-tailed statistics. In cases of multi-dimensional design, tests of interactions were two-tailed.

Two other measures of performance were also noted in the two-odour design. The first was the latency to make a choice, regardless of which odour was chosen. The second measure was the percentage of the animals that oriented upstream and eventually made a choice within the time criterion, as opposed to those animals that persisted in backing out of the olfactometer, or those that moved about within the apparatus without making a choice, or those that were inactive. Pooling of the data was necessary to evaluate the effects of the various treatments on these measures. This was accomplished by comparing a group composed of animals from the lower end of the range of the independent variable (days of experience, age, etc.) with a group of animals from the higher end of the range. For example, the latencies of animals with 0, 1, 2 and 3 days of starvation were pooled and compared with the latencies pooled from starvation days 4, 5, 6 and 7. The *t*-test was used for the comparison of latencies, and the χ^2 -test was

used for comparison of the number of animals in each group that oriented upstream. Two-tailed comparisons were used for both measures due to the absence of a priori directional predictions.

In the single-odour experiments, one of the odourant vials attached to the olfactometer contained shredded lettuce while the other vial contained a small amount of water. A trial was completed either by the snail making a choice, or by the snail backing out of the apparatus, or by the passing of the time criterion. Each snail was tested four or five times over a period of two successive days, and its orientation score equalled the number of lettuce choices divided by the total number of trials completed. The latencies of the trials ending in a lettuce choice were pooled and analyzed in the manner described above. In this paradigm, no separate analysis was performed on the percentage of trials resulting in an upwind orientation, as this measure was incorporated in the orientation score.

Results

Experiment I. Acquisition of Food Odour Preferences in Juvenile Snails

The purpose of this first experiment was to determine the effects of brief exposures to experimental diets on the subsequent food odour preferences of juvenile *Achatina*. Two hundred and fifty hatchlings were randomly divided into five pairs of groups. One pair was immediately exposed to the experimental diets, with one group being given cucumber and the other group carrot. Twenty-four hours later, another two groups started their diets of cucumber or carrot. Similarly, the other pairs were introduced to the foods at 36, 42 and 47 h after the first pair's diet began. After 48 h, all of the animals were removed from their containers, washed, and placed in clean containers. This procedure resulted in different groups being exposed to diets of cucumber or carrot for 1, 6, 12, 24 or 48 h and allowed all animals to be tested in the olfactometer on the same day and at the same age. Testing was performed 12–16 h after termination of the diets. They were not fed during the interim.

The effect of the brief diets on later food odour preferences is shown in Fig. 2. It is evident that even short exposures to selected foods can affect subsequent odour choices. With increasing duration of exposure there is a progressive increase in the relative preference for that food with which the snails are experienced. Although the group differences for the 1, 6 and 12 h exposures do not individually show statistical significance, when combined they indicate that a significant preference is formed within the first 12 h of exposure (χ^2 -test, $P < 0.01$).

The latencies to make a choice decreased as a function of the length of experience with the foods. Those snails exposed to cucumber or carrot for short periods (one and six hours combined) had a mean

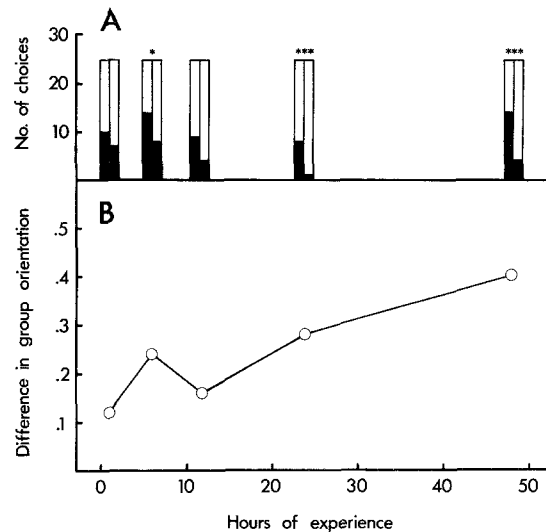


Fig. 2A and B. Effect of brief exposures to food on subsequent odour preferences. Animals were tested 12–16 h after termination of experimental diets. In A, each pair of bars represents the odour choices of two groups of 25 snails, with one group (left bar in each pair) having been fed cucumber and the other group (right bar) having been fed carrot for the indicated period of time. The black, bottom segment of each bar shows the group's choices for cucumber. The remaining white segment shows the choices for carrot. The difference between each pair of groups was analyzed using the χ^2 -test for independence (* $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$). These data are expressed in B as a difference in group orientation at each testing interval. To do this, two ratios were calculated, one for the proportion of cucumber conditioned animals orienting to cucumber, and one for the proportion of carrot conditioned animals orienting to cucumber. Each point represents the difference between these ratios

latency of $13.8 \text{ min} \pm 0.53$ (S.E.) whereas those exposed for longer periods (24 and 48 hours combined) had an average latency of $11.7 \text{ min} \pm 0.55$ (S.E.); (t -test, $P < 0.05$). Thus, increased familiarity with a food resulted in either an increased directness of movement, or an increased speed of locomotion, or a decreased reaction time from first detection of the odour to initiation of locomotion, or a combination of the above. Since these variables can all be interpreted as measures of motivational state, the latency data suggest that experience with a food increases a snail's motivation to orient upwind if that food's odour is present in the airstream. The motivational change, along with the change in selection between the odours, may result from the formation of an association between a specific odour and a proven food source. This possibility is addressed more directly in the following experiment. There was no difference between the long and short exposure periods in the percentage of animals orienting upstream (60.2% vs. 62.8%, respectively; χ^2 -test, $P > 0.5$).

Experiment II. Sensitization vs. Association

The previous experiment demonstrated that brief periods of experience with a novel food will affect the probability of an animal's orientation to the odour of that food. In order to test whether sensitization to airborne chemical stimuli is responsible for this effect, an experiment was performed to determine whether ingestion of the food is necessary for the snails to form an odour preference or whether exposure to the food odour alone is sufficient. One hundred and sixty hatchlings were divided into four groups. One group was placed in a covered container with sand and cucumber. A second group was placed in an identical container with carrot. The third and fourth groups were housed in similar covered containers, again with either cucumber or carrot, however a perforated Plexiglas platform was situated directly over the sand and the food, with the animals placed on top. The animals were separated from the food by less than 1 cm. Since the containers were covered, the enclosed air was nearly saturated with the food odours. Thus, while these snails could not feed, their exposure to airborne chemicals was equivalent to that of the first and second groups. Each group remained in its container for 48 h. After termination of the exposure period all animals were washed and placed in clean containers with moist sand until testing 24 h later. They were not fed during the interim.

Table 1 shows the results of this experiment. The groups that were allowed to feed during the exposure period showed a significant preference for the familiar food odours (χ^2 -test, $P < 0.01$), whereas the groups that were exposed to the odours alone showed no subsequent preferences (χ^2 -test, $P > 0.5$). Thus ingestion is a necessary condition for the formation of food odour preferences. Since the olfactory experiences of the eating and the non-eating animals were very nearly identical, sensitization to the familiar food odour is not a sufficient explanation to account for the phenomenon. The formation of a learned association between the odour of a food and some consequence of its ingestion appears to be the most likely alternative explanation.

There was also a significant difference between the groups' mean latencies (t -test, $P < 0.05$). The groups exposed to the odours alone made choices with an average latency of $9.8 \text{ min} \pm 0.56$ (S.E.). Those allowed to eat during the conditioning period made choices with a mean latency of $11.7 \text{ min} \pm 0.61$ (S.E.). Another difference in performance was the percentage of snails that oriented upstream under the different treatments. Whereas 32.5% of those fed cucumber or carrot oriented upstream during testing, 44.2% of those exposed to the odours alone made choices

Table 1. Necessity of food ingestion for formation of odour preferences. Two groups of 40 juvenile snails were exposed to the odours of either carrot or cucumber for 48 h; two other groups were allowed to feed on one of the two foods during the same period of time. All animals were tested 24 h after termination of the experimental exposure

Stimulus condition	Choices for cucumber	Choices for carrot	χ^2 value (probability)
Cucumber odour alone	18	22	0.2046 ($P > 0.5$)
Carrot odour alone	16	24	
Cucumber as a food	19	21	11.6667 ($P < 0.01$)
Carrot as a food	5	35	

in the olfactometer. This constitutes a significant difference in the behavior of the two groups (χ^2 -test, $P < 0.01$). These results presumably reflect the fact that the animals exposed to the food odours alone were at a higher level of food deprivation at the time of testing than were the animals fed during the conditioning period. Later experiments (VII-IX) specifically examine the effects of food deprivation on olfactory orientation.

Experiment III. Retention of Food Odour Preferences

This experiment was designed to determine the retention time for the food odour preferences acquired during a 48 h conditioning period. Three hundred and fifty juvenile snails were used in this experiment. One half was given a diet of cucumber, the other half was given a diet of carrot. After 48 h, all of the animals were washed and placed in clean containers with sand and lettuce. Twenty-four hours later, the animals were again washed and placed in clean containers with fresh sand and lettuce in order to eliminate the faecal ribbons that contained large quantities of partially digested cucumber or carrot. Groups of 25 carrot-fed snails and 25 cucumber-fed snails were tested at 0, 1, 5, 7, 10, 15 and 20 days following termination of the conditioning diets.

The retention curve for the memory of the food odours after a two day exposure is shown in Fig. 3. Extrapolation of a straight line fitted to the data gives an estimate of 21 days for the length of the memory. Thus, even with brief periods of conditioning, the memory is of long duration.

The latencies to make a choice decreased with increasing delays between conditioning and testing. The mean of the latencies for days 0, 1 and 5 combined was $13.0 \text{ min} \pm 0.45$ (S.E.) while the mean latency for days 10, 15 and 20 combined was $11.2 \text{ min} \pm 0.44$ (S.E.); (t -test, $P < 0.05$). There was

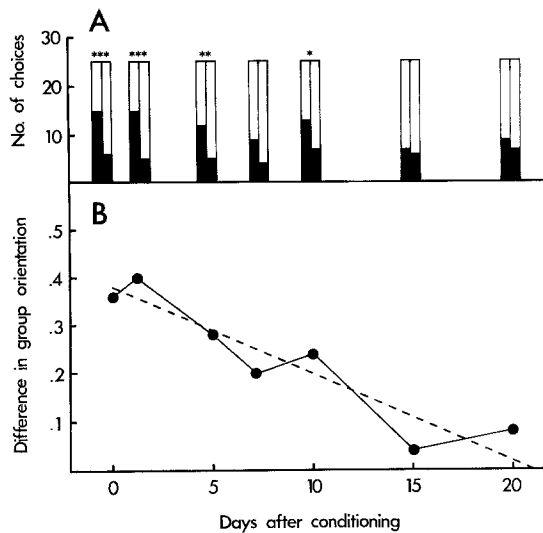


Fig. 3. Retention of food odour preferences. Snails were fed cucumber or carrot for 48 h and then fed lettuce until testing. Format same as in Fig. 2

also a significant difference between groups with short delays and those with longer delays in the number of animals that oriented upwind. The groups tested shortly after conditioning oriented upstream 37.5% of the time, whereas those tested later oriented upstream 51.9% of the time (χ^2 -test, $P < 0.01$). In experiment I weaker preferences for familiar food odours were accompanied by a weaker motivation to orient. If deterioration of the memory for the food odours were the sole factor changing over the time course of the present experiment, one would expect a similar reduction in motivation with increasing intervals between conditioning and testing. This expectation is not supported by the data. However, since the animals in this experiment were of differing ages at the time of testing, the results may, at least in part, be due to age-dependent performance factors. This issue is investigated in the following experiment.

Experiment IV. Effects of Age on Preference Formation and Orientation

Sensitivity to certain forms of learning (for example imprinting) is known to change markedly during early life. The present experiment tested for changes in sensitivity to food odour conditioning in *Achatina* during the first 50 days of life. On the fifth day after hatching, 300 snails from 6 clutches were divided into 10 groups. One group was fed cucumber for 24 h, and another group was fed carrot for 24 h. The two groups were tested for their food odour preferences immediately

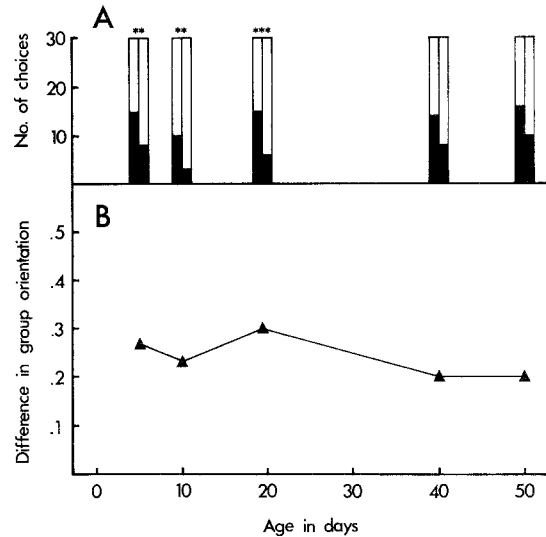


Fig. 4. Effect of age on formation of food odour preferences. After hatching, all snails were maintained on a lettuce diet until various ages at which time they were fed either cucumber or carrot for 24 h. They were tested immediately afterwards. Format same as in Fig. 2

after termination of these experimental diets. The remaining groups were maintained on fresh lettuce until they were either 10, 20, 40 or 50 days old, at which times they were fed either cucumber or carrot for 24 h and tested immediately afterwards.

Figure 4 shows that differences in age have no strong effects on preference formation during the first 50 days of life. As the animals matured, however, they oriented upstream more often (29.9% of the 5–10 day old animals oriented upstream vs. 69.4% of the 40–50 day old animals; χ^2 -test, $P < 0.01$). Also, their latencies decreased from a mean of 14.6 min \pm 0.46 (S.E.) at the age of 5–10 days, to a mean of 9.7 min \pm 0.54 (S.E.) at the age of 40–50 days (t -test, $P < 0.01$).

These results suggest that the measures of food odour preferences in the previous experiments were not confounded by age-dependent factors and may be generalized to various ages within the juvenile range. However, comparisons between age groups must take account of differences in latencies and the percentage of animals orienting upwind in the olfactometer. This latter fact explains the anomalous result reported in Experiment III.

Experiment V. Effects of Brief Periods of Feeding on Subsequent Food Odour Preferences of Adult Snails

From the experiments thus far described it is evident that juvenile *Achatina* form long-lasting food odour preferences following brief periods of experience with

Table 2. Effect of brief periods of restricted diet on odour preferences in adult snails. 6 snails were fed cucumber for 48 h while another 6 snails were similarly fed carrot. All animals were tested five times each 24–48 h after termination of the conditioning diets

Conditioning diet	Mean orientation to cucumber ± standard error	Significance level for difference between means (<i>t</i> -test)
Cucumber	0.70 ± 0.045	$P < 0.01$
Carrot	0.43 ± 0.053	

selected foods. Croll and Chase (1977a) reported that food odour preferences of adult *Achatina* were not affected by brief periods of experience with hitherto novel foods. However, prolonged regimes of restricted feeding resulted in a memory for the food odours that lasted four months. The present experiment examines in greater detail the question of whether adult specimens form odour preferences following brief periods of experience with novel foods.

Two groups of six snails were used. All animals were first starved for 12 days. Thereafter, one group was fed carrot for 48 h while the other group was simultaneously fed cucumber. At the end of this exposure period both groups of snails were washed and placed in clean containers. After 24 h of food deprivation the animals were tested for their orientation preference between the odours of cucumber and carrot. The testing was performed over a two day period. Each snail was tested until it made five food odour selections.

The results (Table 2) show that a short period of exposure to a food can significantly affect an adult *Achatina*'s preference for the odour of that food one to two days after termination of the diet (*t*-test, $P < 0.01$). Moreover, the animals appeared to be highly motivated to orient in the olfactometer. The mean latency for a choice was 8.1 min ± 0.86 (S.E.). Of the 63 trials run during this experiment, 60 trials (95.2%) ended in an upstream orientation and selection of either of the two airstreams within the time criterion. Thus it appears that brief periods of feeding result in short-term memories for food odours. Longer periods of feeding experience are required for preferences to be evident at later times.

Experiment VI. Incremental Effects of Feeding Experiences on Orientation Preferences in Adult Snails

This experiment demonstrates the effects of increasingly longer periods of an exclusive diet on long term changes in olfactory orientation. Forty-two adult

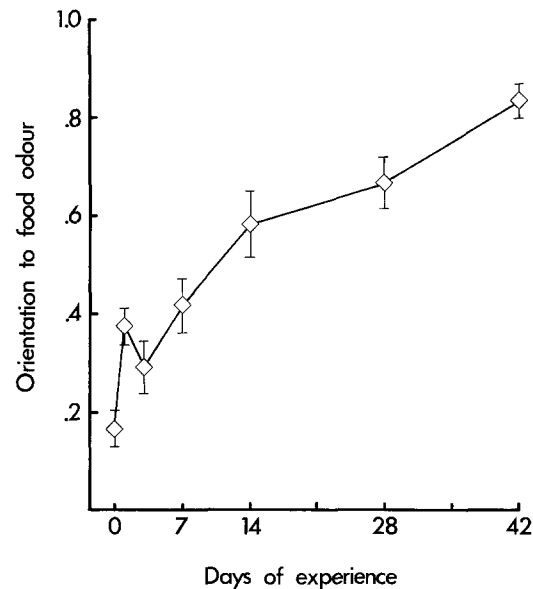


Fig. 5. Effect of restricted feeding on a single food on orientation to the odour of that food. Forty-two snails were divided into 7 groups. The animals were fed exclusive diets of lettuce for varying lengths of time, starved for 10 days, and then tested in the olfactometer. One airstream was odourized with lettuce; the other airstream was unodourized. Each animal was tested four times. The points represent the group means of orientation scores, defined as the number of orientations to lettuce odour divided by the number of trials. Bars: standard errors

snails were divided into seven equal groups. Six of the groups were placed in clean containers and fed lettuce. The seventh group was starved for ten days and then tested in the olfactometer on two successive days for its accuracy in selecting an airstream odourized with lettuce when the alternative airstream was unodourized. The animals were tested four times each. The other six groups of snails were similarly starved and tested after varying lengths of experience with lettuce.

Figure 5 shows the effect of increasing lengths of exposure to lettuce on orientation to lettuce odour after a 10–11 day period of starvation. The accuracy of selecting the lettuce odour increased substantially during the several weeks of the experiment. A correlation analysis of the data yields a coefficient of 0.635, and an analysis of variance for the regression indicates the means were significantly different ($P < 0.001$). As was the case with juvenile snails (Experiment I), the food odour orientations of adult snails became progressively more selective as the duration of the restricted diet increased. However, a comparison of this experiment with Experiment I suggests that the adults require longer periods of dietary experience than the juveniles to form food odour memories of equivalent strength.

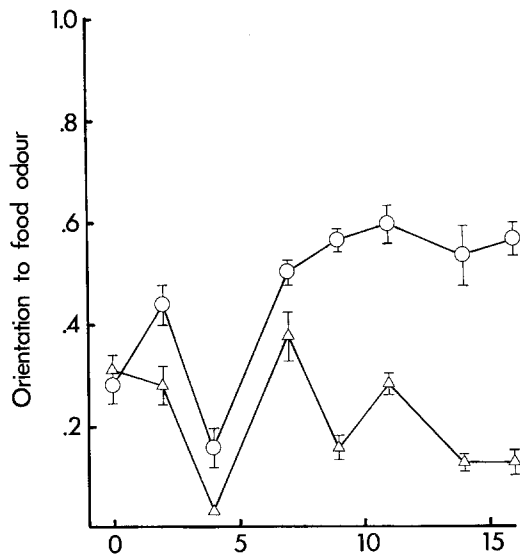


Fig. 6. Effect of food deprivation on orientation to a familiar food odour. 128 snails were fed an exclusive diet of lettuce for 6 weeks prior to day 0. At this time half of the snails were starved (\circ) and the other half continued on the lettuce diet (Δ). On subsequent days 8 animals from each group were tested 4 times each for their orientation to lettuce odour. Group means and standard errors are indicated

The mean latency for lettuce choices of animals combined from 0, 1 and 3 days of lettuce experience was $9.2 \text{ min} \pm 0.68$ (S.E.), whereas the mean for the animals with longer experience (14, 28 and 42 days combined) was $6.7 \text{ min} \pm 0.68$ (S.E.). Although this difference does not reach statistical significance (t -test, $0.1 > P > 0.05$), it does suggest that increased experience with a food results in an increased motivation to orient toward that food, as with the juvenile snails (Experiment I).

Experiment VII. Effects of Food Deprivation on Orientation to a Single Familiar Food Odour

The preceding experiments examined the acquisition and retention of modifications in food-finding behavior as a result of experience with selected foods. This experiment, and those that follow, examines the effects on the same behaviour of changes in motivation induced by starvation. The present experiment investigated the effects of increasing periods of food deprivation on the orientation of adult *Achatina* to a single familiar food odour.

One hundred and twenty-eight snails were fed lettuce exclusively for six weeks. On day 0, 64 snails were taken off the lettuce diet and were starved; the remaining 64 snails continued to feed on lettuce. On various days following day 0, eight starved and eight

non-starved snails were tested four times each for their orientation to lettuce odour in the olfactometer. The alternative airstream was unodourized.

The results are summarized in Fig. 6. There was a significant difference in the overall performance of the starved animals and the satiated animals (ANOVA, $P < 0.001$), with the former orienting more accurately to the lettuce odour. This difference in performance also showed a significant interaction with time (ANOVA, $P < 0.05$). The longer the animals were starved, the more accurately they oriented to the food odour, compared to the non-starved animals. The difference in orientation between the starved and the satiated animals is particularly striking following day 7.

The mean latency for the starved animals on days 0, 2, 4 and 7 combined was $10.4 \text{ min} \pm 1.02$ (S.E.), while the mean latency for the unstarved animals over the same time period was $8.9 \text{ min} \pm 0.90$ (S.E.); (t -test, $P < 0.2$). On days 9, 11, 14 and 16 combined the mean latency increased for the unstarved animals to $12.1 \text{ min} \pm 1.42$ (S.E.) and decreased for the starved animals to $8.1 \text{ min} \pm 0.68$ (S.E.); (t -test, $P < 0.01$). The change in the difference between the starved and the satiated snails from early in the treatment to later in the treatment is statistically significant (ANOVA, $P < 0.01$). Thus, food deprived *Achatina* not only orient more accurately to a familiar food odour than do food satiated animals, they also orient more rapidly.

Experiment VIII. Separation of Effects of Food Deprivation and Food Odour Deprivation

Experiments with other invertebrates have suggested that sensory adaptation to chemical food cues may be involved in the decrement of consummatory feeding behaviours during satiation (Dethier, 1952; Senseman, 1978). Conversely, if similar mechanisms operate upon appetitive feeding behaviours, it could be argued that in Experiment VII the critical factor in starvation was food odour deprivation rather than food deprivation. The present experiment tested this issue.

Four groups of adult snails were used, each composed of eight animals that had been fed lettuce for six weeks. One group continued the lettuce diet until tested in the olfactometer. The second group had the lettuce removed from its cage on day 0 and was thereafter fed cucumber. This group was therefore deprived of the lettuce-odour, but was not food deprived. The third group also had the lettuce removed from its cage on day 0, and was instead provided with a screened enclosure containing fresh lettuce. This group

Table 3. Effects of food deprivation and food odour deprivation on orientation to the familiar food odour. Four groups of eight snails were fed lettuce for six weeks. The experimental conditions were then maintained for 12 days before testing for orientations to lettuce odour. Each animal was tested four times

Experimental condition	Mean orientation score ± standard error	Significance level for difference from lettuce fed group (<i>t</i> -test)
Fed lettuce	0.15 ± 0.094	—
Fed cucumber	0.25 ± 0.074	<i>P</i> > 0.2
Starved/smelled lettuce	0.56 ± 0.113	<i>P</i> < 0.01
Starved/no odour	0.56 ± 0.091	<i>P</i> < 0.01

was food deprived, but not odour deprived. The remaining group had the lettuce removed from its cage on day 0 and did not eat or smell food until tested. On days 12 and 13 each animal was tested a total of four times for its orientation to lettuce odour, using the single odour testing paradigm.

Table 3 shows the relative accuracy of each group in its orientation to lettuce odour. The results are not such as would be expected if olfactory adaptation regulated the snails' performance in the olfactometer. On the contrary, the orientation to lettuce odour was improved by the two treatments which results in food deprivation, but it was not significantly affected by the deprivation of lettuce odour in the absence of food deprivation. Hence, one may reasonably exclude olfactory adaptation as an explanation for the starvation effects reported in Experiment VII. An alternative interpretation of those data must assume the monitoring of internal events subsequent to ingestion, and the linkage of that information with olfactory processes.

Measures of latency support the hypothesis that food deprivation and not food odour deprivation, is the critical factor in the modification of food-finding behaviour during starvation. The pooled latencies of the two food satiated groups yielded a mean of 11.8 min ± 2.16 (S.E.), while the mean latency for the two food deprived groups was 8.9 min ± 1.35 (S.E.). Thus the latency was significantly shorter for the starved snails as compared to non-starved animals, regardless of whether or not they were exposed to the familiar food odour (*t*-test, *P* < 0.05).

Experiment IX. Effects of Food Deprivation on Choice Between Novel and Familiar Food Odours

The two previous experiments demonstrate that snails orient more accurately to a familiar food odour if they are food deprived before testing. Both of these experiments tested for orientation to a single

Table 4. Effect of food deprivation on food odour preferences. Two groups of 42 snails were fed exclusive diets of either cucumber or carrot for 6 weeks. The cucumber experienced animals were then subdivided into two groups. One group continued its cucumber diet while the other was food deprived. The carrot experienced animals were similarly subdivided. On the 12th and 13th days following initiation of the starvation regimes for the food deprived animals all subjects were tested five times each for their food odour preferences

Conditioning diet	Mean orientation score to cucumber ± standard error	
	Satiated animals	Starved animals
Cucumber	0.55 ± 0.033	0.705 ± 0.056
Carrot	0.44 ± 0.053	0.381 ± 0.041

odourized airstream. The present experiment examines the effects of food deprivation on orientation to a familiar food when the animals are presented with a choice between a familiar and a novel food odour.

Four groups of 21 adult animals were used. Two groups had previously been fed cucumber for six weeks and the other two groups had been fed carrot. One cucumber group and one carrot group were then starved. The other two groups continued their diets of cucumber or carrot. On the 12th and 13th days of starvation for the deprived groups, all animals were tested five times each for their orientation to cucumber and carrot odours using the two-odour testing design.

The results of this experiment are summarized in Table 4. Animals showed a significant difference in orientation as a consequence of their experience with either cucumber or carrot (ANOVA, *P* < 0.001). Furthermore, there was a significant interaction between the effects of feeding experience and starvation (ANOVA, *P* < 0.05), reflecting the fact that starved animals oriented more toward the familiar food odours than did non-starved animals. Starved snails increase their selectivity for a familiar food odour when presented with a choice between it and a novel food odour.

Latency and upwind orientation measures did not reach statistical significance. The mean latency for the starved animals was 8.8 min ± 0.44 (S.E.) and for the satiated animals was 9.0 min ± 0.42 (S.E.); (*t*-test, *P* > 0.5). Of the starved animals, 81.4% oriented upwind, while 81.8% of the satiated animals oriented upwind (χ^2 -test, *P* > 0.5).

Discussion

This study demonstrates that the food-finding behaviour of a snail can be influenced by both experiential

and motivational factors. Olfactory orientation to a food is dependent on the animal's previous dietary experience with that specific food and on its nutritional needs. These influences interact and result in variations in behaviour that can be effectively measured by choice and performance criteria in an olfactometer. Taken together, the experiments show that the appetitive phase of gastropod feeding, like the consummatory phase, is plastic and is not solely determined by innate responses to tactile and chemical cues.

Food odour choices in the olfactometer were significantly affected by previous brief encounters with foods. Experiment I showed that juvenile snails, when given an exclusive diet of a food for 12 h or less oriented preferentially toward the odour of that food 12–16 h after termination of the experimental diet. The significance of this finding becomes apparent when one considers its application to an animal in the wild. Since *Achatina* is active nocturnally and during the early morning, this means that 12 h or less of feeding on a plant one night will affect a snail's foraging the next night. Subsequently, the second night's feeding will affect the third night's and so on. As experience with the food increases so does the preference for that food's odour. If the food remains readily available, the snail will increasingly specialize on it. Although the content of the first meal in a snail's life may be determined in large part by the availability of foods within the near proximity of the nest, and by innate responses to food cues, later choices of diet will evidently be influenced by experience with particular foods.

Measures of latency confirm the plasticity of food-finding behaviour resulting from feeding experiences. In Experiments I and VI, where age and hunger motivation were controlled for, it was found that changes in odour selection were accompanied by changes in latency to make a choice. As familiarity with a food increased, not only did selection for the odour of that food increase, but latencies decreased in a corresponding manner. Thus our experiments succeeded in demonstrating two different, and significant, effects of dietary experience on olfactory orientation to foods.

Once established, these food odour preferences are extremely stable over time. After a monophagous diet lasting 48 h, an animal can be expected to retain a memory of the food odour for at least 21 days (Experiment III). This estimate is probably conservative, and the feeding experience may actually influence foraging behaviour for a much longer time. One reason for this belief is that the estimate is based on a linear regression of the retention scores, whereas retention scores in other animals generally approach zero asymptotically (Hulse et al., 1975). The present

study was intended only to demonstrate a long-lasting memory for the food odours after brief periods of experience; therefore the precise shape of the retention curve was not determined. If an asymptotic memory function is operating in the snail, slight odour preferences should persist beyond the point estimated by linear regression. Furthermore, Croll and Chase (1977a) reported that conditioned snails show savings upon re-learning at a time when their olfactometer performance had fallen to a chance level. One can thus conclude that the effects of experience with selected foods last longer than indicated by the responses examined in Experiment III.

Two lines of evidence suggest that the establishment of food odour preferences is based on a learned association between the odour of a food and some consequence of its ingestion. First, Experiment II showed that exposure to the food odours alone, without eating, is insufficient to produce an orientation bias. This result eliminates the possibility that olfactory sensitization might account for the phenomenon. Second, it is clear from Experiments VII and IX that the snails perceive the conditioned odour as a *food* odour, since food-deprived animals oriented more accurately than did satiated animals. Olfactory adaptation has been eliminated as an explanation for this change (Experiment VIII). Experiments II, VIII and IX also control for the possibility that the snails are learning to associate an odour with a home site and that orientation to the conditioned odours represents an olfactory homing response, as has been reported in slugs (Gelperin, 1974).

We have not ascertained the precise event which constitutes reinforcement in the present conditioning paradigm. Ingestion per se might serve this function, or perhaps distension of the gut. A dependency on either of these cues, however, could lead to the reinforcement of maladaptive behaviour since the ingestion of non-nutritious bulk would reinforce olfactory orientation as well as the ingestion of nutritious foods. It therefore seems reasonable to suppose that the post-digestive recognition of the food's nutritive value serves as reinforcement.

For the snail, olfactory orientation to foods represents a large expenditure of time and energy. Hence it might be expected that snails would orient only infrequently to odours deriving from foods that have not yet proven to be worth the expenditure. Experiment VI supports this prediction. Nevertheless, some innate mechanism for food recognition must exist whereby snails can gain the requisite experiences for learning. This mechanism was manifested in Experiment II, where hunger motivated snails encountered two novel food odours. Although the animals oriented non-selectively to the odours, they did so with greater efficiency than did satiated animals (as

judged by measures of latency and percent making a choice). It may be inferred that the snails responded appropriately to one or more volatile chemicals common to both stimuli and which denote a potential food source.

Conversely, since the consummatory phase of feeding represents a minimal expenditure of time and energy, snails should show little reluctance to sample a novel food, if encountered directly. Evidence is available to support this prediction (Croll, 1978). By eating novel foods, animals acquire an orientation bias that will be expressed in subsequent food-finding expeditions. Thus one would expect that although individuals may specialize, the species as a whole should eat a wide variety of foods, where available. Field observations of *Achatina* indicate that this is, in fact, the case (Mead, 1979).

To summarize our interpretation of the conditioning phenomenon, we believe that snails rely heavily on previous experience to maximize the efficiency of their foraging. The present experiments, together with our earlier ones (Croll and Chase, 1977a), show that snails learn that some odours derive from acceptable foods. Gastropods have also been reported to learn negative associations with food cues (Suzuki, 1968; Gelperin, 1975; Mpitsos et al., 1978). Thus, snails possess two complementary mechanisms for optimizing foraging. They select foods associated with favorable nutritional outcomes and avoid foods associated with deleterious outcomes.

Clearly, however, experiential factors do not completely determine food odour selection. An illustration of this is the fact that in most cases the difference between the orientation of the cucumber and the carrot groups could be largely accounted for by an increase in the preference for the cucumber odour by the animals fed cucumber. Little change occurred in the preferences of the animals conditioned to carrot. This may be explained by a ceiling effect whereby the innate preference for the carrot odour was already so great that it could increase only very little. In light of this, the conditioning to food odours might best be interpreted as a device for the modulation of pre-existing food odour preferences.

Hunger motivation had two principal effects in these experiments. One was that it reduced the latencies to make a choice in the olfactometer (Experiments II, VII, and VIII). The second was that hunger motivation interacted with the effects of experience to increase selection for the familiar food odour (Experiment IX). The latter result means that hungry animals are more likely than satiated snails to optimize foraging by orienting to a proven food source.

Experiment VIII shows that sensory adaptation of olfactory receptors plays little or no role as a

mechanism of physiological control over appetitive feeding behaviour. This suggests the involvement of a post-ingestional mechanism. Gut distension appears to regulate the consummatory phase in other gastropod species by producing satiation (Davis et al., 1977; Senseman, 1978; Susswein and Kupfermann, 1975). However, while a distended gut may signal the end of a meal, the search for a new meal is not necessarily initiated by an empty gut. In fact, this does not appear to be the case in *Achatina*. Experiment VII shows that food deprivation has little effect on food-finding behaviour for at least seven days after the last meal. If appetitive behaviour were regulated solely by gut distension, the gut should remain relatively full prior to the time when the effects of starvation are first seen. Our observations in the laboratory reveal, however, that most of the faeces produced after the cessation of an ad lib, diet are passed within the first two days. During that time, orientation changes in the olfactometer are not detectable. This suggests that while gut distension may play a role in the regulation of consummatory components of feeding, additional or alternative mechanisms must be invoked to account for the regulation of appetitive components.

The neurobiological analysis of consummatory components of feeding is now well advanced in several laboratories. Some progress has even been made in understanding the cellular mechanisms by which experience and motivation affect these behaviours (Chang and Gelperin, 1978; Davis and Gillette, 1978; Kupfermann et al., 1979). It is evident, however, that feeding is not a unitary act, but rather a sequence of responses each of which is regulated by different internal and external signals, or by a different set of criteria applied to those signals. Mechanisms involved in the regulation of one component may not be involved in the regulation of other components. The demonstration of plasticities in the appetitive component offers the opportunity to investigate the common and the unique aspects of each phase of feeding and to extend existing models to include temporal sequences of decisions leading to final dietary selection.

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